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Synthesis and characterization of 4-(2-aminoethyl)aniline imprinted polymer as a highly effective sorbent of dopamine

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ABSTRACT

The aim of the study was to develop an efficient sorbent for the separation of dopamine. 4-(2-Aminoethyl)aniline was chosen as a pseudo-template to produce the imprinted polymers from seven different functional monomers in the presence of ethylene glycol dimethacrylate as a cross-linker. The binding capacity showed that the highest binding specificity towards dopamine was achieved when methacrylic acid was used as the monomer in methanol solution to form a polymer matrix. The imprinting factor value was equal to 22.96. Other biogenic amines were bound much more weakly. A simple theoretical model was used to give an insight into the imprinting process and the selectivity of polymer matrix. Two artificial urine samples were used as the complex matrices to show the usefulness of the new sorbent for bioanalysis.

1. Introduction

Dopamine (2-(3,4-dihydroxyphenyl)ethylamine) is an important neurotransmitter involved in motor and cognitive functions [1]. It plays a significant role in cardiovascular, renal, and hormonal systems even at low concentrations [2,3]. Very low levels of dopamine in the brain may result in serious neurological diseases, the clinical manifestations of which would be tremors, muscle rigidity and postural instability [4]. Hence, the essential task was to monitor the fluctuations of dopamine levels in tissues or physiological fluids. The analysis of dopamine in urine is critical for biochemical diagnostics but difficult because the samples are very complex due to the coexistence of dopamine metabolites at micro- or nanomolar levels [5]. The current analytical tools of choice are electrochemical methods or chromatographic techniques, but both of them have major limitations. The main obstacle to the electrochemical determination of dopamine is the presence of electrochemically active compounds in the samples, which deteriorates the sensitivity and selectivity of detection. The chromatographic techniques required time-consuming sample clean-up and preconcentration steps [6–8]. Thus, the development of simple, sensitive, selective and cheap tools for the isolation of dopamine is still a challenge and an important scientific target.

Solid phase extraction (SPE) is the most popular method for isolation and preconcentration of analytes [9]. Although a lot of commercial stationary phases are available, their successful application is limited because of low selectivity. Among the promising modifications used for selective separation is the molecularly imprinted solid phase extraction (MISPE) [10], where the molecularly imprinted polymers produced by the imprinting technique provide the stationary phase with the desired selectivity towards a specific analyte [11,12].

This paper presents the synthesis and characterization of 4-(2-aminoethyl)aniline imprinted polymers as the effective sorbent for MISPE of dopamine. 4-(2-Aminoethyl)aniline was selected as the structural analog of the target analyte, i.e. dopamine, in the polymerization process. The strategy that used structural analogs during the imprinting process (the so-called pseudo-template strategy) is very useful because it allows one to avoid the bleeding of the target analyte from the polymer matrix during the analysis, which could bring about overestimated results [13]. Dopamine was used as the template by several scientific groups [14,15] but the properties of the obtained imprinted materials are not as good as those of the selective dopamine sorbents.

The molecular modeling was employed to rationalize the imprinting process and the dopamine adsorption by analyzing the energies and intermolecular interactions in prepolymerization complexes.

The binding properties towards 4-(2-aminoethyl)aniline and dopamine were measured for seven pairs of polymer sorbents. The adsorption and the surface characteristics were performed for the selected polymers. MISPE of dopamine from two models of

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artificial urines AU1 and AU2 were carried out in order to verify the applicability of the new sorbent. The obtained results were compared with those obtained for three commercial sorbents (C18, Florisil, MCX Oasis). The affinities towards the selected biogenic amines were also defined.

2. Experimental

2.1. Materials and methods

4-(2-Aminoethyl)aniline (the template molecule), 2-(3,4-dihydroxyphenyl)ethylamine (dopamine) hydrochloride (the target analyte), (*R*,*S*)-1-(3,4-dihydroxyphenyl)-2-aminoethanol (D,L-norepinephrine) hydrochloride, (*R*)-4-(1-hydroxy-2-(methylamino)ethyl) benzene-1,2-diol (L-epinephrine) D-hydrogen bitartrate salt, 3-(2-aminoethyl)-5-hydroxyindole (serotonin) hydrochloride, and 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) were purchased from Sigma-Aldrich (Steinheim, Germany). The functional monomers, allylamine (**1**), 4-vinylpyridine (**2**), acrylic acid (**6**) were from Fluka (Steinheim, Germany) and 2-phenylpropene (**3**), 4-vinylbenzoic acid (**4**), itaconic acid (2-methylidenebutanedioic acid, **5**), and methacrylic acid (**7**), were from Sigma-Aldrich (Steinheim, Germany). The cross-linker, ethylene glycol dimethacrylate (EGDMA), was from Fluka (Steinheim, Germany). Two solvents were used: methanol was from POCh (Gliwice, Poland) and acetone was from Chempur (Piekary Śląskie, Poland). Methanol (analytical grade) was purchased from POCh (Gliwice, Poland). The polymerization reaction initiator, 2,2′-azobisisobutyronitrile (2,2′-azobis(2-methylpropionitrile), AIBN), was from Merck (Darmstadt, Germany). The salts, urea, creatinine and methylene blue were from POCh (Gliwice, Poland). The monomers were purified prior to use by standard procedures (vacuum distilled or recrystallized from the appropriate solvents). All other reagents were used without purification. Ultra-pure water delivered from a Milli-Q purification system (Millipore, France) was used to prepare the water solutions.

The stock solutions of the analyzed compounds were prepared by weighting the appropriate amount of each compound and dissolving it in methanol or in water adjusted to pH 3 with 0.04 M perchloric acid [16] to obtain the concentration of 10 mmol L^{-1}. The standard solutions were prepared prior to use by dilution of the appropriate stock solutions with methanol–water (85:15 v/v) to obtain the required concentrations. All stock solutions were stored in dark at +8 °C.

The UV–vis measurements were performed with a UV-1605PC spectrophotometer (Shimadzu, Germany). The calibration lines data as well as the limits of quantification and the limits of detection of all analyzed compounds are given in Supplement S2.1.

