Computational design of dummy molecularly imprinted polymers via hydrogen bonding investigation for oxytetracycline determination

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Abstract. Oxytetracycline (OTC), a banned broad-spectrum antibiotic, currently requires highly selective and specific determination methods to measure its concentration below 200 ppb in foods of animal origin. Molecular imprinting technology could be utilized to construct a highly selective and cost-effective synthetic receptor for OTC. In this work, we investigated different monomers in designing a dummy MIP for OTC detection using TC as the dummy template. Template-monomer complexes of pre-polymerization mixtures were modeled using density functional theory for geometry optimization, intermolecular hydrogen-bonding situation, and interaction energies. O-phenylenediamine (OPD) at TC:OPD molar ratio = 1:7 was shown to be the optimum monomer, forming 11 stable intermolecular hydrogen bonds with TC and having the lowest interaction energy among the complexes. We also presented indole, pyrrole, and carbazole to be plausible monomers for imprinting TC; however, they are energetically less-favored than OPD. This study provides aid in dummy MIP design for OTC concentration measurement using the molecular level interaction of different monomers with the template TC.

1. Introduction

Oxytetracycline (OTC) is a broad-spectrum antibiotic used against gram-positive bacteria, gram-negative bacteria and atypical organisms including mycoplasmas, protozoan parasites, and chlamydiae. However, it is currently an agricultural contaminant, which could lead to serious multi-drug resistance of bacteria, environmental pollution, and chronic toxicity [1]. As a countermeasure, the Codex Alimentarius Commission implemented a maximum residue limit of 200 µg kg⁻¹ for OTC determination in food of animal origin [2]. Traditional method for determining trace amount of OTC utilizes high performance liquid chromatography (HPLC) coupled with either mass spectroscopy or UV/Vis spectroscopy. Unfortunately, traditional assays suffer from expensiveness, laborious sample pretreatment, and complex extraction procedures. In effect, researchers are developing cost-effective and simpler assays, which include immunological methods, electrochemical methods, colorimetric methods, and molecularly imprinted polymer (MIP)-based sensing [3].

MIP-based techniques develop a synthetic receptor for a particular analyte through self-assembled complexes between functional monomers and the target analyte. The active imprint created in the polymer acts as a selective recognition site in the MIP, which the target analyte rebinds to via non-covalent interactions such as hydrogen bonding, electrostatic attraction, and dispersion forces [4].

However, some recognition sites have non-extracted template due to imperfect template removal
via solvent extraction. The limitation of solvent extraction results to template bleeding during the rebinding process and the problem extends to quantification inaccuracies. To address template bleeding, researchers develop a dummy MIP, which uses a dummy template with an analogous structure to the analyte of interest, as reported in cyproheptadine, polybrominated diphenyl ethers, and nevirapine determination [5-7]. OTC has eight structural analog molecules, closest of which is tetracycline (TC) differing only with a single hydroxyl moiety, as shown in figure 1.

Moreover, development of dummy MIP utilizes computational approach through \textit{ab initio} calculations such as density functional theory (DFT). Recent advances in dummy MIPs used the DFT approach for antibiotic detection such as clenbuterol and chloramphenicol [8,9].

DFT methods provide efficient theoretical description of the template molecules, the functional monomers, and the intermolecular hydrogen bonds present in the pre-polymerization complex. Calculated theoretical descriptions include prediction of equilibrium geometry, interaction energy $\Delta E$, and sites of action between molecules [10]. DFT approach selects optimum functional monomers with the lowest predicted $\Delta E$ value of the resulting template-monomer complexes. Currently, researchers attribute low $\Delta E$ values to the presence of intermolecular hydrogen bonding between the template and the functional monomers [11]. Thus, to develop an optimum dummy MIP, we explored the resulting intermolecular hydrogen bonds between TC and functional monomers through their sites of action, equilibrium geometry, and interaction energy using DFT calculations. This paper presents the optimum functional monomer for dummy MIP-based sensing of OTC as determined from DFT calculations, using TC as dummy template.

