12th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-12) 1-30 November 2008
http://www.usc.es/congresos/ecsoc/12/ECSOC12.htm & http://www.mdpi.org/ecsoc-12

Preliminary Evaluation of Molsidomine Imprinted Polymers

[F0003]

Piotr Luliński and Dorota Maciejewska

Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1 Str., 02-097 Warsaw, Poland
Tel./fax: +48 22 5720643, e-mail: piotr.lulinski@wum.edu.pl

Abstract: Preliminary results of the recognition and release of molsidomine from molecularly imprinted polymers are reported. The molecularly imprinted polymers (MIPs) were prepared with molsidomine as a template molecule through radical polymerization. Eight different functional monomers were used with ethylene glycol dimethacrylate as crosslinker and dimethylsulphoxide as porogenic solvent. Non-covalent interactions between molsidomine and each functional monomer in DMSO prior to thermal bulk polymerization was utilized. Parallel stationary and dynamic binding experiments on both MIPs and NIPs (non-imprinted control polymers) were applied to verified imprinting process. Prepared polymers bound and remove molsidomine not selectively, but drug release properties were satisfactory.

Keywords: Molecularly imprinted polymers, molsidomine, controlled release

Introduction

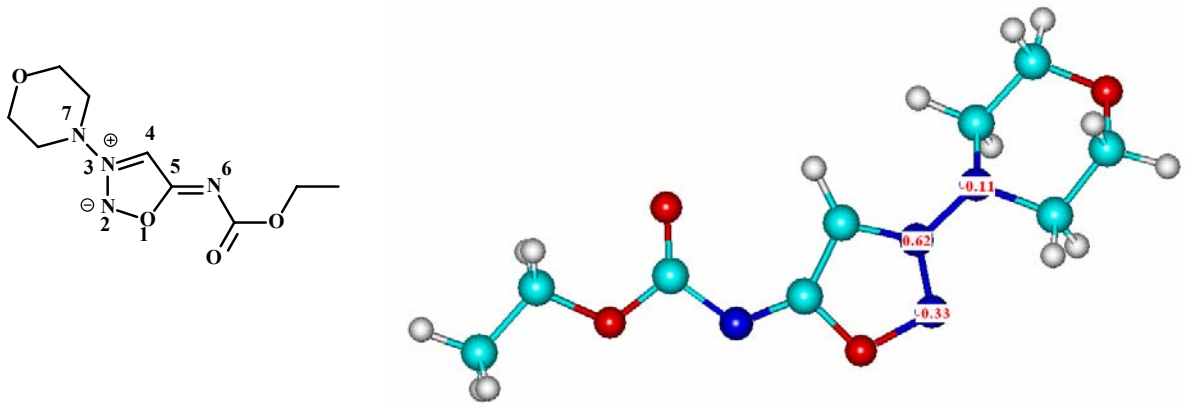
Molecular imprinting is a technique for preparing polymers with desired and predetermined selectivity [1]. Functional groups of template molecule (for which selectivity is desired) were used to create interactions with monomer(s) prior to polymerization process. Template molecules are extracted after polymerization, leaving well-defined, three-dimentional cavities with spatially oriented functionalities in the highly cross-linked polymer network. In their most common form, MIPs are prepared as a monolith, which is than ground and sieved to the appropriate size of particles.

Molecularly imprinted polymers has been used in many areas such chromatography or solid phase extraction (SPE) [2], antibody mimics [3] and catalysis [4]. In the last few years MIPs have been reported to be suitable for drug delivery systems, especially for controlled release devices of drugs with narrow theraupetic index [5-7].

Molsidomine (Figure 1), (*N*-ethoxycarbonyl)-3-(4-morpholino)sydnonimine, is an antiangial prodrug, an alternative to the organic nitrates widely used in the treatment of ischaemia coronary artery disease [8]. Molsidomine is metabolized in the liver to its active metabolite, linsidomine, next transformed into other intermediates, and finally forming a pharmacologically active nitric oxide. Nitric oxide should be release slowly to avoid toxicity [9].

We have used simple PM3 semiempirical computation method to get the Mulliken atomic charges for molsidomine. Geometry of molsidomine was optimized about the expected configuration (X-ray diffraction data were reported) [10]. The charge distribution at molsidomine atoms showed large charge separation as was expected. The nitrogen atom at position N3 in the sydnone ring is an electron-deficient atom, and induces a strong polarization of the accompanying bonds. As a consequence at nitrogen atom at N2 and N7 the negative charges are locatized (Figure 1). This "mesoionic" structure of template molecule could prevent building of selective binding sites in polymer matrix.