The HPLC system consisted of a LC 10AT pump, a CTO 10A oven, and a SPD 10A UV detector operated at λ_{max}=234 nm (Shimadzu, Germany). Chromatographic separation was performed using a Gemini-NX C18 stainless steel column (150 mm × 4.6 mm ID, 5 µm, Phenomenex, North Valley, CA, USA), preceded by a 4 mm × 3 mm ID, Gemini-NX guard column. RP-HPLC was used for the quantitative determination of dopamine. The mobile phase consisted of 0.02 M pH 2.5 NaH_2PO_4 buffer delivered at a flow rate of 1 mL min^{-1}. The five-point calibration lines for dopamine in AU1 and AU2 were constructed as a function of peak area (x) versus concentration (y) in the range of 0.15–4.60 mg L^{-1}. The linearity of calibration lines was good with the correlation coefficients $r^2 > 0.999$. LOQs and LODs values were (in mg L^{-1}) as follows: 0.315, 0.116 and 0.116, 0.042 for AU1 and AU2, respectively.

2.2. Molecular modeling

Seven prepolymerization complexes between functional monomers – allylamine (**1**), 4-vinylpyridine (**2**), 2-phenylpropene (**3**), 4-vinylbenzoic acid (**4**), itaconic acid (**5**), acrylic acid (**6**) or methacrylic acid (**7**) and the template of 4-(2-aminoethyl)aniline – were analyzed by the computational methods at PM3 level of theory. The target analyte dopamine hydrochloride was also taken into account. Three-dimensional structures were drawn using the tools implemented in the software Hyperchem version 7.01 [17]. The structure of the components and the complexes were optimized until the energy gradient was below 0.01 kcal mol^{-1} $Å^{-1}$. The prepolymerization complexes were built up of four monomer molecules and one of 4-(2-aminoethyl)aniline molecule taking into account the molar ratio used in the synthetic procedure. The starting geometries of the complexes were constructed manually by placing the monomer around the template in such a way that the formation of as many hydrogen bonding interactions as possible was allowed between the monomers and the template functional groups. Starting distances between the atoms involved in the interactions were 2.5–3.0 Å. In our discussion we considered two parameters: the enthalpies of formation of prepolymerization complexes ($\Delta H_{complex}$) and the energies of the complexation reaction (ΔE) calculated using the following equation:

$$\Delta E = \Delta H_{complex} - \Delta H_{template} - 4\Delta H_{monomer} \quad (1)$$

2.3. Synthesis of polymers

The experimental quantities of reagents (moles, masses, and volumes) used for the preparation of different types of polymers are listed in Table 1. The molecularly imprinted polymers (MIPs) coded as MIP**1**–MIP**7** were prepared by the radical bulk polymerization. Briefly, 4-(2-aminoethyl)aniline as the template, the appropriate functional monomer, and ethylene glycol dimethacrylate, EGDMA (the cross-linker), were dissolved in methanol (the porogen) in thick-walled glass tubes. Then, the initiator of polymerization, 2,2'-azobisisobutyronitrile (AIBN), was added. The homogeneous solutions were purged with nitrogen for ca. 5 min and then the glass tubes were sealed. Subsequently, the polymerization was carried out under nitrogen atmosphere for 24 h at 64 °C. Non-imprinted polymers, NIP**1**–NIP**7** were prepared under the same polymerization conditions but without the template molecule and were treated in the same way as the corresponding imprinted polymers. The syntheses of MIP**7** and NIP**7** were

Table 1
Selected details of polymerization processes.

No. of MIPs	Template (mg, mmol)	Functional monomer (mg, mmol)	Cross-linker (µL, mmol)	Initiator (mg)	Porogen (µL)
1	27.2, 0.2	Allylamine (**1**), 45.7, 0.8	EGDMA, 754, 4.0	AIBN, 9.5	Methanol, 814
2	27.2, 0.2	4-Vinylpyridine (**2**), 84.1, 0.8			Methanol, 841
3	27.2, 0.2	2-Phenylpropene (**3**), 94.6, 0.8			Methanol, 858
4	27.2, 0.2	4-Vinylbenzoic acid (**4**), 118.5, 0.8			Methanol, 754
5	27.2, 0.2	Itaconic acid (**5**), 104.0, 0.8			Methanol, 754
6	27.2, 0.2	Acrylic acid (**6**), 57.7, 0.8			Methanol, 809
7	27.2, 0.2	Methacrylic acid (**7**), 68.9, 0.8			Methanol, 822

repeated five times. Post-polymerization procedure is described in Supplement S2.3.

2.4. Binding experiments

The stationary binding experiments were performed to evaluate the binding ability of MIPs and NIPs towards 4-(2-aminoethyl)aniline (the template) and dopamine (the target analyte). Polypropylene tubes of 10 mL were filled with 10 mg of MIP**1**–MIP**7** or NIP**1**–NIP**7** particles. To each tube 5 mL of 50 μmol L^{-1} of 4-(2-aminoethyl) aniline methanol–water (85:15 v/v) standard solution or 50 μmol L^{-1} of dopamine methanol–water (85:15 v/v) standard solution were added. The tubes were sealed and oscillated at room temperature for 24 h. Then the tubes were centrifuged for 10 min at 3000 rpm and the aliquots of supernatant (0.7 mL) were used for the analysis. For Scatchard analyses, polypropylene tubes were filled with 10 mg of MIP**7** or NIP**7** particles. Next, 5 mL of different 4-(2-aminoethyl) aniline methanol–water (85:15 v/v) standard solutions or dopamine methanol–water (85:15 v/v) standard solutions (concentrations ranging from 0.015 to 1.2 mmol L^{-1}) were added. For kinetics of dopamine, the tubes were prepared as above with the standard solution of dopamine in methanol–water (85:15 v/v) of the concentrations 50 μmol L^{-1}, but different times of oscillation were employed (0.25, 0.5, 1, 1.5, 3 and 6 h). The binding of dopamine was controlled for every synthesized MIP7 and NIP**7**.

The selectivity studies were performed as non-competitive stationary binding experiments. Polypropylene tubes of 10 mL were filled with 10 mg of MIP**7** or NIP**7** particles. In total, 5 mL of the standard solution (concentration of 50 μmol L^{-1} in methanol–water 85:15 v/v) of the analyzed biogenic compound (dopamine, D,L-norepinephrine, L-epinephrine, serotonin or L-DOPA) was individually added to the tube. Calculations details of the amounts of analytes are included in Supplement S2.4.