2. Experimental

2.1. Instrumentation and computational methods

DFT calculations were performed using Spartan Student Edition Version 7.2.7 (Wavefunction Inc.) on a personal computer with an Intel Core i5 processor, 3.20 GHz CPU, 8 GB memory, and a 1 TB hard disk running on Windows 8.1 operating system [12]. B3LYP was chosen as gradient-corrected functional to provide accurate structural and energetic description of hydrogen bond systems in the template-monomer complex. 6-31G* was used as the basis set to provide accurate results with the minimum computational time [13]. Moreover, basis set superposition error (BSSE) was reported negligible for systems with large clusters of hydrogen bonding and was not considered in this study [10].

2.2. Analysis of reaction sites

During the imprinting and rebinding process, the template molecule and functional monomer interact at specific sites of action via non-covalent bonding [4]. The sites of interaction of the template were analyzed through chemical functional description (CFD) and electrostatic potential (ESP) diagrams. CFD shows proton donors and acceptors of a molecule, aiding in possible hydrogen bonding sites between the template and the functional monomer. ESP diagram shows the concentration of electronegative and electropositive regions of a molecule, assisting in the determination of electrostatic attraction sites between the template and functional monomer.
2.3. Construction of template-monomer complexes

To produce optimum template-functional monomer complex during the imprinting process, the template-monomer complex ratio, was modeled in terms of energy. This work proceeds on the idea that generating the most possible number of stable hydrogen bonds would yield the most stable imprinted complexes [14]. The number of hydrogen bonds, bond length, bond angles, and bond types was further analyzed. Hydrogen bonding strength based on geometry was discussed using Jeffrey’s classification of hydrogen bonding geometry shown in table 1 [13].

| Type              | Strong          | Moderate       | Weak            |
|------------------|-----------------|----------------|-----------------|
| interaction type | strongly covalent | mostly electrostatic | electrostatic/dispersion |
| bond length [Å]  | 1.2 – 1.5       | 1.5 – 2.2      | 2.2 – 3.2       |
| bond angle [°]   | 175 – 180       | 130 – 180      | 90 – 150        |

2.4. Selection of functional monomer

Introduction of conducting functional monomers lead MIP-based sensing to improved MIP processability, stability, and cost effectiveness. This route of MIP production proceeds with electropolymerization, removing the need for the crosslinking agent and initiator [16]. Four conducting functional monomers were screened: o-phenyldiamine (OPD), carbazole (CAR), indole (IND), and pyrrole (PYR). The molecular structures of the functional monomers are shown in figure 2. Each conformation of functional monomer and template was optimized separately to obtain their respective energies. Using the magnitude of $\Delta E$, the optimum functional monomer and template:monomer molar ratio was selected.

![Figure 2. Chemical structures of OPD, CAR, IND, and PYR.](image)

$\Delta E$ was computed using equation (1) [16]:

$$\Delta E = E_C - E_T - nE_M$$  \hspace{1cm} (1)

where $E_C$ is the equilibrium monomer-template complex energy, $E_T$ is the equilibrium template energy, $E_M$ is the equilibrium monomer energy, and n is the number of monomers present.

3. Results and discussion

3.1. Determination of template molecule’s site of action

With the goal of forming stable complex configuration, molecular self-assembly was simulated with the assistance of CFD and ESP to investigate all possible sites of action. Figure 3 shows the CFD and ESP diagrams of TC. There are 10 hydrogen bonding sites in the TC molecule: hydroxyl groups - O1, O2, O3, O5, and O6; carbonyl groups – O4, O7, and O8; and amine groups – N1 and N2. All these functional sites were proton acceptors, while only 7 of these were also proton donor sites for functional monomer adduction (O1, O2, O3, O5, O6, N1, and N2). The ESP diagram shows the electroactive regions of TC. TC has 9 electronegative regions: O1, O2, O3, O4, O5, O6, O7, O8, and N1; and has 2 electropositive regions: H5(N2) and H7(O2). This investigation proceeded to use the 10 sites of action of TC as proton acceptor, and each functional monomer as a single proton donor. No functional monomer was paired with H5(N2) and H7(O2), to avoid steric crowding of functional monomers on O2 and N2, respectively [18,19]. In effect, the selected sites of action for the template
molecule were O1, O2, O3, O4, O5, O6, O7, O8, N1, and N2.