Figure 1. Structure of molsidomine with atomic charges.



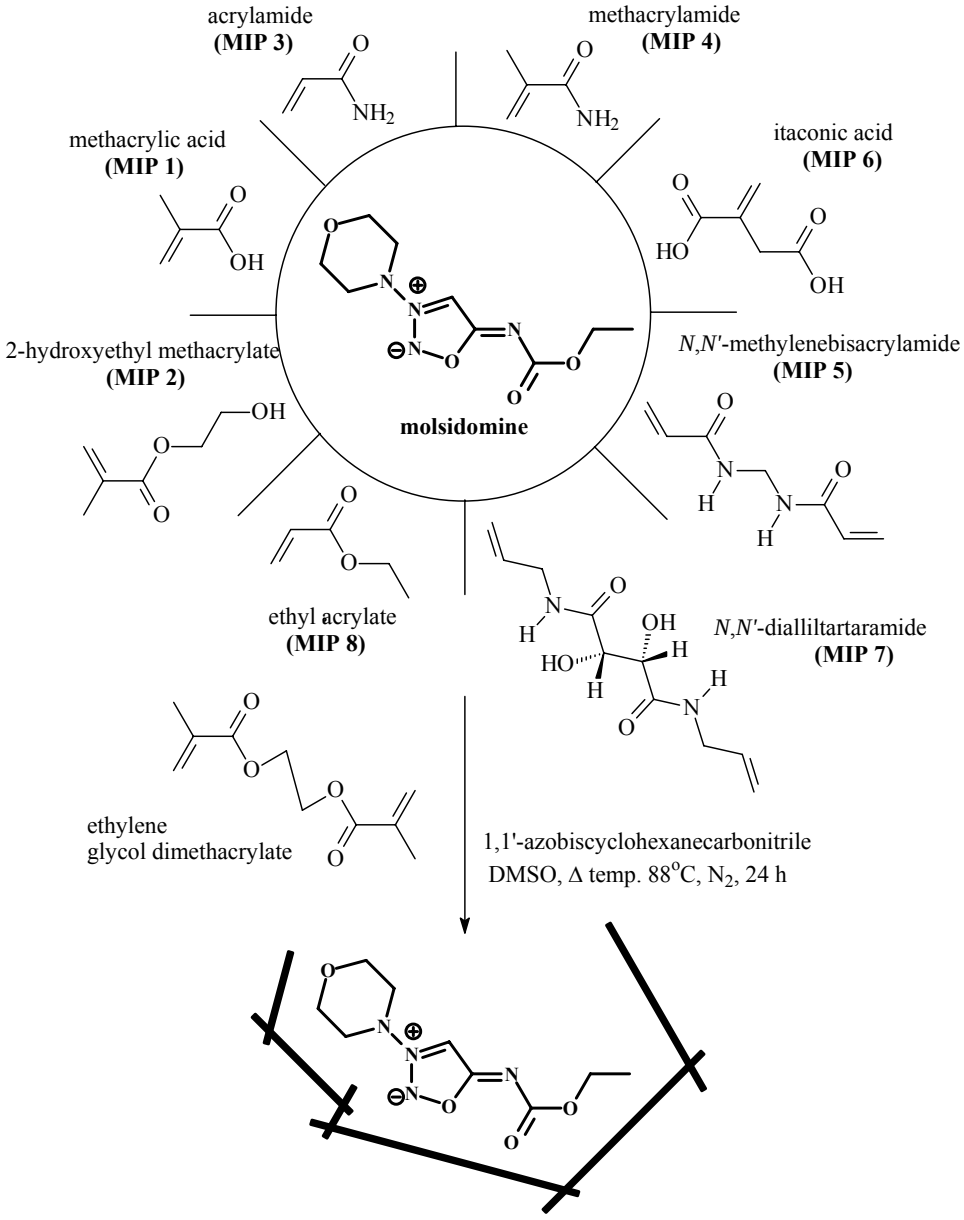
The aim of this short paper is the study of recognition and controlled release of molsidomine from different MIPs. To our best knowledge, these results are the first research trials of synthesis and evaluation of MIPs with molsidomine as a template, compound with sydnone heterocyclic ring, having an imino group in place of exocyclic oxygen atom. There is also first investigation of imprinting process using sydnone "mesoionic" compounds.