2.5. MISPE of dopamine from artificial urines

MISPE of dopamine from two artificial urine samples was carried out on Macherey-Nagel SPE manifold. Two artificial urines were prepared according to the established formulas AU1 [18] and AU2 [19] with minor modifications. The compositions of AU1 and AU2 are given in Supplement S2.5. The sample volumes of 0.1 mL of each artificial urine and 5 μL of stock solution of dopamine were transferred to a 10.0 mL volumetric flask and diluted to volume with methanol–water 85:15 v/v.

Polypropylene SPE columns of 1 mL (Chromabond, Germany) secured by glass-fiber frits were filled with 50 mg of MIP**7** as well as NIP**7** and commercial sorbents: C18 (J.T. Baker, Phillipsburg, NJ, USA), Florisil (Fluka, Switzerland), and MCX Oasis (Waters, MA, USA). The following steps of SPE protocol were applied on each column: conditioning (methanol–water 85:15 v/v, 1 mL), loading (2 mL of spiked artificial urine sample), washing (water, 1 mL), eluting (0.04 M aq. ammonium acetate–methanol, 30:70 v/v, 1 mL). The flow rate of each SPE step was 0.5 mL min^{-1}. The elution fractions were collected and used to analyze the amount of dopamine eluted from MIP**7**, NIP**7**, C18, Florisil, and MCX Oasis cartridges by HPLC. Triplicate cartridges of sorbents were used for each extraction. The loading fractions of AU1 samples spiked with dopamine were also collected and the concentration of dopamine not adsorbed on each particular sorbent was determined. The bound amount of dopamine was calculated by subtracting the unbound amount from the initial amount of dopamine.

2.6. Composition and morphology analyses

Thermogravimetric analyses (TGA) of MIP**7** and NIP**7** were performed at the Department of Chemistry, Warsaw University of Technology, Poland, on a Q600 thermogravimetric analyzer (TA Instruments, United States) in argon atmosphere with a heating rate 5 °C min^{-1}.

The ^{13}C CP/MAS NMR spectrum of MIP**7** in the solid-state was recorded at the Faculty of Pharmacy, Medical University of Warsaw, Poland, on a Bruker Avance DMX 400 spectrometer (Bruker, Germany). The powdered sample of polymer MIP**7** was contained in 4 mm ZrO_2 rotors and was spun at 8 kHz. The 90° pulse length was 2.15 μs. Contact time of 4 ms and repetition time of 10 s were used for the accumulation of 8200 scans. The chemical shifts δ ppm were referenced to TMS.

Surface morphologies of MIP**5**, MIP**7** and NIP**7** were studied on a Merlin FE-SEM (Zeiss, Germany) at the Department of Chemistry, University of Warsaw, Poland. The samples were Au/Pd sputtered-coated before the analysis.

Details of methylene blue adsorption experiments can be found in Supplement S2.6.

3. Results and discussion

3.1. Experimental binding capacity and imprinting factor

In order to determine the binding ability of the prepared polymers, the stationary experiments were carried out as described before. The binding capacities (B, μmol g^{-1}) of MIPs and NIPs were calculated according to the following equation [20]:

$$B = \frac{(C_i - C_f)V}{m} \quad (2)$$

followed by the calculation of distribution ratios (K_D, mL mg^{-1}) for MIPs and NIPs, according the following equation [21]:

$$K_D = \frac{(C_i - C_f)V}{C_f m} \quad (3)$$

where V represents the volume of the solution (mL), C_i stands for the initial solution concentration (mmol L^{-1}), C_f represents the solution concentration after adsorption (mmol L^{-1}) and m is the mass of polymer particles (mg).

The imprinting factors (IF) were calculated as follows [21]:

$$\mathrm{IF} = \frac{K_D\ (\mathrm{MIP})}{K_D\ (\mathrm{NIP})} \quad (4)$$

The binding capacities of MIP**1**–MIP**7**, NIP**1**–NIP**7** distribution ratios K_D, and imprinting factors IF for 4-(2-aminoethyl)aniline (the template molecule) and for dopamine (the target analyte) are presented in Table 2.

First, the binding capacities of polymer pairs MIP**1**/NIP**1**–MIP**7**/NIP**7** synthesized from seven functional monomers were investigated. The polymers were prepared from chemically different compounds: the basic monomers such as allylamine (**1**) and 4-vinylpyridine (**2**), the neutral monomer such as 2-phenylpropene (**3**), and the acidic monomers such as 4-vinylbenzoic acid (**4**), itaconic acid (**5**), acrylic acid (**6**), and methacrylic acid (**7**). Polymerization processes were carried out in methanol acting as the porogen, taking into account the solubility of all components. *The unpublished observations*: the prepolymerization complex formed of 4-(2-aminoethyl)aniline and methacrylic acid precipitated out in toluene and in some halogenated solvents such as chloroform, dichloromethane and 1,2-dichloroethane.

As it could be seen, the binding capacities of the template molecule were low for the polymers prepared from basic and neutral monomers (**1**)–(**3**). In contrast to that, the polymers synthesized from acidic monomers (**4**)–(**7**) were characterized by high or even very high (MIP**5**) binding capacities. The selectivity of all polymers except MIP**7** was very low or the non-imprinted

Table 2
Binding capacities of MIP**1**–MIP**7** and NIP**1**–NIP7 distribution ratios, K_D and calculated imprinting factors, IF towards the template and the target analyte.

No. of polymer	Template molecule (4-(2-aminoethyl)aniline)					Target analyte (dopamine)				
	Binding capacities ± S.D. (B, μmol g^{-1})		Distribution ratio (K_D, mL mg^{-1})		IF	Binding capacities ± S.D. (B, μmol g^{-1})		Distribution ratio (K_D, mL mg^{-1})		IF
	MIP	NIP	MIP	NIP		MIP	NIP	MIP	NIP	
1	1.543 ± 0.005	1.58 ± 0.02	0.030	0.031	0.98	0.458 ± 0.001	0.210 ± 0.001	0.008	0.004	2.20
2	1.511 ± 0.002	1.91 ± 0.02	0.029	0.038	0.78	0.188 ± 0.001	0.146 ± 0.001	0.003	0.003	1.28
3	2.01 ± 0.02	2.96 ± 0.04	0.040	0.061	0.65	0.437 ± 0.002	0.418 ± 0.003	0.008	0.008	1.05
4	8.4 ± 0.2	6.94 ± 0.07	0.282	0.213	1.33	3.26 ± 0.03	1.500 ± 0.009	0.067	0.029	2.33
5	20 ± 2	20.1 ± 0.9	3.011	3.248	0.93	22.6 ± 0.8	20.5 ± 0.6	2.279	1.438	1.58
6	6.42 ± 0.06	6.71 ± 0.05	0.191	0.203	0.94	2.72 ± 0.03	2.03 ± 0.02	0.055	0.040	1.38
7	7.6 ± 0.2	2.15 ± 0.03	0.390	0.070	5.55	9.6 ± 0.4	0.661 ± 0.003	0.308	0.013	22.96

counterparts bound better the template than MIPs. The calculated imprinting factors, IF, were in the range from 0.65 to 1.33. Only the polymer prepared from methacrylic acid (MIP**7**) showed significant affinity towards the template and IF equal to 5.55.

