

Synthesis and Characterization of Molecularly Imprinted Polymers for Phenoxyacetic Acids

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Abstract: 2-methylphenoxyacetic acid (2-MPA), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 4-chlorophenoxyacetic acid (4-CPA) were imprinted to investigate the cross-selectivities of molecularly imprinted polymers (MIPs). The result indicates that 2-MPA, which is similar in shape, size and functionality with phenoxyacetic herbicides, are suitable to be used as a suitable template to prepare the MIPs for retaining phenoxyacetic herbicides. To study the ion-pair interactions between template molecules and functional monomer 4-vinylpyridine (4-VP), computational molecular modeling was employed. The data indicate that the cross-selectivities of MIPs for phenoxyacetic acid herbicides depend on the binding energies of complexes.

Keywords: molecularly imprinted polymers, 2-methylphenoxyacetic acid (2-MPA), phenoxyacetic herbicides, molecular modeling

1. Introduction

Phenoxyacetic acids are widely used in agriculture as herbicides or hormone mimics. This series of compounds include 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 4-chlorophenoxyacetic acid (4-CPA), etc. Residues of these compounds in soil, ground water,

and plants are of concern due to their polarity. Methods typically used to measure these herbicides and their metabolites in environmental samples rely on extraction and analysis via gas chromatography [1-3]. However, due to the physical and chemical properties of these compounds, they tend to be difficult to separate from complex sample matrices. The synthesis of a material that selectively recognizes these serial compounds would be significant.

Figure 1. Synthesis of molecularly imprinted polymers.

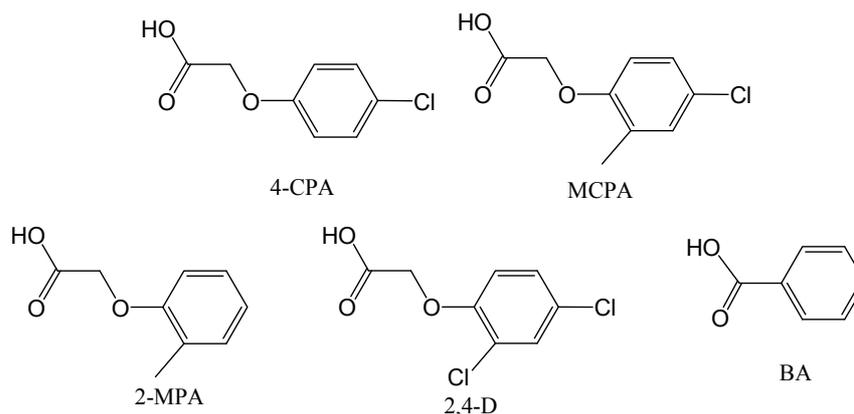


Molecularly imprinted polymers (MIPs) were polymerized as shown in figure 1. In solvent, the aim is to develop a complex in which the target molecule is recognized based on whether the functional group is a carboxyl, amido or acyl to form a complex by hydrogen bonding, and electrostatic interaction or hydrophobic effects. A crosslink polymerization is performed to fix the desired complex. After the template molecules are removed, binding cavities that recognize the applicable template are left to rebind selected molecules. Generally, a functional monomer has an appreciative group to match the active site of the template molecule, which makes the complex stable and improve the selectivity of the MIPs. The strength of the interaction between the template and the monomer determines the accuracy of the binding cavity and its selectivity.

In previous work, 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were imprinted [4-16]. The result indicates that MIPs showed recognition capability for targeted molecules because of the ion-pair and hydrophobic interactions between the template and the monomer 4-vinylpyridine (4-VP). Baggiani's research demonstrates that 2,4,5-T MIPs have a good potential to serve as phenoxyacetic acid herbicides templates in solid-phase extraction (SPE) [7].

However, MIPs are synthesized by using a target molecule as template. When they are used for solid phase extraction (SPE), the leakage of a trace amount of the imprinted molecules remaining in the MIPs has hindered the accurate and precise assay of the target analyte [17]. To overcome this problem, a structural analogue of the analyte is used as a "dummy template" molecule and separated from the target molecules during chromatographic analysis [18, 19]. The MIPs prepared by dummy template are dummy molecularly imprinted polymers (DMIPs). In this work, herbicides MCPA, growth regulator 4-CPA and expectorant 2-MPA (Figure 2) were imprinted to investigate cross-selectivity of these MIPs and choose the suitable dummy template to prepare the MIPs for retaining phenoxyacetic herbicides.

