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A COMPUTATIONAL APPROACH TO STUDYING CIPROFLOXACIN AND METHACRYLIC ACID IN PRE-POLYMERIZATION PHASE

UN ENFOQUE COMPUTACIONAL PARA EL ESTUDIO DE CIPROFLOXACINA Y ÁCIDO METACRÍLICO EN LA FASE DE PRE-POLIMERIZACIÓN

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Abstract
Density-functional theory calculations at the WB97XD/6-311++G** level of theory are presented to characterize the hydrogen-bonding interactions between ciprofloxacin and methacrylic acid during the pre-polymerization stage in molecular imprinting. Ciprofloxacin is an antibiotic characterized by multiple selective sites that can interact with an acid monomer. The reactivity was analyzed using natural bond orbital charges. The nucleophilic and electrophilic centers become more negative and more positive, respectively, after complex formation. A combination of geometrical parameters, atomic charges analysis and theoretical IR spectra are used to predict the hydrogen bond strength. The counterpoise method for the mitigation of basis set superposition error was used. By means of these results, it is possible to better understand these H-bonding interactions between the ciprofloxacin molecule, acting as template, and the methacrylic acid.
Keywords: ciprofloxacin, methacrylic acid, hydrogen bond strength, atomic charges, molecular imprinting, WB97XD functional.

Resumen
Se presentan cálculos con la teoría de funcionales de la densidad a un nivel de teoría WB97XD/6-311++G** para caracterizar las interacciones por enlace de hidrógeno entre ciprofloxacina y ácido metacrílico durante la etapa de pre-polimerización en la impresión molecular. La ciprofloxacina es un antibiótico caracterizado por múltiples sitios selectivos que pueden interaccionar con un monómero ácido. Se analizó la reactividad con las cargas atómicas NBO. Los centros nucleofílicos y electrofílicos se hacen más negativos y más positivos, respectivamente, después de la formación del complejo. La combinación de los parámetros geométricos, análisis de las cargas atómicas y espectros de IR teórico se utilizan para predecir la fuerza del enlace de hidrógeno. Se utilizó el método de la compensación para el error de superposición de bases. Con estos resultados, es posible comprender las interacciones por enlace de hidrógeno entre la ciprofloxacina que actúa como molécula molde y el monómero, ácido metacrílico.
Palabras clave: ciprofloxacina, ácido metacrílico, fuerza del enlace de hidrógeno, cargas atómicas, impresión molecular, funcional WB97XD.

1 Introduction

Molecular imprinting technology is a general protocol to create tailor-made cross-linked polymers that remember a particular target analyte. This molecular memory is created in the presence of a template molecule that is extracted afterwards, thus leaving complementary cavities to shape, size and functionality of the template as schematically shown in Fig. 1. These synthetic matrices are able to show recognition features comparable to those of biological systems (Sellergren 2001) and are stable to physical and chemical treatment.

During the synthesis of a Molecularly Imprinted Polymer (MIP) with a non-covalent approach, the target molecule is dissolved in a porogen, together with one or more functional monomers to produce a pre-organized system. The stability and extent of the template-functional monomer are associated with the number of high affinity binding sites.

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Therefore, it is very important to match the degree of complementarity (e.g. ionic, hydrophobic, π-π, H-bond donor with H-bond acceptor) between the template and functional monomer to maximize the recognition properties (Wackerling & Schirhagl 2015, Yan & Row 2006, Wu *et al*. 2003).

A methodology to find the optimal monomer functional is combinatorial screening (Takeuchi *et al*. 1999). However, this procedure is expensive, laborious and time consuming. With the rapid development of computational chemistry as a predictive tool, molecular modeling methods have been applied in the performance of MIPs (Karim *et al*. 2005). The functional monomers giving the highest binding energy with the template should be the best candidates for the polymer synthesis.

