Talanta 85 (2011) 1680–1688

Contents lists available at ScienceDirect

Talanta

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

Rationally designed molecularly imprinted polymers for selective extraction of methocarbamol from human plasma

Mohammad Bagher Gholivand*, Mehdi Khodadadian

Department of Analytical Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran

ARTICLE INFO

Article history:
Received 10 January 2011
Received in revised form 26 May 2011
Accepted 27 June 2011
Available online 2 July 2011

Keywords:
Molecularly imprinted polymer (MIP)
Methocarbamol
Density functional theory (DFT)
Solid-phase extraction (SPE)
Differential pulse voltammetry (DPV)

ABSTRACT

Molecularly imprinted polymers (MIPs) with high selectivity toward methocarbamol have been computationally designed and synthesized based on the general non-covalent molecular imprinting approach. A virtual library consisting of 18 functional monomers was built and possible interactions between the template and functional monomers were investigated using a semiempirical approach. The monomers with the highest binding scores were then considered for additional calculations using a more accurate quantum mechanical (QM) calculation exploiting the density functional theory (DFT) at B3LYP/6-31G(d,p) level. The cosmo polarizable continuum model (CPCM) was also used to simulate the polymerization solvent. On the basis of computational results, acrylic acid (AA) and tetrahydrofuran (THF) were found to be the best choices of functional monomer and polymerization solvent, respectively. MIPs were then synthesized by the precipitation polymerization method and used as selective adsorbents to develop a molecularly imprinted solid-phase extraction (MISPE) procedure before quantitative analysis. After MISPE the drug could be determined either by differential pulse voltammetry (DPV), on a glassy carbon electrode modified with multiwalled-carbon nanotubes (GC/MWNT), or high performance chromatography (HPLC) with UV detection. A comparative study between MISPE-DPV and MISPE-HPLC-UV was performed. The MISPE-DPV was more sensitive but both techniques showed similar accuracy and precision.

1. Introduction

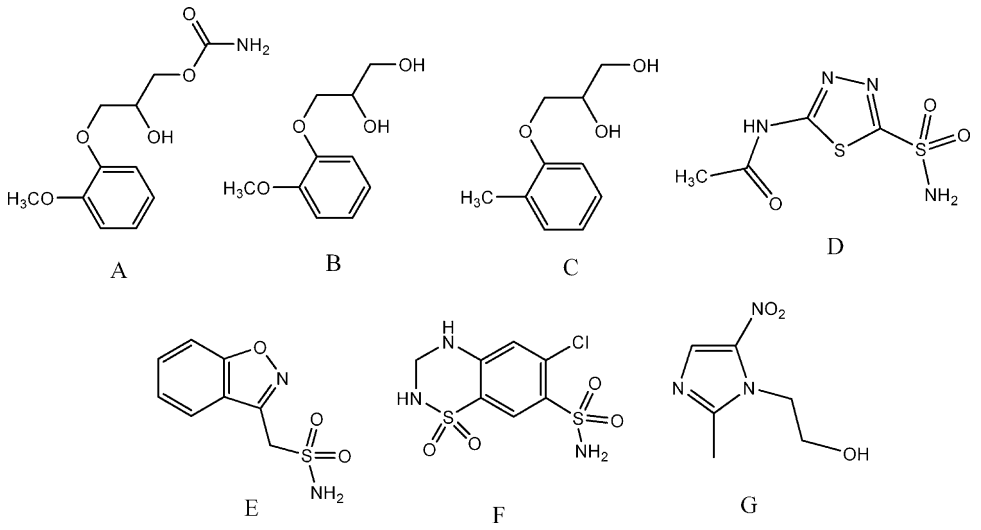
Methocarbamol (Scheme 1), a carbamate structural analogue of the aryl glycerol ethers, is a centrally active muscle relaxant and widely used for the treatment of skeletal muscle conditions of pain and discomfort [1,2]. Methocarbamol is easily absorbed from the intestine and widely distributed in all body tissues [3]. Because of potential for severe side effects, this drug is on the list for high risk medications in the elderly [4].

The sensitive and fast monitoring of methocarbamol in body fluids is of great interest from both pharmacokinetic and forensic aspects [4–6]. The determination of methocarbamol in biological fluids is usually carried out by chromatographic techniques [6–9]. Compared to chromatography, voltammetric techniques have several advantages such as their low cost, sensitivity and short analysis time. However, one of the drawbacks of conventional voltammetric techniques is their low selectivity for target molecules in complex matrices such as biological samples [10]. To overcome this problem, a clean-up procedure such as liquid–liquid extraction (LLE) or solid-phase extraction (SPE) should be applied to reduce matrix complexity prior to quantitative analysis. In the past, LLE has played a major role in sample clean-up and preconcentration of the sample components to be measured. However, recovery of sample components by liquid extraction is seldom complete. Liquid extraction tends to be slow and labor-intensive. Additionally, more stringent environmental concerns are making the use and disposal of large amounts of organic solvents more difficult. On the other hand, the popularity and use of SPE are growing at a fast rate. SPE is easily automated, faster, and in general more efficient than LLE. However, the selectivity of commercial SPE sorbents is low and this makes a problem when a selective extraction from a complex matrix has to be performed.