Low binding capacities of MIP**1**/NIP**1**, MIP**2**/NIP**2**, and MIP**3**/NIP**3** could result from weak interactions between basic groups of the template and those in the polymer matrices. The higher binding capacity for the polymers MIP**4**/NIP**4**–MIP**7**/NIP**7** prepared from the acidic monomers could be explained by strong interactions between the acidic groups existing in the polymer matrix and the amine groups in the template molecule. The very high binding capacity of the polymers prepared from itaconic acid (**5**) could be the result of the presence of a double amount of carboxyl residues in the polymer matrix.

The imprinting effect will be discussed in more detail on the basis of the results of theoretical computations in the next part of the paper.

The binding capacities towards the target analyte, dopamine, of the tested polymers were more diversified. For all imprinted polymers except MIP**5** and MIP**7** the obtained values were much lower than those for the template molecule, 4-(2-aminoethyl) aniline. For non-imprinted counterparts the binding capacities of dopamine were lower, which could be explained by higher specificity of polymer matrices towards dopamine. The polymer prepared from methacrylic acid (**7**) presents particularly promising properties: high binding capacity of dopamine (9.6 ± 0.4 μmol g^{-1}) on the imprinted matrix MIP**7** with simultaneously much lower binding capacity detected for the respective non-imprinted counterpart NIP**7** (0.661 ± 0.003 μmol g^{-1}). These resulted in a large increase of specificity towards dopamine with the IF value equal to 22.96. This is the evidence that the strategy involving the use of a structural analog of dopamine in the imprinting process allowed for the successful production of a highly specific polymer. The MIP**7**/NIP**7** polymers were synthesized five times, and their binding properties were preserved.

On the basis of the results obtained for dopamine adsorption, the polymer prepared from methacrylic acid synthesized in methanol (MIP**7**) was selected as the most appropriate candidate for further investigations and optimization of analytical protocols for dopamine isolation.

3.2. Theoretical analysis of polymer properties

During the theoretical investigations of MIP properties we assumed that the polymer which had the highest affinity to a given template should afford the highest interaction energy between the template and respective monomer molecules in the energy computations of the prepolymerization complexes [22,23]. This means that the complex structure formed in the prepolymerization solution should be preserved in the polymer matrix. In our discussion we considered two parameters of the energy: the enthalpies of formation of prepolymeric complexes ($\Delta H_{complex}$) and the energies of the complexation reaction (ΔE).

Details of the computation procedure are given in Section 2.2. The theoretical analysis showed that three prepolymerization complexes formed from allylamine (**1**), 4-vinylpyridine (**2**), and 2-phenylpropene (**3**) were unstable because they were characterized by the positive enthalpy of formation ($\Delta H_{complex}$) equal to 57, 186, 126 kcal mol^{-1}, respectively. These results suggest that the basic or aromatic monomers (**1**)–(**3**) should not be good candidates to form imprinting sites in the polymer matrix when 4-(2-aminoethyl)aniline is used as the template. The experimental studies confirmed the above statement: the determined imprinting factors were below 1 (see Table 2).

Indeed, in the weakest prepolymerization complex formed between the template and 4-vinylpyridine (**2**), the monomer (**2**) molecules did not interact with 4-(2-aminoethyl)aniline. Only the weak intermolecular interaction length of 2.9 Å was detected between H atoms of the amino group of the template and N atom of the pyridine ring.

The prepolymerization complexes formed from acidic monomers were stable and were characterized by negative values of $\Delta H_{complex}$ (on average −376 kcal mol^{-1}), but the calculations of the complexation energies ΔE revealed that only the reactions with itaconic acid (**5**), acrylic acid (**6**), and methacrylic acid (**7**) were characterized by favorable negative values of −13.4, −0.9, and −16.1 kcal mol^{-1}, respectively. The reaction with 4-vinylbenzoic acid (**4**) was thermodynamically unfavorable, and ΔE was found to be equal to 12.6 kcal mol^{-1}. The positive value for complexation with (**4**) and the value close to 0 for complexation with acrylic acid (**6**) could indicate that both monomers (**4**) and (**6**) were not suitable to form imprinting sites. Those findings agreed with the experimental data for all but one of the polymers, because only for the polymer formed from methacrylic acid (MIP**7**) the imprinting effect was observed. The polymer synthesized from itaconic acid (MIP**5**) had the highest binding capacity but its selectivity was low (IF=0.93), despite the negative values of $\Delta H_{complex}$ and ΔE energies. This discrepancy could be explained by the structure of the prepolymerization complex formed between itaconic acid and the template. The intermolecular interactions were dominated by strong hydrogen bonds formed between both carboxyl groups of the monomer, and this could be the reason why only non-selective binding sites were formed in the polymer matrix.

For the polymer formed from methacrylic acid (MIP**7**) we measured the highest IF for the template and even better for the target analyte – dopamine. In the prepolymerization complex formed between methacrylic acid (**7**) and the template, four hydrogen bonds of the length of 1.8–2.6 Å stabilized the complex, giving the opportunity to create imprinting sites. To explain the high affinity of dopamine to MIP**7** matrix we compared the

structural parameters of both molecules. Fig. 1 shows the overlay of dopamine on the template molecule in the prepolymerization complex formed by the template and methacrylic acid (**7**). Dopamine occupies the same position as the template molecule. The amino group of dopamine is located closer to the carboxylic group of the monomer than the amino group of the template forming stronger hydrogen bonds. In addition, the molecular volumes of both molecules are close: 505 for 4-(2-aminoethyl)aniline and 519 Å^3 for dopamine, which proved that dopamine can penetrate into cavities formed during imprintation. The above observations can explain the high adsorption of dopamine on the polymer matrix MIP**7**.

