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Preliminary Evaluation of Molecularly Imprinted Polymer Synthesized with Dopamine Hydrochloride as a Template

Piotr Luliński[a], Dorota Maciejewska[a], Magdalena Bamburowicz-Klimkowska[b] and Mirosław Szutowski[b]

[a] Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1 Str., 02-097 Warsaw, Poland.
Tel./Fax: +(4822) 5720643. E-mail: lulinski93@yahoo.com
[b] Department of Toxicology, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1 Str., 02-097 Warsaw, Poland.
Tel./Fax: +(4822) 5720730.

Abstract: A new easy approach to separation of dopamine hydrochloride is reported. The molecularly imprinted polymer (MIP) was used as the adsorbent material. The MIP was prepared employing dopamine hydrochloride as a template and methacrylic acid (MAA) with ethylene glycol dimethacrylate (EDMA) in co-polymerization process. Non-covalent prearrangement between dopamine hydrochloride and methacrylic acid in organic porogen prior to bulk polymerization was utilized. Preliminary chromatographic results showed that dopamine hydrochloride could be retained by the polymer and then eluted using optimized elution protocol. The interactions between template and monomers were proposed of the basis of semiempirical PM3 theoretical calculation.

Keywords: Molecularly imprinted polymers, Dopamine hydrochloride, Solid Phase Extraction

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Introduction

In our investigation related to selective enrichment and clean-up of the analytes containing catecholamines we found a new easy approach to separation of dopamine hydrochloride using molecularly imprinted polymer (MIP) as the adsorbent material.

Molecular imprinting is a method for preparing polymers with desired and predetermined selectivity [1, 2]. First, the molecule for which selectivity is desired, called template or target molecule, is allowed to prearrange prior to polymerization in presence of monomer(s) with different functional group(s) that can interact with template. After polymerization the target molecules are removed, leaving well-defined three-dimentional cavities with spatially oriented functional groups in the highly cross-linked polymer network.

Covalent and/or non-covalent interactions can be employed in prepolymerization prearrangement between template and functional monomer. Covalent bonds are enough strong, but non-covalent interactions require use of functional monomers which form stable self-assembled complexes. In the later approach different interactions are involved to give prepolymerization complexes, such as hydrogen bonding, electrostatic and hydrophobic interactions. Traditionally, free radical bulk polymerization is utilized to prepare rigid monolith following grinding and sieving. Simplicity and availability are the major advantages of this method. Unfortunately, particles obtained during grinding and sieving process are heterogenous due to size, shape and internal morphology. Recently, several other methods were established to give more homogenous products like nanobeads or thin imprinted films, but they have some disadvantages, i.e. use of large amount of template [3].

Molecularly imprinted polymers are synthetic materials that have some important advantages over biomolecules i.e. thermal and chemical stability, sample load capacity, reproducibility, and low cost of preparation [4, 5]. MIPs have been applied in wide range of analytical techniques as adsorbents for solid phase extraction (SPE) [6, 7], chiral separation [8], artificial antibodies [9], biosensor components [10], and they are use as synthetic mediators [11].

The possibility of employing molecularly imprinted polymers for solid phase extraction has been evaluated in many papers. Increasing interest is observed in the application of molecularly imprinted solid phase extraction (MISPE) in pharmaceutical science, and lot of drugs was separated, e. g. local anesthetics [12], β_2-antagonists [13], antibiotics [14]. We have found up to now only few reports concerned to separation of dopamine. Dopamine is an important neurotransmitter. Decrease of dopamine concentration in human brain leads to many serious diseases. Makote and Collinson [15] prepared dopamine templated nanoporous silica gel films. Results showed that obtained material is not selective and have an affinity for other catecholamines. Due to our best knowledge no one used non-covalent approach to prepared molecularly imprinted polymer capable to separation of dopamine hydrochloride.

Experimental

Materials and Instruments

Dopamine hydrochloride, methacrylic acid, ethylene glycol dimethacrylate, and 2,2'-azobis(izobutyronitryl) were purchase from Fluka (Germany). Ammonium formate was

purchase from POCh Gliwice (Poland). Acetonitryle was from Riedel-de-Haën (The Netherlands), methanol and chloroform were from Chempur (Czech Rep.), aceton and glacial acetic acid were purchase from POCh Gliwice (Poland). All reagents were analytical grade or equivalent, and were used without further purification.