The rational design of a MIP requires knowing the structural and functional properties (Nicholls *et al*., 2015, Nicholls *et al*., 2013). Density-Functional Theory (DFT) is an appropriate quantum mechanical (QM) method for defining and elucidating important universal concepts of molecular structure and molecular reactivity (Parr & Yan 1989).

Ciprofloxacin (CIPRO), the most prescribed synthetic fluoroquinolone antibiotic used to prevent and treat a wide variety of infectious diseases was chosen as print molecule characterized by carboxylic and amino groups to interact with methacrylic acid (MAA) as functional monomer due to the presence of carboxyl group as a hydrogen donor and a hydrogen acceptor at the same time. Different authors have synthetized ciprofloxacin-imprinted polymers using MAA as functional monomer without a theoretical study of the pre-assembly formation (Mirzajani & Kardani 2016, Wang *et al*. 2014, Oliveira *et al*. 2011, Prieto *et al*. 2011, Chen *et al*. 2011, Díaz-Alvarez *et al*. 2009, Yan *et al*. 2008, Yan & Row 20 08, Turiel *et al*. 2007, Caro *et al*. 2006). Energy calculations between CIPRO and functional monomers at a semi-empirical level were performed (Marestoni *et al*. 2016). Therefore, our purpose in this paper is elucidate the H-bonding interactions between CIPRO and MAA by further exploring geometrical parameters, atomic charges analysis, theoretical IR spectra and binding energy using DFT at WB97XD/6-311++G** level of theory.

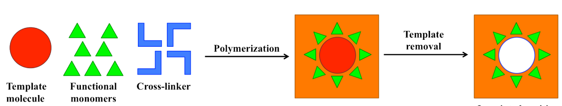


Figure 1. Creating a molecular memory in an artificial receptor.

2 Methods

All DFT calculations were carried out in the gas-phase with the WB97XD functional (Chai & Head-Gordon 2008) and a 6-311+G** basis set, using *Gaussian 09* software (Frisch *et al*. 2013). The minimum energy conformation has been identified and confirmed by frequency calculations. NBO charges were used for atomic charge analysis.

Finite difference approximations have been used to estimate the chemical potential (μ), hardness (η), softness (S) and electrophilicity index (ω) for a system with ionization potential IP and electron affinity EA (Cuán *et al*. 2005):

$$\mu = -\frac{IP + EA}{2} \tag{1}$$

$$\eta = \frac{IP + EA}{2} \tag{2}$$

$$S = \frac{1}{IP - EA} \tag{3}$$

$$\omega = \frac{\mu^2}{2\eta} \tag{4}$$

The binding energy of pre-organized systems was calculated as the difference between the sum of monomers energy and that of the corresponding complex. The basis set superposition error was corrected by means of the counterpoise method (Boys & Bernardi 1970).

3 Results and discussion

3.1 *Reactive centers*

Figure 2 shows the optimized structures for CIPRO and MAA. The most stable isomer of ciprofloxacin showed an intramolecular hydrogen bond formation (21H—1O). To predict the interacting groups of the isolated molecules, NBO charges have been employed. Table 1 summarizes the atomic charges in CIPRO and MAA. From these results and spatial considerations, it can be deduced that in CIPRO the nucleophilic reactive centers are the fluorine atom (8F), the carbonyl oxygen (1O), the carboxyl oxygen (17O) and the piperazinyl nitrogen (37N), while the electrophilic reactive center is carboxyl group hydrogen (21H). For MAA the proton donor is the carboxyl hydrogen (12H) and the proton acceptor is the carboxyl oxygen (11O).

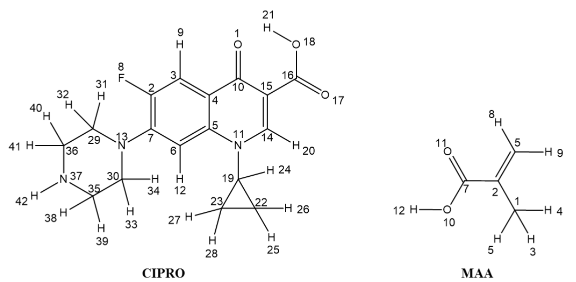


Figure 2. Optimized geometries for the isolated molecules.