To enhance the molecular selectivity in SPE, molecularly imprinted polymers have been developed [11–15]. MIPs are cross-linked macromolecules bearing “tailor-made” binding sites for target molecules. They are prepared by the complexation, in solution, of a target compound (template) with functional monomers, through either covalent or non-covalent bonds, followed by polymerization with an excess of cross-linker to form a highly cross-linked polymer network. Upon removal of the template molecule from the polymer network, specific recognition sites that are complementary to the template in terms of their size, shape, and functionality are exposed [16,17]. As a result of their chemical and physical robustness, in combination with the

* Corresponding author. Tel.: +98 831 4274557; fax: +98 831 4274559.
E-mail address: mbgholivand@yahoo.com (M.B. Gholivand).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.06.066



Scheme 1. Chemical structures of: (a) methocarbamol, (b) guaifenesin, (c) mephenesin, (d) acetazolamide, (e) zonisamide, (f) hydrochlorothiazide and (g) metronidazole.

polymer's selectivity, MIPs have proven to be good adsorbents for MISPE applications [18,19]. Nevertheless, general molecular imprinting protocols are tedious and time-consuming because they are based on trial and error method to find the best conditions for imprinting process.

Recently the combinatorial and computational methods have been considered as alternative approaches for the rational design of MIPs [20–22]. The characterization of molecular complexes formed between templates and monomers, with the aim of achieving a clearer picture of the interactions that are the basis of MIP technology, has been the goal of numerous theoretical studies [23]. Over the past few years, a number of studies have been reported describing the application of *ab initio* and DFT computational methods in the rational design of MIPs [21,24,25]. However, the long computation times required for geometry optimization is one of the drawbacks of such calculations. One way of reducing computation time, however, is to combine the fast computational methods such as molecular mechanics (MM) or semiempirical calculations with *ab initio* or DFT methods. For example, a large library of functional monomers can be screened fast by applying a semiempirical method followed by a DFT optimization on the most stable structures. This will reduce the computation time by the fast rejection of functional monomers which do not interact with the template molecule intensively.

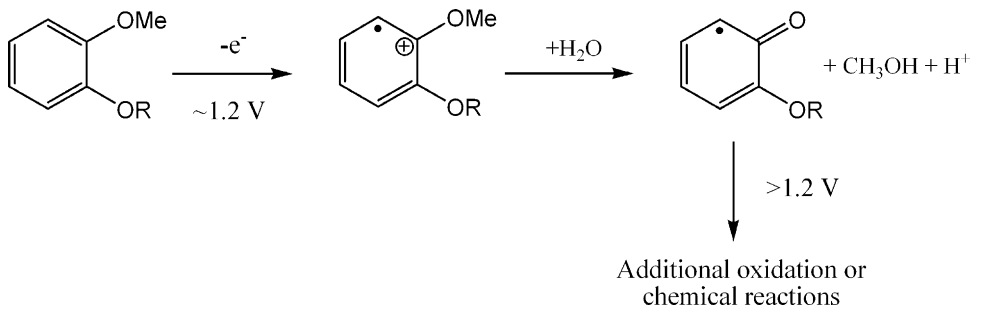
Electroanalytical techniques have some important advantages including speed, high sensitivity, relative simplicity and low costs compared to other techniques. In recent years, the application of carbon nanotubes in electrode modification has received remarkable attention in electrochemistry [26–32]. The modification of electrode substrates with MWNTs would result in low detection limits, reduced overpotentials and resistance to surface fouling and therefore MWNTs have been claimed as electrocatalysts [26,28,29,32].

Herein, the semiempirical and DFT-quantum mechanical calculations were used for the rational design of methocarbamol-imprinted polymers. The MIPs were then synthesized to develop a MISPE procedure for the selective extraction of methocarbamol from human plasma before quantitative analysis by DPV or HPLC-UV.

2. Experimental

2.1. Materials

Methocarbamol (99.8%), mephenesin, guaifenesin, zonisamide, hydrochlorothiazide, metronidazole, acetazolamide and 4-vinylpyridine (4-VP) all were purchased from Sigma (Madrid, Spain). A standard solution of 100 $\mu g\,mL^{-1}$ of methocarbamol was prepared by dissolving an appropriate amount of the drug in methanol. This solution was stored at dark and 4 °C. Other diluted solutions were prepared by dilution from the standard solution. Acrylic acid (AA), ethylene glycol dimethacrylate (EGDMA), 2,2′-azobis(isobutyronitrile) (AIBN), triethylamine (TEA), trifluoroacetic acid (TFA) and HPLC grade solvents such as methanol (MeOH) and acetonitrile (MeCN) were purchased from Merck (Darmstadt, Germany). All monomers were distilled under the reduced pressure to remove their stabilizers before use. Human plasma samples were obtained from healthy volunteers and stored at −20 °C until use. All other chemical were of analytical reagent grade and used without further purification.



Scheme 2. Possible electrode reaction of methocarbamol.