Computer modeling was used previously to design MIPs by calculating the binding energy between the template and the functional monomers [20-22]. In order to investigate the recognition mechanism of ion-pair interactions, computational molecular modeling is employed here to simulate the combination of a 1:1 template: monomer complex.

Figure 2. The chemical structures of template and analogy compounds.

2. Results and Discussion

2.1 HPLC and Cross-selectivity

MIPs for 2,4-D have been polymerized and characterized by radioligand binding assay [4, 5], SPE [7] or HPLC previously [6, 9, 10]. However, the bleeding template phenomenon could result in inaccurate quantification, especially at low concentration residue levels. In this work, a series of MIPs for MCPA, 4-CPA and 2-MPA are studied by HPLC to investigate the cross-selectivities of these MIPs and choose the suitable dummy template to prepare the MIPs for retaining phenoxyacetic herbicides. The selectivity of MIPs and blank polymers columns were evaluated by eluting 4-CPA, MCPA, 2-MPA, 2,4-D and benzoic acid (BA) with acetonitrile/water/acetic acid (93+5+2, v/v). Their molecular structures are shown in Figure 2. The structure of BA differs from those of the other templates to investigate the imprinting effect by this polymerization. Shown in Tables 1 - 3 are the capacity factors (k'), separation factors (α) and retention indices of various analytes on blank polymers and MIP columns. The k' of the prepared MIPs are evaluated as:

$$\text{capacity factors: } k' = (t - t_0) / t_0, \quad (1)$$

Where t is the retention time of the analyte and t_0 is the retention time of the void marker [23]. The t_0 is indicated by the methanol signal. The separation factor and retention index are evaluated as:

$$\text{Separation factor: } \alpha = k'_{\text{template}} / k'_{\text{analog}} \quad (2)$$

$$\text{Retention index: } \text{RI} = \alpha_{\text{blank}} / \alpha_{\text{MIPs}} \quad (3)$$

The k' of 4-CPA, MCPA and 2-MPA on MIPs are higher than those on a blank column (Table 1-3), but the improvement of k' of BA is subtle. The higher k' value means a higher retention capability of the MIP. The fact that BA displays higher affinity than 2-MPA on the blank polymers but is opposite on the 2-MPA MIP demonstrates that the oxygen bridge sub-structure of 2-MPA is imprinted successfully. Without the oxygen bridge sub-structure, BA whose phenyl is too big to fit into the narrow part of the cavity matching the oxygen bridge sub-structure, shows poor retention on the MIPs due to the weakened interaction between the carboxyl and the basic nitrogen of the pyridyl. The

retention index of 4-CPA on MCPA and that of MCPA on 4-CPA imply that the selective affinity of MIPs for template is improved by shape selectivity.

Although generally DMIPs are inferior to MIPs in terms of selectivity for the target analyte [17], past research indicates that the property of the probe molecule can disturb the shape selectivity of the MIPs when the acidity of molecule is stronger than that of the template [21]. In this case, the retention indices of 2,4-D, 4-CPA and MCPA are higher than those of 2-MPA on 2-MPA MIP columns. Thanks to the absence of a strong active site on the aromatic parts of the templates, exact imprints are not possible. The binding cavities can't discriminate between 2,4-D, 4-CPA, MCPA and 2-MPA when the interaction between the analogs and the binding site is too strong. The k' of 2,4-D, MCPA or 4-CPA on blank polymers also indicate their natural affinities with 4-VP.

The chromatographic results (Figure 3, 2-MPA MIPs (a), blank polymer (b)) indicate that the MIPs prepared using 2-MPA as a dummy template show selective affinity for phenoxyacetic herbicides. On the DMIPs, BA can be separated from the phenoxyacetic acids and 2-MPA can be separated from phenoxyacetic herbicides such as 4-CPA, MCPA or 2,4-D. So, 2-MPA, which is similar in shape, size and functionality with phenoxyacetic herbicides, is suitable to be used as a dummy template to prepare MIPs for retaining group phenoxyacetic herbicides.