Results and Discussion

Effect of functional monomers

Eight kinds of MIPs (MIP 1-8) and corresponding NIPs (NIP 1-8) were prepared using eight monomers with different functionalities: methacrylic acid and itaconic acid with acidic groups, 2-hydroxyethyl methacrylate and ethyl acrylate with ester groups, acrylamide, methacrylamide, *N*,*N'*-methylenebisacrylamide and *N*,*N'*-diallyltartaramide with amide functionalities.

Scheme 1. Reagents and conditions of MIPs synthesis.



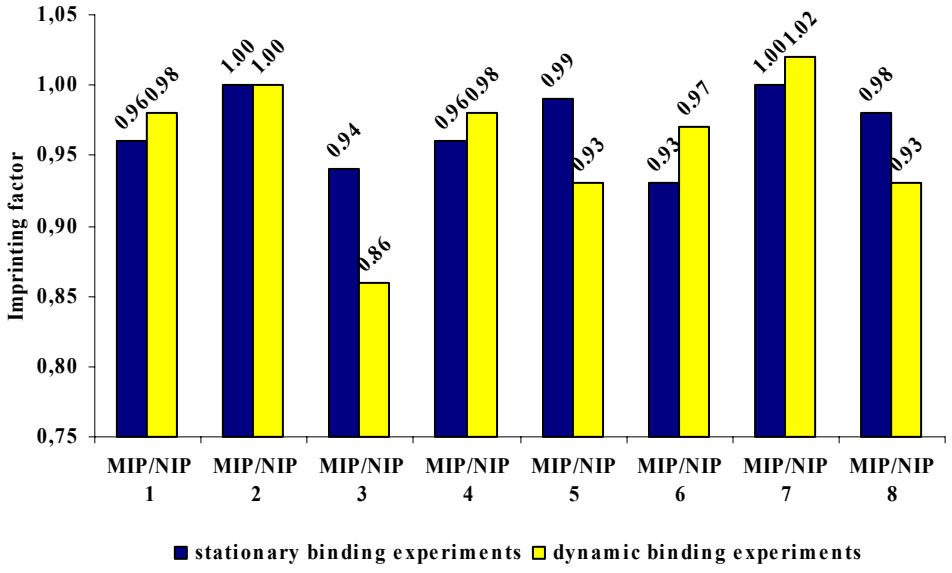
The template molecule was dissolved in DMSO. The chosen functional monomer, the cross-linker and initiator were added to DMSO (Scheme 1). DMSO (as porogen) was chosen

due to solubility of molsidomine and functional monomers. Molsidomine is soluble in DMSO, chloroform and methanol and practically insoluble in other organic solvents, *N*,*N*'-diallyltartaramide shows low solubility in chloroform and methanol. Acrylamide, methacrylamid and *N*,*N*'-methylenebisacrylamide are only slightly soluble in chloroform. Molar ratio of template to functional monomer and cross-linker was 1:4:16. The solution was saturated with nitrogen, sealed and incubated. Non-imprinted polymers were prepared in the same way, except addition of template. After the grinding, sieving and suspending processes, recognition and released properties of obtained particles were evaluated.

MIPs and NIPs recognition and release properties

Two parallel trials of recognition for obtained polymers were performed. Stationary and dynamic binding experiments (see *Experimental*) showed imprinting factors for all obtained polymers between 0.86 and 1.02 (Figure 2). Both experiments were performed in aqueous media. Similar values of imprinting effect were obtained when experiments were performed in molsidomine aqueous solutions adjusted to pH 3 and pH 7 (data not showed). Range of pH values was limited by stability of molsidomine [8]. The small difference between the quantity of molsidomine bound by MIPs and NIPs is an evidence of high non-specific binding of the template in water.