3.2 *Hydrogen bonding*

Table 2 contains the global reactivity parameters for isolated molecules. The chemical potential is a function of the escaping tendency of an electron cloud (Shenghua *et al*. 2004). The value of chemical potential for methacrylic acid is lower than for ciprofloxacin which implies that electron transfer will be from CIPRO to MAA during the formation of the pre-arrangement. It is important to note that MAA is a better electrophile and CIPRO is a better nucleophile. CIPRO and MAA have similar global softness values, which will allow a strong interaction according to the HSAB principle (Chattaraj *et al*. 1991). Based on the global electrophilicity, the fluoroquinolone will behave as a nucleophile and the acid monomer as an electrophile when approaching each other.

As introduced earlier, there are four interacting regions for MAA in CIPRO. Based on these, the possible conformations of the 1:1 complexes of CIPRO and MAA were optimized (see Fig. 3). The hydrogen bonding distances have been obtained, and the results are presented in Table 3 where D, H, A indicate the donor, bridging hydrogen and acceptor atoms, respectively. The atoms involved in the hydrogen bond are referred to Figure 2. The formation of the H-bond elongates the O-H bond lengths compared to the corresponding bonds in the isolated molecules (Lv *et al*. 2010). The elongation of bond 10O-12H in complex 1 is 0.0145 Å, 0.0203 Å in complex 2, 0.0033Å in complex 3 and 0.0412 Å in complex 4 while the bond 18O-21H is shorter by 0.0067 Å in complex 1. The variation of the bond length in Complex 3 is relatively small, indicating that the hydrogen-bonding interaction is weak.

Table 1. NBO charges distribution for CIPRO and MAA. Labels are referred to Fig. 2.

CIPRO		MAA	
Atom	NBO charge	Atom	NBO charge
1O	**-0.65528**	1C	-0.60778
2C	0.39244	2C	-0.13818
3C	-0.20216	3H	0.22145
4C	-0.14523	4H	0.20800
5C	0.20002	5H	0.22147
6C	-0.21983	6C	-0.29040
7C	0.15456	7C	0.78882
8F	**-0.34836**	8H	0.21663
9H	0.26162	9H	0.19116
10C	0.51154	10O	-0.69065
11N	-0.41037	**11O**	**-0.60809**
12H	0.23885	**12H**	**0.48757**
13N	-0.59361		
14C	0.16781		
15C	-0.32555		
16C	0.80492		
17O	**-0.61971**		
18O	-0.68159		
19C	-0.03168		
20H	0.2477		
21H	**0.50147**		
22C	-0.40149		
23C	-0.40851		
24H	0.21938		
25H	0.22457		
26H	0.22649		
27H	0.22667		
28H	0.2202		
29C	-0.19084		
30C	-0.19104		
31H	0.20258		
32H	0.17615		
33H	0.17583		
34H	0.20323		
35C	-0.19025		
36C	-0.19027		
37N	**-0.67148**		
38H	0.19911		
39H	0.18846		
40H	0.18835		
41H	0.19892		
42H	0.34668		

Table 4 lists the atomic charges for the interaction sites upon complexation. The parameter λ is calculated as the difference of the atomic charge before and after of the interaction and represents the effective number of valence electrons participating in the process (Chandrakumar & Pal 2002). The proton donor, 12H, is the most positive after complexation.

Table 2. Global reactivity descriptors for CIPRO and MAA in atomic units.

System	Chemical potential	Global hardness	Global softness	Global electrophilicity
CIPRO	-0.14442	0.16827	2.97142	0.061978
MAA	-0.20425	0.18370	2.72188	0.113553

Table 3. Hydrogen bonding for CIPRO-MAA (Å, º).