Table 1. Capacity factors(k'), separation factors (α) and retention indices (RI) on MCPA MIPs and blank polymer.

	k'_{blank}	k'_{MIPs}	α_{blank}	α_{MIPs}	RI
MCPA	0.668	1.411	1	1	1
4-CPA	0.778	1.581	0.859	0.892	0.962
2-MPA	0.468	0.864	1.427	1.633	0.874
BA	0.504	0.535	1.325	2.637	0.503
2,4-D	0.982	2.404	0.680	0.587	1.158

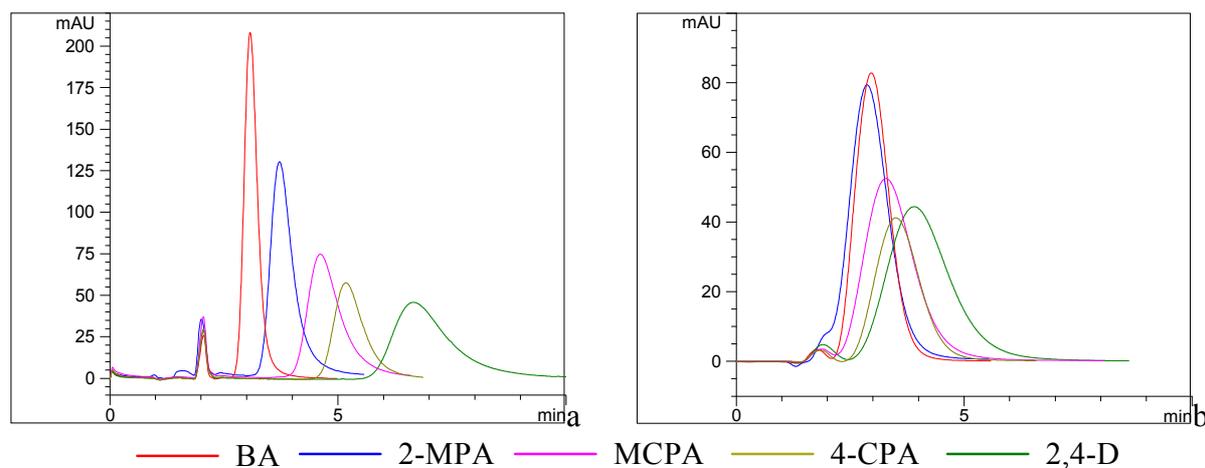
Table 2. Capacity factors(k'), separation factors (α) and retention indices (RI) on 4-CPA MIPs and blank polymer.

	k'_{blank}	k'_{MIPs}	α_{blank}	α_{MIPs}	RI
4-CPA	0.778	2.241	1	1	1
MCPA	0.668	1.732	1.165	1.294	0.900
2-MPA	0.468	1.072	1.662	2.090	0.795
BA	0.504	0.590	1.544	3.798	0.406
2,4-D	0.982	3.170	0.792	0.707	1.121

Table 3. Capacity factors(k'), separation factors (α) and retention indices (RI) on 2-MPA MIPs and blank polymer.

	k'_{blank}	k'_{MIPs}	α_{blank}	α_{MIPs}	RI
2-MPA	0.468	0.943	1	1	1
4-CPA	0.778	1.702	0.602	0.554	1.086
MCPA	0.668	1.410	0.700	0.669	1.048
BA	0.504	0.605	0.928	1.559	0.596
2,4-D	0.982	2.475	0.476	0.381	1.251

Figure 3. Chromatograms of BA, 2-MPA and phenoxyacetic herbicides on 2-MPA MIPs (a) and the blank polymer (b) column.



2.2 Computational molecular modeling

Computational molecular modeling is employed to study the selectivity of these MIPs. Previous studies have shown that pbe1pbe [24, 25], a hybrid functional which contains a portion of Hartree-Fock exchange, yields the best performance for predicting hydrogen binding energies [26]. So we chose this method in our study. The basis set 6-311g (d, p) is also good enough to provide the best results.

For each template the most stable template–monomer complexes are located. The optimized geometries for the most stable complexes are shown in Figure 4, which are the same as we expected. Their electronic stabilization energies relative to isolated fragments, ΔE , are calculated through the equation:

$$\Delta E = E(\text{template–monomer}) - [E(\text{template}) + E(\text{monomer})] \quad (4)$$

The interaction energies obtained by docking the template and the monomer structures minimized in vacuum are reported in Table 4. According to the binding energies and the bond length of H...N, the ion-pair interactions between 4-VP and 4-CPA or MCPA are higher than that of 2-MPA. This evidently leads to 4-CPA and MCPA retaining on 2-MPA MIPs. When the binding energies of the complexes are the same, the retention behavior of the templates on each MIP column depends on the shape selectivity, e.g., 4-CPA and MCPA. The bond length changes shown in Table 4 indicate that the acidic protons leave the oxygen atom and approach the nitrogen atom to form an ion-pair.