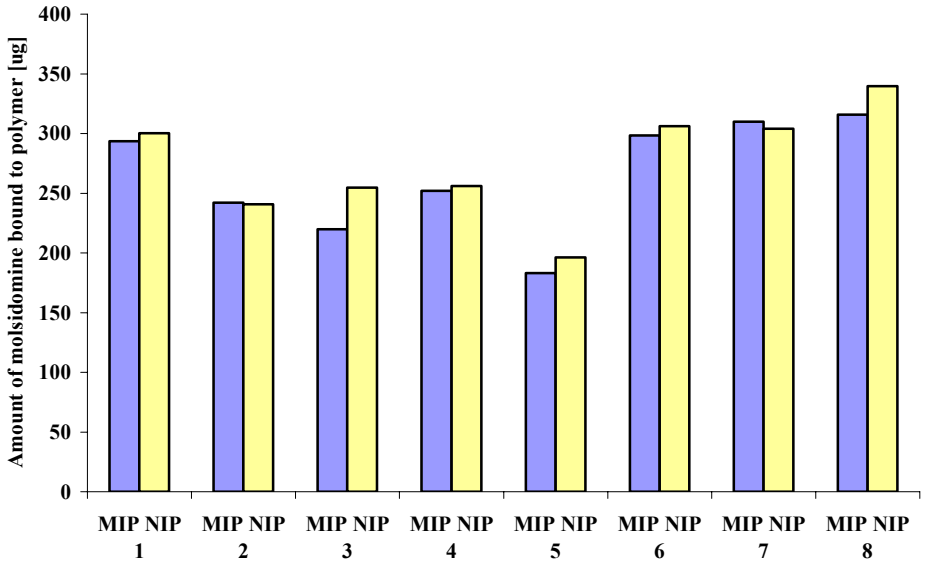
Figure 2. Imprinting factors of MIPs and NIPs pairs of polymers in aqueous media.



Organic solvent (methanol) facilitated recognition properties, and for MIP 3 we could observed slightly higher value of imprinting factor (1.1). Evaluation of capacity of matrices (performed as dynamic binding experiments) showed that amount of molsidomine retained on

the polymer is significantly higher in aqueous media than in organic solvent and this value highly depends on type of polymer (Figure 3). The highest values of adsorption in aqueous molsidomine standard solution (0.6 μmol/mL) were observed for MIP 8 (315.9 μg) and NIP 8 (339.7 μg) and lowest values for MIP 5 (183.0 μg) and NIP 5 (186.3 μg). The greatest difference of binding in aqueous and organic media were found for polymers prepared with acrylamide as a funtional monomer: in aqueous media – 219.7 μg (MIP 3) and 254.7 μg (NIP 3), imprinting factor 0.86, but in organic media – 61.2 μg (MIP 3) and 55.6 μg (NIP 3), imprinting factor 1.10.

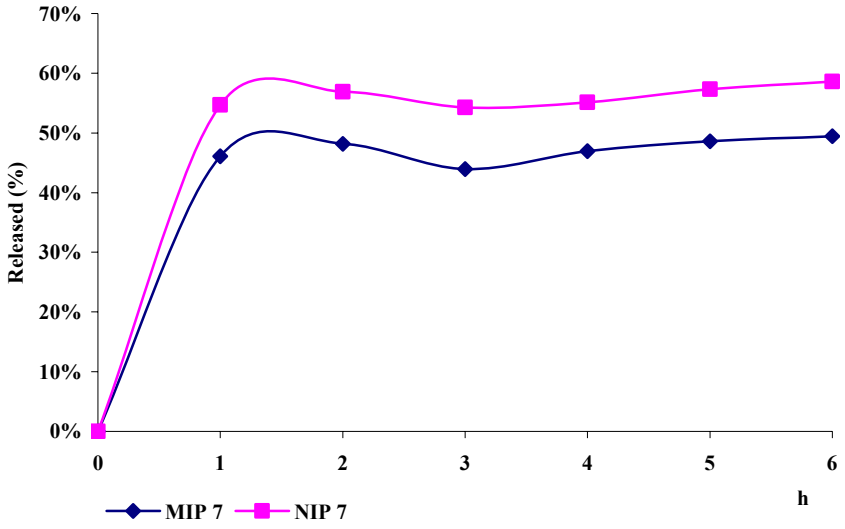
Figure 3. Amount of molsidomine bound to polymers in aqueous media (n = 3, RSD = 10%).



All polymers (except MIP 2 and NIP 2) saturated with molsidomine (after dynamic binding experiments in aqueous media) were tested for release of molsidomine. We observed comparable release profiles for each pair of MIPs and NIPs. Such behaviour is in accordance with binding experiments and propably depends on non-specific adsorption on matrices.

The example results of release profiles as percentage of initial amount of molsidomine release in time (0-6 h) for MIP 7 and NIP 7 are shown in Figure 4. MIP 7 and NIP 7 released a considerable amount of molsidomine already after 1 h (46% and 55%, respectively) and these values were constant for next 5 h (± 4%). Similar released profiles of tetracyclines from MIPs were reported [11].

Figure 4. Release profiles of molsidomine from MIP 7 and NIP 7.