3.3. Selectivity of MIP7 towards dopamine

In order to characterize the adsorption profile of MIP**7**, the selectivity of MIP**7**/NIP**7** was evaluated on the basis of non-competitive stationary binding experiment carried out for biogenic compounds. The chemical formulas of biogenic compounds used in the experiment are shown in Fig. 2.

The binding capacities (B, μmol g^{-1}) of dopamine, serotonin, norepinephrine, epinephrine and L-DOPA on MIP**7** and NIP**7** were calculated according to Eq. (1). The selectivity factors for the imprinted as well as for the non-imprinted polymer were calculated as follows [24]:

$$\alpha = \frac{B_{dopamine}}{B_{analyte}} \quad (5)$$

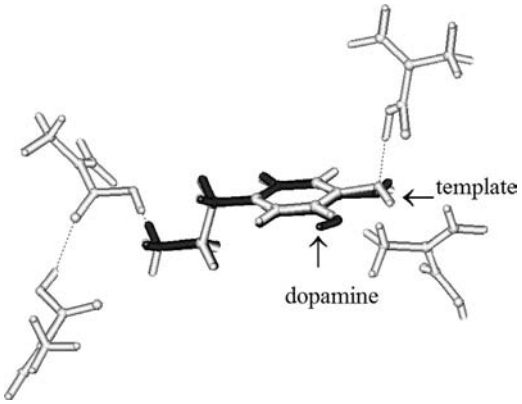

Fig. 1. The target molecule, dopamine, overlaid on the template in the prepolymerization complex.

where $B_{dopamine}$ represents the respective binding capacity of dopamine and $B_{analyte}$ stands for the binding capacity of the analyzed biogenic compound. The results are provided in Table 3.

Out of five biogenic compounds, MIP**7** showed the highest affinity towards dopamine. The adsorbed amounts of serotonin, norepinephrine, epinephrine and L-DOPA were significantly lower, and the high selectivity towards dopamine was observed. In our previous papers [25], we described the imprinted polymers that had similar affinity to dopamine as serotonin. Here, the 4-(2-aminoethyl)aniline imprinted polymer has well defined cavities which are available only to the phenylethylamine system. The bulk indole ring, additional functional groups such as hydroxyl, carboxyl or the N-methyl substituents, limited the adsorption on polymer matrix. All these findings indicated that MIP**7** should be a promising sorbent designed for dopamine isolation.

3.4. Characterization of binding sites of MIP7/NIP7

To analyze the adsorption parameters of MIP**7** and NIP**7** the stationary procedure was used towards the template and the target analyte as described before. The binding characteristics of the imprinted materials were characterized by the Langmuir model transformed to the Scatchard equation (Eq. (6)) [26]:

$$\frac{B}{F} = \frac{(B_{max} - B)}{K_d} \quad (6)$$

where B_{max} is the total number of binding sites, K_d is the dissociation constant, B is the bound amount of the analyte, and F is the unbound concentration of the analyte. The system which fits well the Langmuir model gives a straight line on the Scatchard plot with a slope equal to $(-1/K_d)$ and y-intercept equal to B_{max}/K_d. The binding isotherms were determined by adding a fixed amount of the polymer to various concentrations of the template molecule, 4-(2-aminoethyl)aniline or the target analyte, dopamine. The binding isotherms and Scatchard plots for MIP**7** and NIP**7** for

Table 3
Binding capacities of each biogenic compound bound to MIP**7** and NIP**7** in the non-competitive binding experiments.

Compound	Binding capacities ± S.D. (B, μmol g^{-1})		Selectivity factor (α)	
	MIP**7**	NIP**7**	MIP**7**	NIP**7**
Dopamine	9.6 ± 0.4	0.661 ± 0.003	–	–
Serotonin	2.50 ± 0.05	0.695 ± 0.009	3.86	0.95
Norepinephrine	1.87 ± 0.03	1.53 ± 0.02	5.17	0.43
Epinephrine	1.34 ± 0.02	1.615 ± 0.003	7.19	0.41
L-DOPA	1.27 ± 0.03	1.65 ± 0.05	7.61	0.40

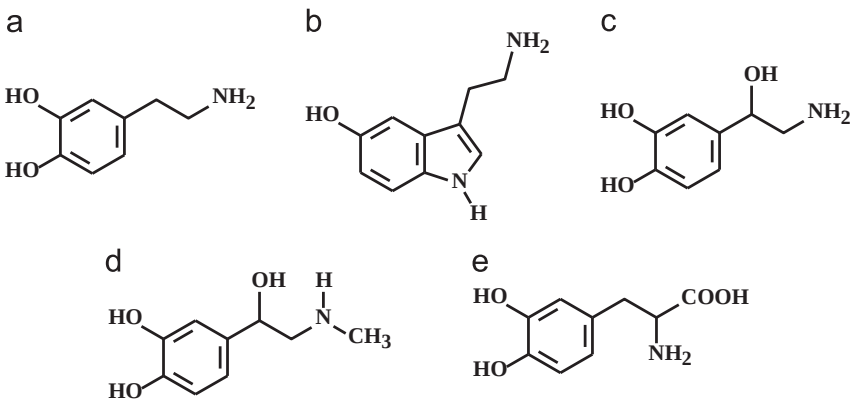

Fig. 2. The chemical formulas of biogenic compounds used in experiment: dopamine (a), serotonin (b), norepinephrine (c), epinephrine (d), and L-DOPA (e).

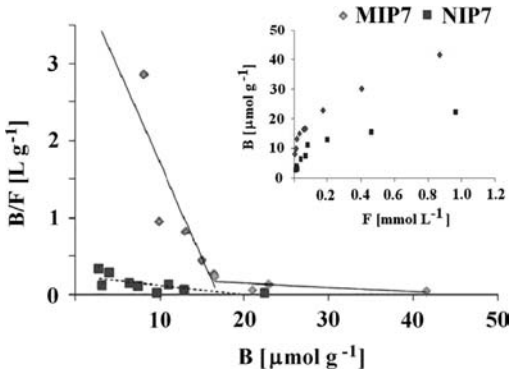

Fig. 3. Dopamine binding isotherms (upper part of figure) and Scatchards plots for MIP**7** and NIP**7**.

Table 4
The values of K_d and B_{max} of MIP**7** and NIP**7** for 4-(2-aminoethyl)aniline and for dopamine.