3. Experimental Section

3.1 Chemicals and apparatus

Compounds 4-CPA(99%), MCPA(95%), 2,4-D(99%) and 2-MPA(99%) were supplied by the Pesticide Analysis Laboratory, China Agricultural University 4-VP. Ethylene glycol dimethacrylate (EGDMA) and chromatographic solvents were obtained from J&K Chemical Ltd. All other reagents were from Beijing Chemical Reagents Company. 4-VP was distilled at reduced pressure and stored in the dark at $-18\text{ }^{\circ}\text{C}$. EGDMA was purified by basic alumina column immediately before use. Standard

samples were prepared by dissolving 10 mg templates and analogs into 10 mL methanol. HPLC analyses were performed using an Agilent Model 1100 Series liquid chromatograph.

Table 4. Computational modeling data: binding energy ΔE (kcal/mol) and bond length r (Å) in vacuum

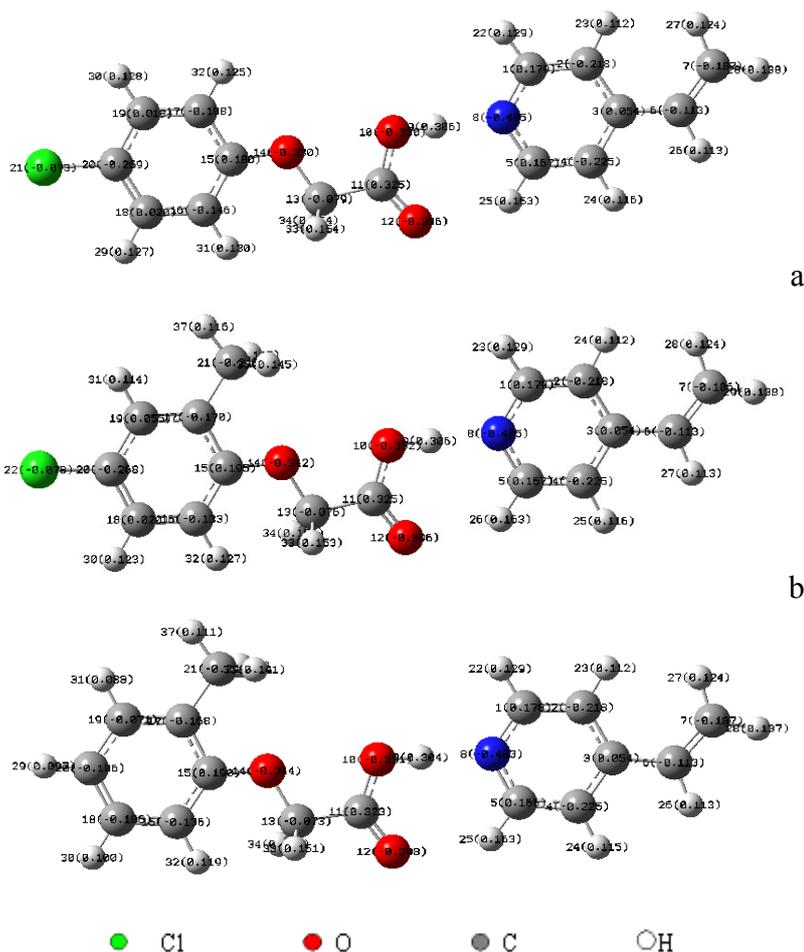
	ΔE (kcal/mol)	r^A (Å)	r^B (Å)	r^C (Å)
4-CPA	-14.47979	0.967	1.016	1.664
MCPA	-14.56388	0.967	1.016	1.663
2-MPA	-14.15600	0.967	1.013	1.666

^A r: bond length of O-H on carboxyl of template

^B r: bond length of O-H on carboxyl of complex

^C r: bond length of H...N of complex

Figure 4. Optimized geometries for the most stable complexes of 4-VP with 4-CPA (a), MCPA (b) and 2-MPA (c) separately. Mulliken atomic charge Q in e.