Conclusions

This short paper presents preliminary results of recognition and release of molsidomine from MIPs. The particles were able to bound molsidomine in aqueous media, but this was a highly non-specific adsorption. By changing the solvent to methanol the slightly better recognition properties were observed (imprinting factor 1.1) for polymer prepared with acrylamide as functional monomer. However, in organic medium the capacity of polymer matrix was significantly lower. The release profiles are similar for each pair of MIPs and NIPs. Some matrices could be considered as promising systems. Further investigations are under way aimed to work up procedure to controlled release of molsidomine.

Experimental

General

Molsidomine and followed functional monomers: itaconic acid, methacrylamide, *N*,*N*'-methylenebisacrylamide, ethyl acrylate and 2-hydroxyethyl methacrylate were obtained from Sigma-Aldrich (Germany), and *N*,*N*'-diallyltartaramide was from Serva (Germany). Acrylamide, methacrylic acid and ethylene glycol dimethacrylate were from Fluka (Germany). Initiator of polymerization reaction, 1,1'-azobiscyclohexanecarbonitrile, was provided by Fluka (Germany). Citric acid monohydrate and disodium hydrogen orthophosphate were from POCh (Poland). DMSO was obtained from Park Scientific Ltd.

(United Kingdom). Methanol and acetone were from POCh (Poland). The monomers were purified prior to use by standard procedures (vaccum distilled or recrystallized from appropriate solvent). All others reagents were used without further purification. Ultra-pure water was delivered from a Milli-Q purification system (Millipore, France). Dynamic binding experiments were perfomed in home-made solid-phase extraction vaccum manifold. Polypropylene 1 mL SPE columns with glass-fiber frits were used (Chromabond, Germany). UV-VIS spectrophotometry measurements of molsidomine content were performed with spectrophotometer UV-1605PC (Shimadzu, Germany), with detection at wave length of λ = 311.1 nm. The calibration curve (absorbance (y) versus concentration (x) was performed in the range 0.01 μmol/mL to 0.15 μmol/mL. The calibration equation was $y = 14.75x + 0.01$, $r^2 = 0.992$, and the limit of quantification (LOQ) was 3.01 μg/mL and limit of detecton (LOD) was 1.0 μg/mL.

Stock solution of molsidomine

In a 10 mL volumetric flask an accurately weighed amount of molsidomine (0.0242 g) standard was dissolved in methanol. The obtained concentration was 10 μmol/mL. The stock solution of molsidomine was stored in dark at -20^{o}C. The stock standard solutions, prepared prior to use, were diluted with ultra-pure water or methanol to give different final concentrations of molsidomine.

Preparation of molecularly imprinted and control polymers

The MIPs and NIPs were prepared by bulk polymerization. Briefly, molsidomine as a template (0.048 g, 0.2 mmol), chosen functional monomer: methacrylic acid (0.069 g, 0.8 mmol), 2-hydroxyethyl methacrylate (0.104 g, 0.8 mmol), acrylamide (0.057 g, 0.8 mmol), methacrylamide (0.068 g, 0.8 mmol), *N*,*N*'-methylenebisacrylamide (0.123 g, 0.8 mmol), itaconic acid (0.104 g, 0.8 mmol), *N*,*N*'-diallyltartaramide (0.183 g, 0.8 mmol), ethyl acrylate (0.080 g, 0.8 mmol), ethylene glycol dimethacrylate (0.634 g, 3.2 mmol) and 1,1'-azobiscyclohexanecarbonitrile (0.012 g, 1.2% mol) were dissolved in DMSO (0.671 mL) in a thick-walled glass tube. The appropriate homogenous solutions were purged with nitrogen for ca. 3-5 min. Then the mixtures were incubated under a nitrogen atmosphere at 88^{o}C for 24 h. The final bulk rigid polymers were ground in a mortal with a pestle and wet-sieved into particles below 100 μm diameter. Fine particles were removed by repeated precipitation in acetone. Finally the particles were extracted to remove the molsidomine in continuous extraction process in Soxhlet apparatus (24-36 h, 80 mL methanol), and dried under vaccum

at room temperature. Level of molsidomine extracted from polymers was determined by UV-VIS measurements. The non-imprinted polymers were prepared and treated in the same way as corresponding imprinted polymers, except addition of molsidomine.