	4-(2-Aminoethyl)aniline		Dopamine	
	MIP**7**	NIP**7**	MIP**7**	NIP**7**
K_d (μmol L^{-1})	0.087 3.52	18.2	0.004 0.19	0.08
B_{max} (μmol g^{-1})	0.067 1.17	2.62	16.9 47.6	20

dopamine are presented in Fig. 3. The numerical data for 4-(2-aminoethyl)aniline and dopamine are collected in Table 4.

The Scatchard analysis revealed two straight lines for MIP**7** and only one for NIP**7** in adsorption experiments made with 4-(2-aminoethyl)aniline as well as made with dopamine. These results are typical of a pair of the imprinted and non-imprinted polymers obtained by the non-covalent preparation approach (Table 4), and well illustrated the imprinting process. The lower values of dissociation constants K_d and the higher values of B_{max} for dopamine than for the template are in good agreement with the experimentally determined binding capacities of the respective molecules.

In order to better characterize the affinity of MIP**7** towards dopamine, the kinetics of adsorption was examined. The adsorption occurred rapidly and increased considerably during the first 15 min, and then slowed down. The equilibrium was reached after 15 min and no significant changes were observed later. The kinetics satisfied the pseudo-second-order equation (Eq. (7)) which was employed [27] in the analysis:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e{}^2} + \left(\frac{1}{q_e}\right) t \tag{7}$$

where k_2 is the second-order-rate constant. The straight line ($y = 0.000163x + 0.000034$; correlation coefficient $r^2 = 0.991$) obtained for t/q_t vs. t is the evidence that adsorption can be described by a pseudo-second-order k_2 constant. The value of k_2 was determined graphically from the slope and intercept of the linear function against t. The calculated values k_2 and q_e were 7.81×10^{-4} g mg^{-1} min^{-1} and 6.13 g g^{-1}, respectively.

3.5. Morphology of particles

Another important parameter related to the binding capability of MIPs and NIPs is the particles morphology described as the macro- and microporous structure and specific surface area.

Table 5
Specific surface areas of MIP**1**–MIP**7** and NIP**1**–NIP**7**.

No. of polymer	Specific surface area ± S.D. (m^2 g^{-1})	
	MIP	NIP
1	4.6 ± 0.1	7.2 ± 0.2
2	2.99 ± 0.09	7.3 ± 0.5
3	8.9 ± 0.2	10.0 ± 0.2
4	102 ± 9	109 ± 7
5	277 ± 20	280 ± 20
6	83 ± 4	104 ± 4
7	99 ± 16	63 ± 3

The specific surface area of MIP**1**–MIP**7** and NIP**1**–NIP**7** was determined by the methodology proposed by Kaewprasit and co-workers, and successfully applied to the imprinted material [28,29]. The specific surface area is measured by methylene blue adsorption, and calculated using Eq. (8):

$$A_s = \frac{G N_{AV} \phi \times 10^{-20}}{M M_W} \tag{8}$$

where A_s is the imprinted and non-imprinted polymer specific surface area (m^2 g^{-1}), G is the amount of methylene blue adsorbed (g), N_{AV} is Avogadro's number (6.02×10^{23} mol^{-1}), ϕ is the methylene blue molecular cross-section (197.2 $Å^2$), M_W is the molecular weight of methylene blue (319.86 g mol^{-1}) and M is the mass of the imprinted and non-imprinted polymer (g). The values of specific surface areas of MIP**1**–MIP**7** and NIP**1**–NIP**7** are presented in Table 5.

The specific surface areas of polymers were strongly dependent on the composition of the polymer matrix. The polymers made up of basic and neutral monomers (**1**)–(**3**) have low specific surface areas, and NIPs were characterized by higher specific surface areas than respective MIPs. Low specific areas correlate well with low binding capacities of the polymers MIP**1**–MIP**3** and NIP**1**–NIP**3** (see Table 2). The polymer matrices produced from acidic monomers (**4**)–(**7**) had much higher specific surface areas. The highest specific surface areas of the polymers were determined for MIP**5** and NIP**5** as close to 280 m^2 g^{-1}. Both these polymers had the highest binding capacities towards the template molecule and the target analyte but they had no selectivity. Only a pair of polymers MIP**7**/NIP**7** prepared from methacrylic acid showed favorable differences in the specific surface areas. The experimental specific surface areas showed good correlation with the binding capacity.

To observe the texture of the particles, the field emission scanning electron microscopy (FE-SEM) was employed. The particles prepared from itaconic acid, MIP**5**, with the highest binding capacity but the imprinting factor close to 1, and the particles prepared from methacrylic acid of MIP**7**/NIP**7** with the highest imprinting factor were selected for the test. The obtained micrographs are presented in Fig. 4.

As it could be seen, all particles were typical of bulk polymerization with irregular shape and size of ca. 10–20 μm (Fig. 4a, c, e). Further magnification showed the differences in surface structure of MIP**5** and MIP**7**/NIP**7**. MIP**5** has a well-developed surface with a large number of holes and pores with the pore diameter of 200 nm and below (Fig. 4b). The micrographs of MIP**5** well correlate with the specific surface area analysis and the high binding capacity of MIP**5**. The surface of MIP**7** (Fig. 4d) is more diversified with numerous small and more or less spherical entities (see the arrow in Fig. 4d). The surface of NIP**7** (Fig. 4f) is much smoother than the surface of MIP**7** (see Fig. 4d). The micrographs illustrated the difference between specific surface areas of MIP**5**, MIP**7** and NIP**7** and different binding capacities.