3.2 Preparation of Polymers

The following procedure was used for the preparation of all MIPs. In a glass bottle, 1mmol of template was dissolved in water/methanol (1 mL+4 mL). To this solution was added 4mmol 4-VP, 20mmol EGDMA, and 0.51mg 2,2'-azobisisobutyronitrile (AIBN). The blank polymer was formulated in a similar method, without template molecule. The bottle was subjected to sonication, purged by nitrogen gas, and sealed. The sample was placed in a 60 °C water bath for 24 h.

3.3 HPLC experiments

The polymers were ground by a machine mill, the particles were sized using wet sieving by acetone, and the particles between 20 and 80 µm were collected. The particles were slurry packed into stainless steel columns (length, 15.0 cm, i. d. 4.6 mm) for the chromatographic experiments. The templates were removed online by acetonitrile/acetic acid 90+10 (v/v), then by mobile phase acetonitrile/water/acetic acid 93+5+2 (v/v) until baseline equilibrated. The flow rate in all cases was 1.0 mL/min, with a UV detector at 270 nm for 2-MPA and BA, 285 nm for 2,4-D, 4-CPA and MCPA. Sample injections were 5 µL of a 1.0 g/L mixed solution of analytes.

3.4 Computational molecular modeling

The theoretical binding energies of templates 4-CPA, MCPA and 2-MPA with 4-VP in vacuum were calculated using Gaussian03 [27]. The geometry of each structure has been fully optimized by using density functional method (pbe1pbe) with 6-311g (d, p) basis set. All the geometric parameters of the possible stationary points that we found have been located and characterized by the number of imaginary frequencies. All the energies of the structures located have Zero-point Energy (ZPE) correction.

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References and Notes

1. Garrison, A. W.; Schmitt, P.; Martens, D.; Kettrup, A. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high performance capillary electrophoresis. *Environ. Sci. & Technol.* **1996**, *30*, 2449-2455.
2. Nilsson, T.; Baglio, D.; Galdo-Miguez, I.; Ogaard-Madsen, J.; Facchetti, S. Derivatization/solid-phase microextraction followed by gas chromatography-mass spectrometry for the analysis of phenoxy acid herbicides in aqueous samples. *J. Chromatogr. A* **1998**, *826*, 211-216.
3. Eitzer, B. D.; Chevalier, A. Landscape care pesticide residues in residential drinking water wells. *Bull. Environ. Contam. Toxicol.* **1999**, *62*, 420-427.
4. Haupt, K.; Dzgoev, A.; Mosbach, K. Assay system for the herbicide 2,4-dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Anal. Chem.* **1998**, *70*, 628-631.

