Computational screening of functional monomers for bitertanol sensing using molecularly imprinted polymer

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Abstract. Pesticide residue monitoring in agricultural products is required by governments and organizations to minimize its toxic effects of pesticides in humans. Bitertanol is a fungicide used in various crops to control plant diseases but also poses harmful consequences in human health when misused. Molecularly imprinted polymers (MIPs) are artificial materials that can be used to selectively isolate and recover this substance from contaminated crops. In this work, we determined the best monomer to imprint bitertanol by evaluating the interaction between different functional monomers and bitertanol in the pre-polymer complex. Density functional theory used to optimize the structure of the complex and evaluate the interaction. The computational results showed that hydroquinone produces the most stable complex at 1:3 template-monomer ratio. The hydroxyl functionalities of hydroquinone can effectively form hydrogen bonds with the triazole ring and the hydroxyl group of bitertanol. Furthermore, parameters such as Mulliken atomic charges, bond lengths, and frontier molecular orbitals were also evaluated to confirm the formation of stable hydrogen bonds. This study can be used as a theoretical foundation for the preparation of MIPs for bitertanol using.

1. Introduction
Molecularly imprinted polymers (MIPs) are synthetic materials that contain cavities that are specifically designed to bind a target analyte. Similar to biological enzymes, these materials are capable of selective binding due to the highly specific structure and functionality of the active site cavities. The superior selectivity of MIP is owed to the interaction of the functional monomer and analyte formed in the pre-polymer complex. Non-covalent interactions such as hydrogen bonding, electrostatic attraction, and van der Waals forces are desired over covalent interactions for ease of analyte removal in the MIPs. Non-covalent interactions should be maximized in the pre-polymer complex in order to produce MIPs with high affinity over the target analyte [1]. Nowadays, computational chemistry has been exploited in the design of MIPs by performing molecular simulations to evaluate the nature and energetics of the interactions present between the functional monomer and analyte in the pre-polymer complex. Density functional theory (DFT), an \textit{ab-initio} quantum chemical calculation, is applied to screen functional monomers to determine the best monomer for the design of highly selective MIPs. This type of calculation predicts the formation of favorable interactions between monomer and analyte, which is dictated by the energy associated with them. Several studies have used DFT to design MIP systems for a wide range of analytes such as disulfoton, amoxicillin, and tinidazole [2-4].

Bitertanol (BIT) is a triazole fungicide (structure shown in figure 1) that inhibits the production of ergosterol, a hormone responsible for fungal growth, that prevents fungi from proliferating. This pesticide is used in banana, apple, tea, and wheat. Studies have shown that large amounts of BIT residue
in crops can result in different human health diseases such as reproductive disorder, cancer and endocrine dysfunction [5]. Hence, different government organizations established a maximum residue limit (MRL) on BIT. The European Food Safety Authority only allows 0.01 mg of BIT per kg of sample for plant and animal commodities while the European Union established an MRL of 0.05 mg BIT per kg of sample [6]. The traditional way of monitoring BIT residue in crops is performed via a chromatographic technique using high-performance liquid chromatography (HPLC) and gas chromatography (GC). This technique can provide accurate measurements and is considered the golden standard for analysis but the tedious and expensive operation of this process is inconvenient when monitoring a large volume of samples. On the other hand, MIPs have been prevalently used as a sensing material for pesticides like disulfoton, endosulfan, and methyl parathion [2,7,8]. In this study, we screened different monomers using DFT to design an MIP for BIT sensing. The monomers were allowed to interact with the template BIT in vacuum until the optimized geometry, the structure with the lowest energy, was achieved.

![Chemical structure of bitertanol](image)

**Figure 1.** Chemical structure of bitertanol.

2. Methodology

2.1. Instrumentation

All computer simulations were performed on a workstation equipped with Intel Core i5 CPU with a clock speed of 3.20 GHz and 8 GB RAM.

