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New sorbent materials for selective extraction of cocaine and benzoylecgonine from human urine samples☆

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ABSTRACT

An increase in cocaine consumption has been observed in Europe during the last decade. Benzoylecgonine, as a main urinary metabolite of cocaine in human, is so far the most reliable marker of cocaine consumption. Determination of cocaine and its metabolite in complex biological samples as urine or blood, requires efficient and selective sample pretreatment. In this preliminary study, the newly synthesized sorbent materials were proposed for selective extraction of cocaine and benzoylecgonine from urine samples. Application of these sorbent media allowed to determine cocaine and benzoylecgonine in urine samples at the concentration level of 100 ng/ml with good recovery values as 81.7% ± 6.6 and 73.8% ± 4.2, respectively. The newly synthesized materials provided efficient, inexpensive and selective extraction of both cocaine and benzoylecgonine from urine samples, which can consequently lead to an increase of the sensitivity of the current available screening diagnostic tests.

1. Introduction

Illicit drug consumption constitutes incalculable socioeconomic consequences, including health treatment costs, economic damage and growing crime rate [1]. Increase in drug consumption, mainly of cocaine (COC), cannabis and amphetamine-type stimulants, has been occurred in Europe during the last decade [2]. After consumption, COC is metabolized to benzoylecgonine (BE) and ecgonine methyl ester (EME). BE and EME, as the primary human metabolites, are excreted in urine in amounts to 45% and 40% of the consumed dose, respectively [3]. Therefore, determination of BE in urine has been confirmed to be a reliable marker of COC consumption. The routinely used immunoassay screening tests are able to detect COC and BE in urine at the concentration level of 300 ng/ml [4]. Due to complexity and presence of many interfering compounds in biological samples, such as urine, efficient and selective sample treatment is required prior to analytical determination [5]. This approach could improve sensitivity of currently applied diagnostic methods which aim to confirm COC consumption.

In this study, newly synthesized molecularly imprinted polymer sorbents were proposed for selective extraction of COC and BE from urine samples. As template molecules, atropine (ATRO) and scopolamine (SCOP) were suggested based on theoretically proved similarity of their structures to target analytes. Newly synthesized sorbents were applied for extraction of COC and BE from urine samples. Regarding preliminary results obtained in this study it could be concluded that newly developed media may provide an efficient, inexpensive and selective sample preparation before COC and BE estimation in biological matrices.

2. Material and methods

2.1. Computational design of molecularly imprinted polymers

Prior to synthesis of new sorbent materials molecular calculations were performed using the Gaussian 03 program [6]. The computational calculations conducted in this study include procedure composed of four steps. Firstly, selection of the suitable template and functional monomers was performed. Afterwards,

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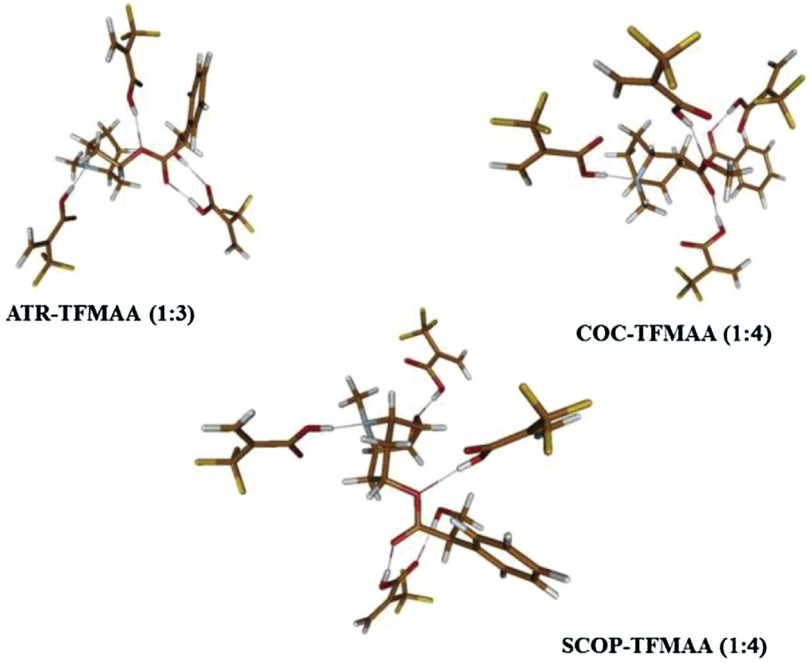