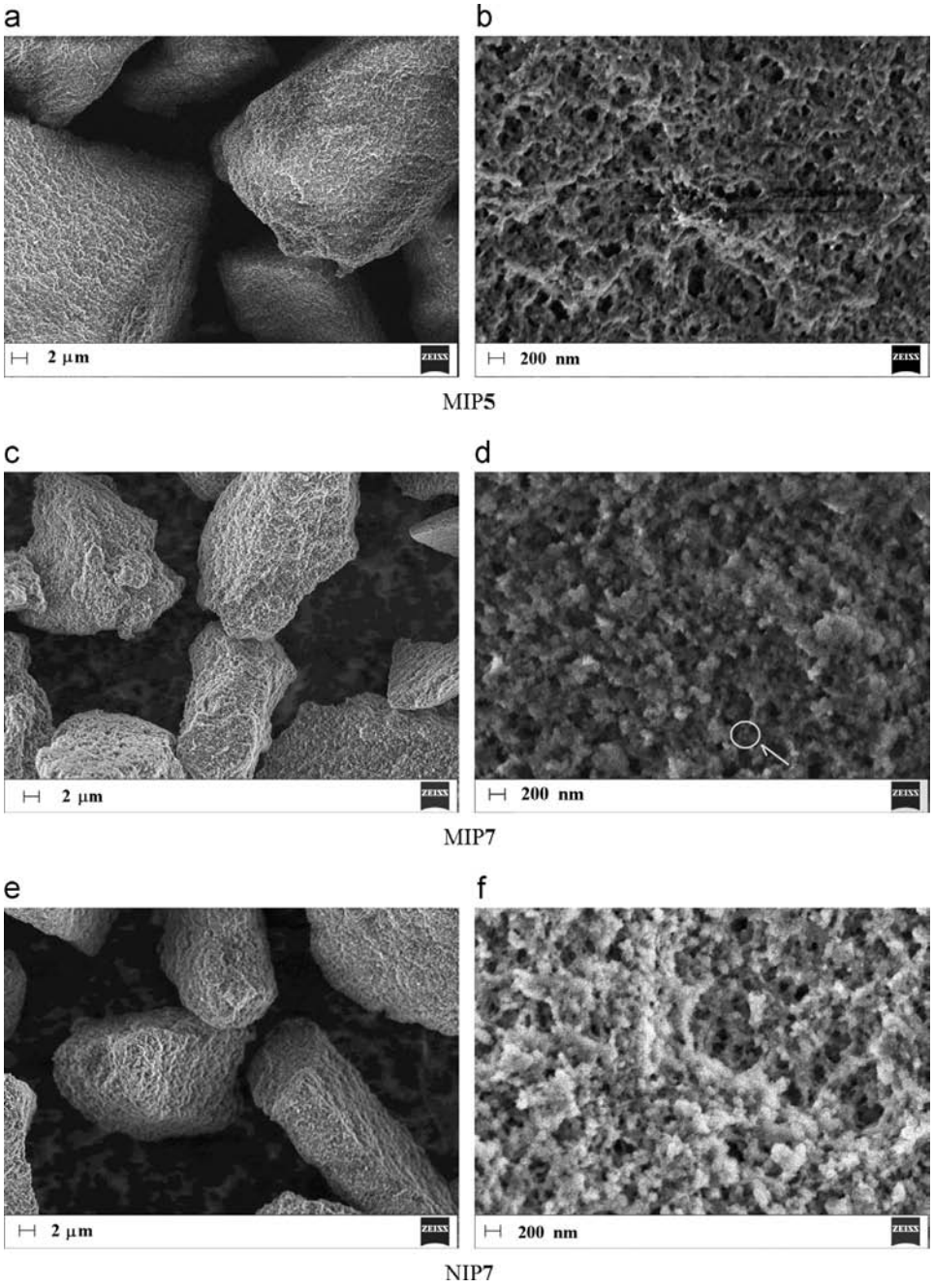

Fig. 4. Micrographs of MIP**5** (a,b), MIP**7** (c,d) and NIP**7** (e,f) particles.

3.6. Thermogravimetry and NMR solid-state spectroscopy measurements

The thermogravimetric analysis (TGA) and ^{13}C CP/MAS NMR spectroscopy could reveal information about the composition (or structure) of the imprinted and non-imprinted polymers. Hence, the TGA analysis of MIP7 and NIP7 was carried out to observe the difference between the degrees of degradation of each material as the function of temperature, and the NMR spectrum was measured to check the composition of polymer particles. The thermographs of MIP**7** and NIP**7** are presented in Fig. 5. Line (a) shows weight loss as the function of temperature and line (b) is a derivative of weight loss as the function of temperature.

Certain negligible differences were detected in the process of thermal decomposition of both polymers. The initial decomposition process of both polymers started at about 200–230 °C and continued until 450 °C. The decomposition of MIP**7** consists of two stages with the first maximum of weight loss at 254.9 °C and the loss of nearly 10% of total mass of the imprinted polymer, and the second maximum at 410.3 °C and the loss of nearly 83% of initial mass of the imprinted material. The total mass loss was 93%. The decomposition stages for NIP**7** were very similar, with the first maximum of weight loss at 231.4 °C and the loss of nearly 5% of total mass of the imprinted polymer, and the second twin maximum at 389.4 °C and 411.9 °C with the loss of nearly 91% of initial mass of the non-imprinted material. The total mass loss was 96%. It could be supposed that the initial decomposition is attributed to short chain degradation as well as the decarboxylation process which is also responsible for stable decomposition in the temperature range of 300–450 °C. The short maximum of weight loss at

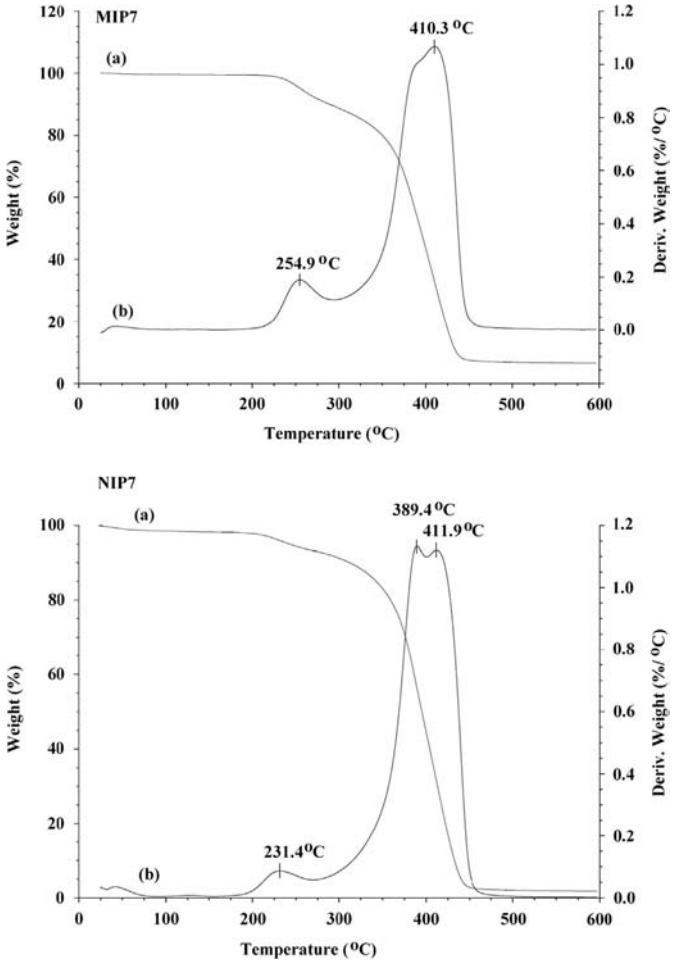

Fig. 5. Thermographs of MIP**7** and NIP**7**.