5. Haupt, K.; Dzgoev, A.; Mosbach K. Assay system for the herbicide 2,4-Dichlorophenoxyacetic acid using a molecularly imprinted polymer as an artificial recognition element. *Anal Chem.* **1998**, *70*, 3936-3939.
6. Baggiani, C.; Giraudi, G.; Giovannoli, C. Chromatographic characterization of molecularly imprinted polymers binding the herbicide 2,4,5-trichlorophenoxyacetic acid. *J. Chromatogr. A* **2000**, *883*, 119-126.
7. Baggiani, C.; Giovannoli, C.; Anfossi, L.; Tozzi, C. Molecularly imprinted solid-phase extraction sorbent for the clean-up of chlorinated phenoxyacids from aqueous samples. *J. Chromatogr. A* **2001**, *938*, 35-44.
8. Baggiani, C.; Giraudi, G.; Giovannoli, C.; Tozzi, C.; Anfossi, L. Adsorption isotherms of a molecularly imprinted polymer prepared in the presence of a polymerisable template: indirect evidence of the formation of template clusters in the binding site. *Anal. Chim. Acta* **2004**, *504*, 43-52.
9. Baggiani, C.; Anfossi, L.; Giovannoli, C.; Tozzi, C. Binding properties of 2,4,5-trichlorophenoxyacetic acid-imprinted polymers prepared with different molar ratios between template and functional monomer. *Talanta* **2004**, *62*, 1029-1034.
10. O'Mahony, J.; Molinelli, A.; Mizaikoff, B. Towards the rational development of molecularly imprinted polymers: ¹H NMR studies on hydrophobicity and ion-pair interactions as driving forces for selectivity. *Biosens. Bioelect.* **2005**, *20*, 1884-1893.
11. Kroger, S.; Turner, A P F.; Mosbach, K.; Haupt, K. Imprinted polymer-based sensor system for herbicides using differential-pulse voltammetry on screen-printed electrodes. *Anal Chem.* **1999**, *71*, 3698-3702.
12. Jakusch, M.; Janotta, M.; Mizaikoff, B. Molecularly imprinted polymers and infrared evanescent wave spectroscopy. A chemical sensors approach. *Anal. Chem.* **1999**, *71*, 4786-4791.
13. Liang, C.; Peng, H.; Nie, L H. Bulk acoustic wave sensor for herbicide assay based on molecularly imprinted polymer. *Fresenius J. Anal. Chem.* **2000**, *367*, 551-555.
14. Leung, M.; Chow, C.; Lam M. A sol-gel derived molecularly imprinted luminescent PET sensing material for 2,4-dichlorophenoxyacetic acid. *J. Mater. Chem.* **2001**, *11*, 2985-2991.
15. Svitel, J.; Surugiu, I.; Dzgoev, A. Functionalized surfaces for optical biosensors: applications to in vitro pesticide residual analysis. *J. Mater. Sci. - Mater. Med.* **2001**, *12*, 1075-1078.
16. Weetall, H H.; Rogers, K R. Preparation and characterization of molecularly imprinted electropolymerized carbon electrodes. *Talanta* **2004**, *62*, 329-335.
17. Kawaguchi, M.; Hayatsu, Y.; Nakata, H.; Ishii, Y.; Ito, R.; Saito, K.; Nakazawa, H. Molecularly imprinted solid phase extraction using stable isotope labeled compounds as template and liquid chromatography-mass spectrometry for trace analysis of bisphenol A in water sample. *Anal. Chim. Acta* **2005**, *539*, 83-89.
18. Theodoridis, G.; Kantifes, A.; Manesiotis, P.; Raikos, N.; Tsoukali-Papadopoulou, H. Preparation of a molecularly imprinted polymer for the solid-phase extraction of scopolamine with hyoscyamine as a dummy template molecule. *J. Chromatogr. A* **2003**, *987*, 103-109.

19. Wang, J.; Guo, R.; Chen, J.; Zhang, Q.; Liang, X. Phenylurea herbicides-selective polymer prepared by molecular imprinting using *N*-(4-isopropylphenyl)-*N'*-butyleneurea as dummy template. *Anal. Chim. Acta* **2005**, *540*, 307-315.
20. Chianella, I.; Karim, K.; Piletska, E. V.; Preston C.; Piletsky, S. A. Computational design and synthesis of molecularly imprinted polymers with high binding capacity for pharmaceutical applications-model case: adsorbent for abacavir. *Anal. Chim. Acta* **2006**, *559*, 73-78.
21. Breton, F.; Rouillon, R.; Piletska, E. V.; Piletsky, S. A. Virtual imprinting as a tool design efficient MIPs for photosynthesis-inhibiting herbicides. *Biosens. Bioelect.* **2006**, *22*, 1948-1954; DOI: 10.1016/j.bios.2006.08.017.
22. Dineiro, Y.; Menendez, M. I.; Blanco-Lopez, M.C.; Lobo-Castanon, M. J.; Miranda-Ordieres, A. J.; Tunon-Blanco P. Computational predictions and experimental affinity distributions for a homovanillic acid molecularly imprinted polymer. *Biosens. Bioelect.* **2006**, *22* 364–371.
23. O'Mahony, J.; Molinelli, A.; Mizaikoff, B. Anatomy of a successful imprint: Analysing the recognition mechanisms of a molecularly imprinted polymer for quercetin. *Biosens. Bioelect.* **2006**, *21*, 1383-1392.
24. Ernzerhof, M.; Perdew, J. P.; Burke, K. Coupling-constant dependence of atomization energies. *International Journal of Quantum Chemistry* **1997**, *64*, 285-295.
25. Ernzerhof, M.; Scuseria G. E. Assessment of the Perdew–Burke–Ernzerhof exchange-correlation functional. *The Journal of Chemical Physics* **1999**, *110*, 5029-5036.
26. Rabuck, A. D.; Scuseria, G. E. Performance of recently developed kinetic energy density functionals for the calculation of hydrogen binding strengths and hydrogen-bonded structures. *Theoretical Chemistry Accounts* **2000**, *104*, 439-444.
27. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, Jr., T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S.S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *GAUSSIAN03, Revision B.02*; Gaussian Inc.: Pittsburgh, PA, 2003.