![CFD and ESP diagrams of TC](image)

**Figure 3.** CFD (a) and ESP (b) diagrams of TC.

### 3.2. Formation of the template-monomer complexes

The template-monomer molar ratio must be optimized for high selectivity of the imprinted polymer. In this study, each imprinting system was simulated to start at template-monomer ratio = 1:10. Each type of functional monomer was initially placed at a hydrogen bond distance of 1.6 Å and angle of 180° with each site of action of the template molecule to simulate the strongest intermolecular hydrogen bond according to Jeffrey [15]. Conformational search was implemented in each constructed complex using Merck Molecular Force Field calculation. Resulting non-hydrogen bonded functional monomers from each run were removed from the complex, using a 3.2 Å and 120° cut-off hydrogen bond distance and angle, until all functional monomers have stable hydrogen bond with the template molecule [12]. After which, hydrogen bonds were verified using equilibrium geometry calculation via DFT at B3LYP level, with a basis set of 6-31G*. This top-down procedure eliminates the ambiguity of comparing ΔE values for 1:1 template-monomer molar ratio for template molecules with multiple sites of action.

Figure 4 shows the final configuration of each template-monomer complex. The optimum conditions were as follows: TC:OPD = 1:7, TC:CAR = 4, TC:IND = 5, and TC:PYR = 5. Differences in the optimum molar ratio were accounted to steric crowding between the functional monomers. Bulky, tricyclic functional monomer CAR has its maximum monomer limit at 4, compared to smaller molecules: bicyclic functional monomer IND, and single ring functional monomers, PYR and OPD. The number of electroactive sites of functional monomers also affect the results. IND, PYR, and CAR have only 1 active amine group to act as a proton donor and proton acceptor, compared to OPD with 2 active amine groups, providing four proton donors and two proton acceptors.

Table 2 illustrates a quantitative analysis of the formed hydrogen bonding networks. Considering the 4 equilibrium complexes, all formed hydrogen bond lengths ranged from 1.9 - 2.5 Å and hydrogen bond angles are within 121.5° - 173.5°. The mean hydrogen bond length of each complex was 2.1, 2.1, 2.2, and 2.0 Å for TC + 7OPD, TC + 4CAR, TC + 5IND, and TC + 5PYR, respectively. The mean hydrogen bond angles of each complex was 152.0°, 158.2°, 145.4°, 154.8° for TC + 7OPD, TC + 4CAR, TC + 5IND, and TC + 5PYR, respectively. The mean hydrogen bond lengths and angles of the
formed complexes indicate moderate hydrogen bond strength, where intermolecular hydrogen bonds were mostly electrostatic in nature, as shown in table 1 [13].

![Complexes](image)

**Figure 4.** Final conformation of template-monomer complexes after DFT optimization: TC + 7OPD (a), TC + 4CAR (b), TC + 5IND (c), and TC + 5PYR (d).