2.2. Molecular Modelling

Equilibrium geometry optimization and property calculation of each monomer (structures shown in figure 2): aniline (ANI), diaminonapthalene (DAN), hydroquinone (HYQ), indole (IND), pyrrole (PYR), and vinyl pyrrole (VP); the template BIT, and template-monomer complexes at different template-monomer ratios (1:1, 1:2, and 1:3 for the top three complexes of 1:2) were performed using Spartan Student Edition Version 7.2.7 (Wavefunction Inc.) at the quantum mechanical level of theory. At first, 3D molecular models of the compounds involved in this study were built in the graphical user interface of the software. An initial global minimum conformational search was performed by implementing a Monte Carlo Search using Merck Molecular Force Field (MMFF). Pre-optimization of equilibrium geometry of all the conformations found using MMFF was done using the PM3 Semi-Empirical (SE) calculation using the Pople type basis set 6-31G* in the vacuum-phase. The three lowest energy conformations were further optimized using DFT with the same basis set used in the pre-optimization stage. The conformations with the lowest energy for each monomer (\(E_M\)), template (\(E_T\)), and each monomer-template complex (\(E_C\)), defined as BIT-MONOMER\(_\text{ratio}\) in the text, at different template-monomer ratios (\(n\)) were used to calculate the energy of interaction (\(\Delta E\)) of the pre-polymer complex using equation (1).

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\Delta E = E_C - [E_T + nE_M]
\]

Hydrogen bond distances and angles were also optimized using DFT. Other properties such as HOMO-LUMO energies and Mulliken atomic charges were also determined using DFT.
Figure 2. Chemical structures of: ANI, DAN, HYQ, IND, PYR, VP.

3. Results and discussion

The design of highly selective MIP for BIT relies heavily on the interaction between the functional monomer and BIT. By observing the electrostatic potential map of BIT molecule (see figure 3), possible interaction sites will occur at the regions in red (proton acceptor) and blue (proton donor) which are in the space of the atoms O1, O2, N1, N2, and H1. In this study, we only considered monomers that contain functionalities such as 1° (−NH2) amine, 2° (−NH) amine, or hydroxyl (−OH) group that can both act as proton donors and acceptors. These monomers are also electropolymerizable which allows the formation of thin polymeric films that is advantageous when extracting the template from the MIP. The geometry of the complexes formed by BIT and functional monomers were optimized in order to determine the nature of its interaction and ΔE. Based on the calculated values of interaction energies in vacuum reported in table 1, all complexes at different template-monomer ratios formed stable complexes (negative ΔE) which imply the ability of the functional monomers to effectively interact with BIT. At 1:1 ratio, HYQ obtained the most negative ΔE value of -47.1 kJ mol⁻¹ followed by IND and DAN with -34.9 kJ mol⁻¹ and -30.6 kJ mol⁻¹ respectively. Complexes formed at the ratio of 1:2 were observed to form a more stable complex than at 1:1 ratio. The order of ΔE in increasing magnitude is as follow: ΔE (BIT-HYQ2) > ΔE (BIT-IND2) > ΔE (BIT-VP2) > ΔE (BIT-PYR2) > ΔE (BIT-DAN2) > ΔE (BIT-ANI2).
Hydrogen bonds play an important role in the formation of stable complexes. Systems containing a good number of short hydrogen bonds are found to have stronger interactions [9]. Furthermore, increasing the number of monomers per molecule of template in the pre-polymer complex increases the chance of more hydrogen bonds being formed. For this reason, we explored the possibility of improving the ΔE value by increasing the ratio of the three monomers that formed the most stable complexes at the ratio of 1:2. The top three monomers were only considered for further analysis to save computation time. Upon observation, there was indeed a significant improvement in the ΔE value of the monomer-template complex as the ratio is increased to 1:3. HYQ retained to form the most stable complex with ΔE of -145.6 kJ mol\(^{-1}\) which indicates that BIT will strongly interact with this functional monomer. Figure 4 shows the optimized structures of BIT-HYQ complexes at the three different ratios. The geometries show that the hydroxyl groups of HYQ function as a proton donor to form hydrogen bonds with the proton acceptors of BIT (N2, N3, and O2). The location of these hydrogen bonding interaction sites will serve as the binding sites of the MIP.