Evaluation of polymers

Stationary binding experiments. The binding batch-mode experiments were performed in aqueous solvent (in non-buffered ultra-pure water and in ultra-pure water buffered to pH 3 and pH 7, phosphate-citrate buffer). The obtained MIPs and NIPs particles (25 mg) were dried-packed into Eppendorf vials. Then 1 mL of molsidomine standard solution was added to each vial. Then the vials were sealed and oscillated by a shaker (Heidolph, Germany) at room temperature for 6 h. Then the mixtures were centrifuged for 10 min. and an aliquot of solvent (0.1 mL) was used to analyzed the amount of molsidomine not bound to polymer by UV-VIS spectrophotometry. Amount of molsidomine bound to the particles was calculated by subtracting the amount determined after the experiment from starting amount of molsidomine in standard solution.

Dynamic binding experiments. The dynamic adsorption experiments were performed in both: aqueous and organic (methanol) solvents. The obtained MIPs and NIPs particles (25 mg) were dried packed into 1 mL polypropylene SPE columns secured by glass-fiber frits. The particles were prewashed with methanol, 1 mL, and conditioned with buffered water (pH 5, phosphate-citrate buffer). Then molsidomine standard solutions (concentrations: 0.15, 0.6, 3 and 6 μmol/mL) were loaded, 1 mL, with flow rate 1 mL/min. An aliqout of supernatant (0.1 mL) was used to analyzed the amount of molsidomine not bound to polymer by UV-VIS spectrophotometry. Amount of molsidomine bound to the particles was calculated by subtracting the amount determined after the experiment from starting amount of molsidomine in standard solution.

Loaded columns were then dried under vaccum and stored in dark (no more than 48 h) for followed controlled release experiments.

Controlled release experiments. The dried MIPs and NIPs particles loaded with molsidomine in dynamic binding experiments (*described above*) were quantitively transferred to 10 mL polypropylene vials, containing magnetic bar. The particles were dispersed with 8 mL of ultra-pure water. Then the vials were sealed and solutions were stirred for 6 h. Samples (0.5 mL) were withdrawn from the dissolution medium at appropriate time intervals to determined the amount of molsidomine released. The medium was replaced with the same

volume of fresh ultra-pure water. An aliquot of solvent (0.2 mL) was used to analyzed the amount of molsidomine released from particles by UV-VIS spectrophotometry.

References

1. Komiyama, M.; Takeuchi, T.; Mukawa, T; Asanuma H (Eds.) *Molecular Imprinting: From Fundamentals to Applications*; Wiley-VCH: Weinheim, **2003**.
2. Qiao, F. X.; Sun, H. W.; Yan, H. Y.; Row, K. H. Molecularly imprinted polymers for solid-phase extraction. *Chromatographia* **2006**, *64*, 625-634.
3. Haupt, K. Imprinted polymers – tailor-made mimics of antibodies and receptors. *Chem. Commun.* **2003**, 171-178.
4. Alexander, C.; Davidson, L.; Hayes, W. Imprinted polymers: artifitial molecular recognition materials with applications in synthesis and catalysis. *Tetrahedron* **2003**, *59*, 2025-2057.
5. Alvarez-Lorenzo, C.; Concheiro, A. Molecularly imprinted polymers for drug delivery. *J. Chromatogr. B* **2004**, *804*, 231-245.
6. Sellergren, B.; Allender, C. J. Molecularly imprinted polymers: a bridge to advanced drug delivery. *Adv. Drug Deliv. Rev.* **2005**, *57*, 1733-1741.
7. Puoci, F.; Iemma, F.; Cirillo, G.; Picci, N.; Matricardi, P.; Alhaique, F. Molecularly imprinted polymers for 5-fluorouracil release in biological fluids. *Molecules* **2007**, *12*, 805-814.
8. Streel, B.; Ceccato, A.; Peerboom, C.; Zimmer, C.; Sibenaler, R.; Maes, P. Determination of molsidomine and its active metabolite in human plasma using liquid chromatography with tandem mass spectric detection. *J. Chromatogr. A* **1998**, *819*, 113-123.
9. Schönafinger, K. Heterocyclic NO prodrugs. *Il Farmaco* **1999**, *54*, 316-320.
10. Improta, R.; Santoro, F.; Barbier, C.; Giordano, F.; Del Re, G. On the geometry of 3-amino-sydnones. *J. Mol. Struct. (THEOCHEM)*, **1998**, *433*, 291-299.
11. Cai, W.; Gupta, R. B. Molecularly imprinted polymers selective for tetracycline binding. *Sep. Pur. Technol.*, **2004**, *35*, 215-221.