System	D-H--A	*d*(H---A)	*d*(D---A)	∠DHA
Complex 1	10O-12H---1O	1.7337	2.7079	173.098
	18O-21H---11O	2.4630	3.1504	126.964
Complex 2	10O-12H---7O	1.6982	2.6784	173.270
Complex 3	10O-12H---8F	1.9832	2.9488	175.641
Complex 4	10O-12H---37N	1.7192	2.7148	169.899

Table 4. Variation of the atomic charges upon complexation.

System	Atom	q_0	q_{eq}	λ
Complex 1	1O	-0.65528	-0.71398	-0.05818
	21H	0.50147	0.52246	0.02099
	11O	-0.60809	-0.62583	-0.01774
	12H	0.48757	0.52040	0.03283
Complex 2	17O	-0.61971	-0.68870	-0.06799
	12H	0.48757	0.52688	0.03931
Complex 3	8F	-0.34836	-0.38965	-0.04129
	12H	0.48757	0.49837	0.01080
Complex 4	37N	-0.67148	-0.70505	-0.03357
	12H	0.48757	0.50989	0.02232

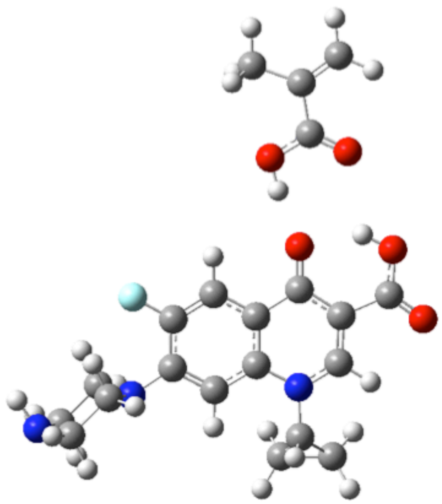
3.3 *IR analysis*

The hydroxyl group in the methacrylic acid is the most important site for analyzing the bridging hydrogen. The IR spectra were also calculated at the WB97XD/6-311+G** level of theory. The MAA OH stretch is located at 3848 cm^{-1} and 91.5 intensity, whereas this peak is 3580 cm^{-1} and 1374.9 intensity in complex 1, 3457 cm^{-1} and 1479.1 intensity in complex 2 and 3788 cm^{-1} with 457.7 intensity in complex 3 and 3002 cm^{-1} with 3124.5 intensity. MAA C=O stretching bond was shifted from 1839 cm^{-1} and 291.4 intensity to 1823 cm^{-1} and 278.9 intensity after complex 1 formation. The OH stretching vibrational after pre-arrangement adduct formation moved to lower frequencies and it is more noticeable in complex 4. However, we think that the spatial orientation of the piperazinyl group does not allow the free access of the monomer. Therefore, the complex 2 is expected to be stronger that the other complexes.

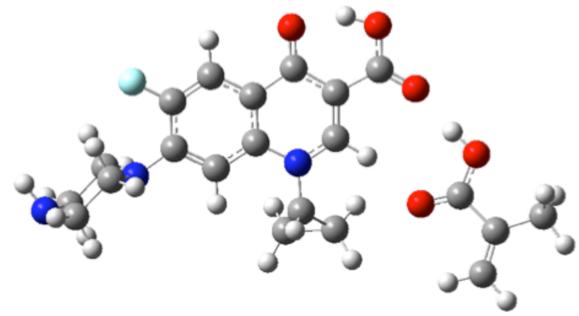
3.4 *Binding energy*

The calculated values for complexation energy of the possible 1:1 complexes are reported in Table 5. One can observe significant differences in the hydrogen bond strength. The complex 2 is more stable than complexes 1, 3 and 4 in the order of 8.0, 12.4 and 1.4 kcal mol^{-1} as expected. The hydrogen bond interaction is strong (> 15 kcal/mol) in complex 2, moderate (4 to 15 kcal/mol) in complexes 1 and 4 and weak (< 4 kcal/mol) in complex 3.