**Table 2.** Geometrical parameters of intermolecular hydrogen bonding in each template-functional monomer complex. Double hydrogen bonds observed in specific sites have superscripts \(^a\) and \(^b\).

| Complex   | Type | Length [Å] | Angle [°] | Complex   | Type | Length [Å] | Angle [°] |
|-----------|------|------------|-----------|-----------|------|------------|-----------|
| TC + 7OPD |      |            |           | TC + 5IND |      |            |           |
|           | N2\(^a\) | 2.3        | 144.9     | N1       | 2.2  | 173.5      | 160.1     |
|           | N2\(^b\) | 2.1        | 167.3     | O2       | 2.0  | 130.0      | 121.5     |
|           | O1    | 2.2        | 142.6     | O3       | 2.5  | 149.3      | 166.4     |
|           | O3    | 2.0        | 153.2     | O7\(^a\) | 2.5  | 153.4      |           |
|           | O4    | 1.9        | 152.6     | O8\(^a\) | 1.9  |           |           |
|           | O5\(^a\) | 2.2        | 139.3     | O8\(^b\) | 2.1  | 153.4      |           |
|           | O5\(^b\) | 2.0        | 152.8     |          |      |            |           |
|           | O7\(^a\) | 2.1        | 142.6     |          |      |            |           |
|           | O7\(^b\) | 2.1        | 148.3     |          |      |            |           |
|           | O8\(^a\) | 2.0        | 169.3     |          |      |            |           |
|           | O8\(^b\) | 2.2        | 158.6     |          |      |            |           |
| TC + 4CAR |      |            |           | TC + 5PYR|      |            |           |
|           | N1    | 2.2        | 173.5     | O4       | 2.0  | 169.5      | 146.9     |
|           | O2    | 2.1        | 160.2     | O7\(^a\) | 2.0  | 145.0      |           |
|           | O7\(^b\) | 2.1        | 154.1     | O8\(^a\) | 2.0  | 158.3      |           |
|           | O8\(^b\) | 2.0        | 154.1     |          |      |            |           |

Double hydrogen bonds were present in each template-monomer complex at the following sites of action: carbonyl groups O7 and O8, hydroxyl group O5, and amine group N2 for TC + 7OPD; carbonyl group O7 for TC + 4CAR; carbonyl groups O7 and O8 for both TC + 5IND and TC + 5PYR. Double hydrogen bonding promotes the stability of the template-monomer complex and increase the affinity of the recognition site during the rebinding process [20].
3.3. Theoretical screening of functional monomer

Functional monomers interact uniquely with a template molecule and in each site of action during the imprinting process. Using DFT optimization, equilibrium geometry and interaction energy were calculated for each complex. All functional monomers have negative $\Delta E$ values, indicating their feasibility during the imprinting process as shown in table 3. Among the functional monomers, OPD has shown the most stability with the most negative $\Delta E$ value. The significant $\Delta E$ value of OPD was attributed to its high template-monomer molar ratio and to the presence of double hydrogen bonds in N2, O5, O7, and O8 [17]. Contrary to the prior hypothesis, the number of stable hydrogen bonds does not necessary determine the electronic stability of a template-functional monomer complex. The results suggest that the molecular size and available functional groups in a functional monomer are key factors in the resulting magnitude of electronic stability of a template-functional monomer complex.

Table 3. Interaction energies of complexes formed with the description of the hydrogen bonding situation.

| Complex   | $\Delta E$ [kJ/mol] | Hydrogen | Double hydrogen | Mean | Mean |
|-----------|---------------------|----------|----------------|------|------|
| TC + 7OPD | -5.112              | 11       | 4              | 2.087| 151.95 |
| TC + 4CAR | -8.131              | 5        | 1              | 2.096| 158.19 |
| TC + 5IND | -7.573              | 7        | 2              | 2.213| 145.37 |
| TC + 5PYR | -2.593              | 4        | 2              | 2.040| 154.76 |

4. Conclusion

In this study, geometry optimization, bonding situation, and binding energies of template-monomer complexes were presented using TC as the dummy template molecule. Through DFT methods, OPD was presented as the best functional monomer with a template-monomer molar ratio = 1:7. The complex TC + 7OPD formed produced 11 stable intermolecular hydrogen bonds and 4 double hydrogen bonds in distinct sites of action. This work provides the theoretical basis for the development of dummy MIP for OTC sensing.

Acknowledgments

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