Fig. 1. The most stable complex TFMAA with ATR, SCOP and COC, respectively.

ground state geometry of the template molecule, functional monomer and complex were obtained by the DFT [7] approach utilizing B3LYP method. Finally, the model of the template–monomer complexes were set up. For each selected functional monomer, the most stable template–monomer complexes were searched and their interaction (binging) energy, ΔE [8,9], were calculated using following formula:

$$\Delta E = |E_{(\text{complex})} - E_{(\text{template})} - E_{(\text{monomer})}|$$

2.2. Chemicals

2-(Trifluoromethyl) acrylic acid (TFMAA, purity 98%), methacrylic acid (MAA, purity 99%), ethylene glycol dimethacrylate (EGDMA, purity 98%), trimethylolpropane trimethacrylate (TRIM, purity 98%) were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). 2,2′-Azobis(2-methylpropionitrile) (AIBN, purity 98%) was purchased from Fluka (Buchs, Switzerland). Anhydrous solvents for synthesis of polymers (toluene, dimethyloformamide) were obtained from POCh (Gliwice, Poland). HPLC grade acetonitrile, methanol and acetic acid came from J.T. Baker (Mallinckrodt Baker, Deventer, The Netherlands). Water was obtained using a Milli-Q ultrapure water producing system (Millipore, Bedford MA, USA). The standard solutions of COC and BE were purchased from Cerilliant (Round Rock, Texas, USA).

2.3. Polymer syntheses

The classical polymerization approach was applied to synthesize molecularly imprinted polymer (MIP) media. The template (atropine or scopolamine) was added to porogenic solvent (toluene or dimethyloformamide) When the template was fully dissolved, appropriate functional monomer (TFMAA or MAA) was added. The solution was stirred for 12 h at room temperature until homogenous prepolymerization complex was formed. Then crosslinking monomer (EGDMA or TRIM) and initiator (50 mg of AIBN) were added. The prepolymerization mixture was sonicated and deoxygenated with nitrogen for 15 min while it was cooled in an ice bath. The tubes were sealed and transferred into a thermostated water bath (60 °C) for 24 h. After polymerization, the tubes were crushed, and the template was removed from polymer by applying the acetic acid/methanol (1/9 v/v) as solvent in Soxhlet apparatus for 24 h. Finally, polymers were dried in vacuum overnight at 60 °C. The obtained polymer block was initially broken into pieces and then ground in the laboratory grinder. To obtain appropriate particle sizes, the ground polymer was sieved through a set of sieves: 63, 90 and 120 μm. Particles larger than 120 μm were grounded again. The fractions purified through decantation were tested under a microscope. Non-imprinted polymers (NIPs) were prepared under identical conditions except that there was no template present during polymerization.

2.4. Binding efficiency of newly synthesized sorbent materials

The binding experiments have been performed in tap water to check binding efficiency and consequently choice the most suitable sorbent for selective extraction of COC and BE from urine samples. Particles of prepared polymers (20 mg) were mixed with 1 ml of standard solution of COC and BE in an eppendorf microtube. Sam-

Table 1
Results of the binding energy ΔE calculations of the complexes [kcal/mol] of atropine, scopolamine and cocaine with TFMAA and MAA, respectively.

Prepolymerization mixture	Complex (1:1) ΔE [kcal/mol]	Complex (1:2) ΔE [kcal/mol]	Complex (1:3) ΔE [kcal/mol]	Complex (1:4) ΔE [kcal/mol]
Atropine–TFMAA	19.1	33.4	39.6	–
Scopolamine–TFMAA	18.8	35.4	42.9	49.6
Cocaine–TFMAA	14.3	27.0	41.0	45.6
Atropine–MAA	18.0	31.4	36.0	–
Scopolamine–MAA	18.4	31.9	40.3	46.6
Cocaine–MAA	10.2	17.3	27.4	32.5