Solid phase extraction was carried out on Bakerbond SPE glass column, 1 ml (J. T. Baker, The Netherlands).

Chromatografic studies were performed using a Shimazu liquid chromatograph system with pump (LC-10 AT, Japan), spectrofluorimetric detector (RF 551, Japan), and 20 µl injector (Rheodyne 7725, Japan). Separation was carried out on a stainless steel column: 150 mm x 4,6 mm i. d., 5 µm (Discovery HS F5, Supelco, Bellefonte, PA, USA). The column was run at a flow rate: 1.0 ml/min (temp. 40^{o}C) using 0.05 M ammonium formate – methanol (3:1 v/v) as a mobile phase, and the detector was set at λ_{EX} = 280 nm and λ_{EM} = 315.

Preparation of Molecularly Imprinted Polymer

Dopamine hydrochloride (0.095 g, 0.5 mmol), methacrylic acid (0.344 g, 4 mmol), ethylene glycol dimethacrylate (5.95 g, 30 mmol) and 2,2'-azobis(izobutyronitryle) (65 mg) were mixed in 12.5 ml of chloroform and the mixture was purged with nitrogen for polymerization at 60^{o}C for 24 h. The resulting monolith was ground with mortal and pestle and put into Soxhlet apparatus to extract template (6 cycles, methanol –acetic acid, 1:1 v/v) followed by continuous wash to remove remains of unreactants and/or byproducts (6 cycles, acetonitrile). Then the polymer was wet-sieved into particles below 45 µm diameter (Retsch, Germany). Fine particles were removed by repeated decantation in acetone (10 x 5 min). The remain particles were put into column and washed with ammonium formate – methanol till no template was found in the washing solution. Finally particles were dried under vacuum and used for following studies. The control polymer was synthesized and treated in the same way as corresponding imprinting polymer except that no target molecule was added and no extraction of template was performed.

Molecularly Imprinted Solid Phase Extraction Protocol

The polymer particles, 56.2 mg were dry packed into SPE columns. During the solid phase extraction protocol we prewashed the particles with 1 ml of water followed by conditioning with 2 ml of phosphorate buffered saline, pH 8. The column was loaded with 2 ml of 1 µg/ml solution of dopamine hydrochloride and washed with 1 ml of water. Elution

stages were performed using 0.5 ml of 0.05 M ammonium formate (3:1 v/v) in the pH range from 6 to 3.

Results and Discussion

Preparation of dopamine imprinted polymer was not easy task. Dopamine and its solution are unstable even in room temperature and when exposed to the light. Compound is available only as a stable hydrochloride salt. We have decided to prepare MIP in a single step by bulk polymerization at 60^{o}C with dopamine hydrochloride as a template. The several systems were tested, including different ratios between template and monomers (unpublished results). Here, we present results of most appropriate system for separation of dopamine hydrochloride containing methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), and chloroform as a porogenic solvent. We also synthesized a control polymer following the same procedure but without dopamine hydrochloride.

Methacrylic acid was extensively studied as a functional monomer and is widely used for various target molecules [16, 17]. It is well-known that this functional monomer shows strong non-specific interactions with templates [18]. On the basis of the PM3 calculation we evaluated the ability of dopamine cation and methacrylic acid to form two types of complexes, one with three molecules of monomer, named A (Fig. 1) and second one with six molecules, named B (Fig. 2).