| Complexes | ΔE (kJ mol\(^{-1}\)) | H-bonds |
|-----------|-----------------|--------|
| BIT-ANI   | -21.8           | 1      |
| BIT-DAN   | -30.6           | 1      |
| BIT-HYQ   | -47.1           | 1      |
| BIT-IND   | -34.9           | 1      |
| BIT-PYR   | -30.4           | 1      |
| BIT-VP    | -21.5           | 1      |
| BIT-ANI\(_2\) | -46.6        | 1      |
| BIT-DAN\(_2\) | -52.1         | 2      |
| BIT-HYQ\(_2\) | -86.9         | 2      |
| BIT-IND\(_2\) | -60.9         | 1      |
| BIT-PYR\(_2\) | -54.8         | 2      |
| BIT-VP\(_2\) | -58.6         | 2      |
| BIT-HYQ\(_3\) | -145.6        | 3      |
| BIT-IND\(_3\) | -90.9         | 2      |
| BIT-VP\(_3\) | -94.6         | 2      |

Hydrogen bonding parameters such as X-H bond length, hydrogen bond length, and angle are presented in table 2. The formation of hydrogen bond was observed to elongate and weaken the O-H bond (from 0.969 Å) of HYQ due to the outflow of electron density from the H atom to O atom. This resulted in the strengthening of the C-O bond, evidenced by bond shortening, connected to O-center to protect its octet structure [10]. Furthermore, these also led to changes in Mulliken atomic charges as a consequence of the redistribution of electron density. At 1:3 ratio, the Mulliken atomic charges before complexation are: -0.650 au on O in HYQ, 0.405 au on H available for hydrogen bonding in HYQ, -0.274 au on N42 in BIT, -0.434 au on N47 in BIT, and -0.629 au on O26 in BIT. After complexation, these atoms changed to -0.733 au on O59, 0.468 au on H60, -0.695 au in O26 for BIT. The following changes in the atomic charge suggest that there are hydrogen bonding interactions present between HYQ monomer and BIT molecule [11,12]. This charge transfer interplay is further supported by the location of the frontier molecular orbitals, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). These orbitals point the direction of charge transfer (from HOMO to LUMO) in molecular systems. We observed from the simulation of BIT-(HYQ)\(_3\) complex that the HOMO is located at HYQ monomers while the LUMO resides at BIT molecule which indicates that the charge transfer occurs from the functional monomers to the template molecule [13].
Figure 4. Optimized structures of the most stable complexes at different ratios between BIT and functional monomer in the gas phase: (a) BIT-HYQ, (b) BIT-HYQ$_2$, and (c) BIT-HYQ$_3$.

Table 2. Hydrogen bonding parameters of BIT-HYQ complexes at different template-monomer ratios.

| Complex | Hydrogen bonding position | X-H bond length (Å) | H - bond length (Å) | Angle (°) |
|---------|---------------------------|---------------------|---------------------|-----------|
| BIT-HYQ | O59-H60-N42               | 0.987               | 1.87                | 161.8     |
| BIT-HYQ$_2$ | O73-H74-O26               | 0.979               | 1.95                | 153.9     |
|          | O59-H60-N47               | 0.990               | 1.90                | 162.9     |
| BIT-HYQ$_3$ | O73-H74-N42               | 0.983               | 1.93                | 158.1     |
|          | O59-H60-N47               | 0.989               | 1.88                | 169.0     |
|          | O89-H90-O26               | 0.976               | 1.97                | 145.3     |

4. Conclusion
The result of computational screening shows that HYQ is the best monomer to imprint BIT among the monomers considered in this study at all different template-monomer ratios. This monomer has the best interaction with the template molecule at 1:3 ratio forming multiple hydrogen bonds (at O2, N2, and N3) based on the interaction energy and optimized structure of the corresponding complex calculated using DFT. These hydrogen bonding sites will serve as the adsorption site during template rebinding. The computational results from this study can be useful in the actual preparation of highly selective MIP for BIT.

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