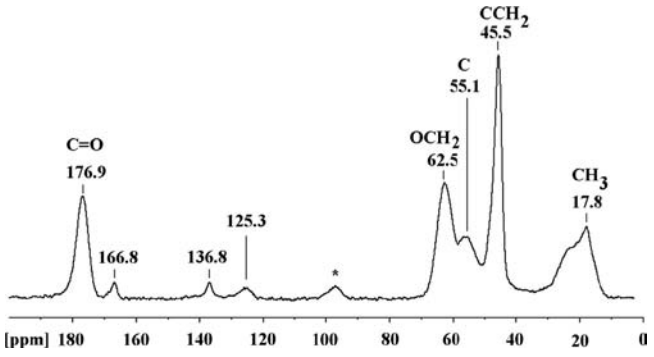

Fig. 6. The ^{13}C CP/MAS NMR spectrum of MIP**7** (sideband is marked as an asterisk).

about 30–40 °C is associated with the loss of intrinsically bound water [30]. The imprinting process does not change dramatically the stability of polymer matrix.

Fig. 6 presents the ^{13}C CP/MAS NMR spectrum of MIP**7** as an example to characterize the composition of polymer particles (the differences between NMR spectra of NIP**7** and MIP**7** are negligible – the composition is the same).

In the spectrum the resonances of all types of C atoms can be observed. Various CH_3 groups are represented by broad peaks located in the range 17–24 ppm. Methylene groups in OCH_2CH_2O linkers are found in the proximity of 62.5 ppm and those in C–CH_2–C groups at 45.5 ppm. Quaternary C atoms can be seen as broad peaks at 55.1 ppm. The carbon atoms from C═O groups are represented by a signal at 176.9 ppm. Low intensity resonances at 167, 137, and 125 ppm could be assigned to fragments with double bonds $CH_2{=}C(CH_3)CO_2R$. The ^{13}C CP/MAS NMR spectroscopy supported the composition of polymer matrix.

Table 6
SPE of dopamine from artificial urine samples spiked with dopamine (concentration of 0.74 mg L^{-1}, loading volume of 2 mL) on MIP**7**, NIP**7**, C18, Florisil, and MCX Oasis (n=3).

	MIP**7**	NIP**7**	C18	Florisil	MCX Oasis
SPE from AU1					
Elution, 1 mL (μg)	0.85 ± 0.05	0.20 ± 0.02	< L.Q.[a]	< L.Q.[a]	< L.Q.[a]
Total recovery	57.7%	13.7%	–	–	–
SPE from AU2					
Elution, 1 mL (μg)	0.92 ± 0.06	0.27 ± 0.02	< L.Q.[a]	0.116 ± 0.004	< L.Q.[a]
Total recovery	61.8%	18.2%	–	7.8%	–

[a] Below limit of quantification.

3.7. MIPSE of dopamine from artificial urine

In order to show the ability of the imprinted polymer to isolation of dopamine from complex samples we applied MIP**7** as a stationary phase in molecular imprinted solid phase extraction. The SPE protocol was optimized in loading, washing and elution steps based on the procedure that we worked out earlier [31]. The artificial urine was used as a complex matrix for the sample loading step because it is widely applied for in vitro cellular studies and assays [32,33]. The impact of two different formulas of artificial urine, *viz*. AU1 [18] and AU2 [19], was investigated. Both selected AU formulas have different compositions. AU1 contains urea, creatinine and inorganic salts, but AU2 contains various inorganic salts at the concentrations of selected salts above the physiological limits, but does not contain urea and creatinine. The total recovery of dopamine after MISPE from MIP**7** was determined. For comparison, SPE protocol was carried out on NIP**7** as well as on the commercial sorbents: C18 (non-polar sorbent), Florisil (polar sorbent), and MCX Oasis (ion-exchange sorbent). The obtained results are presented in Table 6.

The results show that the presented solid phase extraction procedure was appropriate for separation of dopamine from the artificial urines on the 4-(2-aminoethyl)aniline imprinted polymer. The total recoveries ranged from 57.5% to 61.8% depending on the composition of the multicomponent sample matrix. The non-imprinted polymer extracted only 13.7–18.2% of dopamine and commercial sorbents were unable to extract dopamine. In order to explain the reason of low extraction values for commercial sorbents we analyzed the amounts of dopamine bound on each particular sorbent when AU1 samples were loaded. We found that the amounts of dopamine bound to each sorbent were as follows: MIP**7**: 1.01 ± 0.06 μg, NIP**7** 0.74 ± 0.06 μg, C18: 0.47 ± 0.06 μg, Florisil: 0.61 ± 0.07 μg, and MCX Oasis: 1.2 ± 0.2 μg of dopamine. Those results revealed that the highest adsorptions of dopamine were observed on ion-exchange sorbent as well as MIP**7** but only the imprinted polymer was able to desorb dopamine when the proposed SPE protocol was applied.

4. Conclusions

The imprinted material obtained from 4-(2-aminoethyl)aniline as the pseudo-template and methacrylic acid as the monomer in methanol turned out to be a very good sorbent for dopamine isolation, and its high selectivity towards dopamine was observed

(the adsorbed amounts of serotonin, norepinephrine, epinephrine and L-DOPA were significantly lower). The presented solid phase extraction procedure was appropriate for the extraction of dopamine from different model multicomponent samples of artificial urine. The imprinted particles were more appropriate sorbents than the commercial materials of C18, Florisil and MCX Oasis. The adsorption characteristics of dopamine are described by the pseudo-second-order-rate constant $k_2 = 7.81 \times 10^{-4}$ g mg^{-1} min^{-1}, and the Scatchard equation parameters: K_d (0.004 and 0.19 μmol L^{-1}) and B_{max} (16.9 and 47.6 μmol g^{-1}).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.060.

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