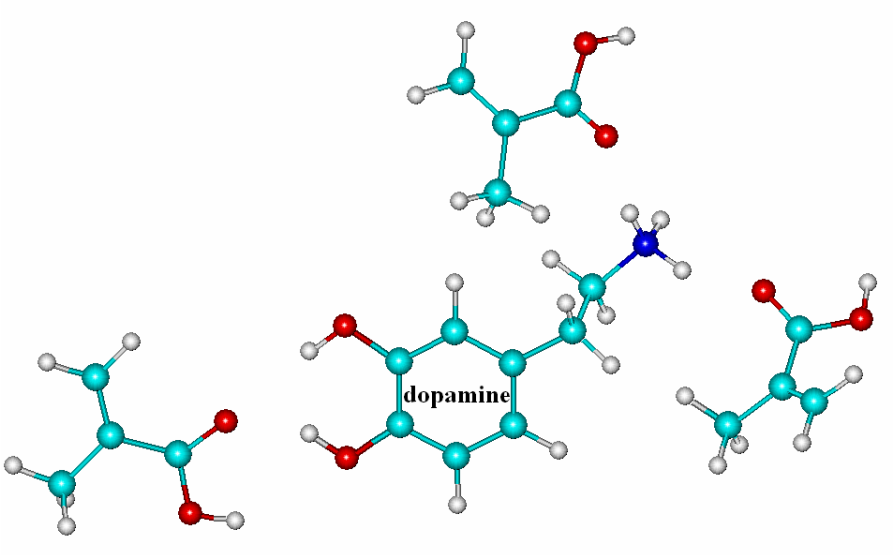

Fig. 1. Theoretical complex of A.

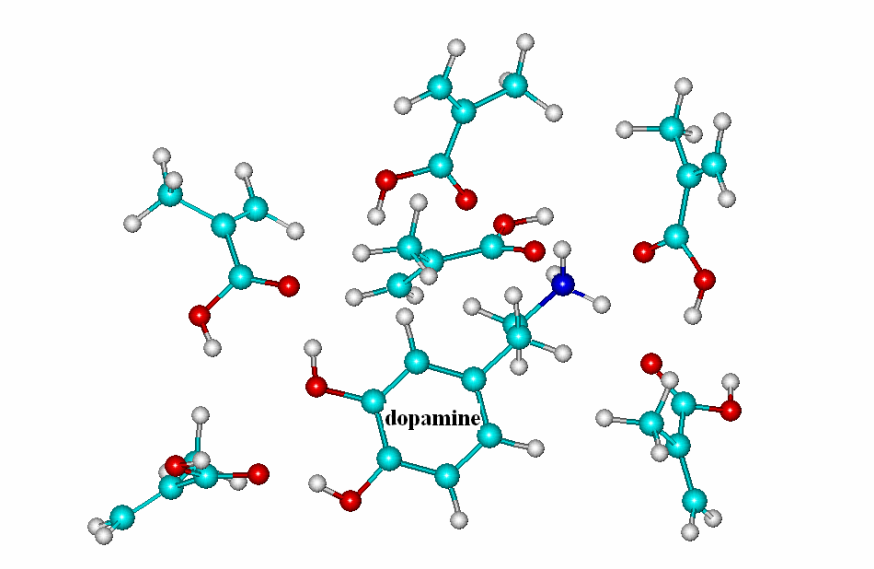

Fig. 2. Theoretical complex of B.

The starting geometries of these complexes were generated by replacing optimized components in the same file. The energies of complexes A and B are smaller by -43.1 and -66.6 kcal/mol, respectively. Energies were calculated as: $\Delta E = \Delta H_{complex} - n\Delta H_{MAA} - \Delta H_{dopamine\ cation}$. Apparently, both complexes represent favourable structures in solution.

Both polymers, imprinted and blank, were employed for solid phase extraction of dopamine hydrochloride and then were evaluated by HPLC for their imprinting effects. Experiments were performed in duplicate. The polymers were tested using water as a washing solvent and ammonium formate – methanol (3:1 v/v) as an eluent. It was also necessary to introduce gradient pH elution to improve recovery of dopamine hydrochloride.

Table. Recovery of dopamine hydrochloride using ammonium formate – methanol (3:1 v/v) as an eluent, eluted in gradient pH.

Fractions	Recovery (%)	
	Control polymer	Imprinted polymer
Load (2 ml)	9.6	0
Water wash (1 ml)	2.2	0
Ammonium formate – methanol (3:1) pH 6 elution (0.5 ml)	3.6	34.1
Ammonium formate – methanol (3:1) pH 5 elution (0.5 ml)	5.3	26.5
Ammonium formate – methanol (3:1) pH 4 elution (0.5 ml)	2.9	7.1
Ammonium formate – methanol (3:1) pH 3 elution (0.5 ml)	2.3	13.1
Total recovery	25.9	80.8

Results show (Table) that dopamine hydrochloride was retained by the imprinted polymer through the load and washing steps. Total recovery from imprinted polymer was high compared to blank polymer. This result is probably related with high non-specific binding of methacrylic acid. It will be the aim of our further investigations.

Conclusions

These preliminary results show that appropriate MIP adsorbent was synthesized and optimal SPE protocol was performed. Further investigations are under way.

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