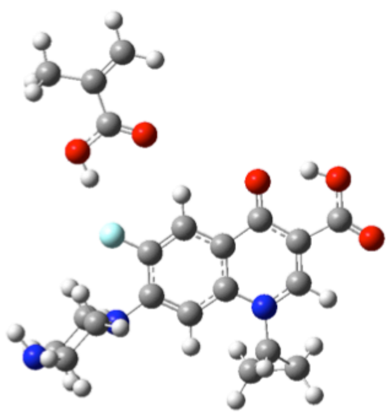
Two H-bonding coexist in the complex 1 formation. The binding distances for 10O-12H—1O and 18O-21H—11O are 1.7337 Å and 2.4630 Å, respectively. Thus, the hydrogen bond 10O-12H—1O is the highest contributor to the binding energy, whereas 18O-21H—11O mostly stabilizes the complex (Saloni *et al*. 2010).



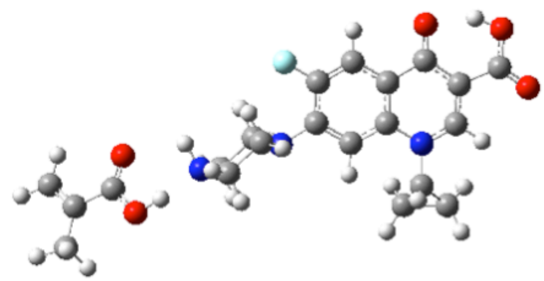
Complex 1



Complex 2



Complex 3



Complex 4

Figure 3. Optimized structures of all possible 1:1 complexes between CIPRO and MAA.

Table 5. Binding energy for 1:1 complexes between CIPRO and MAA

System	ΔE (kcal mol^{-1})	ΔE^* (kcal mol^{-1})
Complex 1	- 8.2	8.0
Complex 2	-16.2	0.0
Complex 3	-3.8	12.4
Complex 4	-14.8	1.4

ΔE^* = Complex *i* – Complex 2, where *i* corresponds to the complex under study.

3.5 Solvent effect

The solvent has an important role in the binding between template and functional monomer. The relative energy with the solvent correction of the CIPRO and MAA was investigated by using the polarizable continuum method (Miertus *et al*. 1981, Tomasi & Persico, 1994). Dichloromethane (DCM) and methanol have been experimentally used (Turiel *et al*. 2007). For the isolated molecules, methanol is the solvent most suitable for stabilization (energy becomes more negative). Polar groups in the solvent can compete with the template-monomer interactions or in some cases favor the interactions (Vasapollo *et al*. 2011). Ciprofloxacin is slightly soluble in methanol and practically insoluble in DCM. It is expected that a MIP synthesized in methanol exhibits better molecular recognition ability.

The experiments carried out by solid-phase extraction, the MIP prepared in methanol using MAA as monomer showed the best performance (Turiel *et al*. 2007). In addition, the authors showed that there was no imprint effect with 4-vinylpyridine as functional monomer. Based on computed reactive centers, it is found that MAA is able to interact with four sites from CIPRO, whereas 4-VP would have a single interaction through its nitrogen atom with carboxyl group hydrogen (21H). Thus, MAA is an effective functional monomer for the molecular imprinting.

Conclusions

A density functional QM method was employed as a predictive tool for describing the hydrogen bonding strength between ciprofloxacin and methacrylic acid. This study showed that CIPRO has four-recognition sites for MAA. Individually, the MAA interacts more strongly with the CIPRO carboxyl group. After complex formation for the reactive sites, the bond length of O-H elongated and the atomic charges increases as a result of electron density flow. The analysis of the changes of OH stretching vibrations upon pre-assembly revealed the presence of the hydrogen-bonded interactions. WB97XD/6-311+G** level of theory is sufficient for study of hydrogen-bonding interactions. The binding energies are calculated taking into account the basis set superposition error.

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Nomenclature

μ	chemical potential
η	hardness
S	softness
ω	electrophilicity index
IP	ionization potential
EA	electron affinity
ΔE	complexation energy

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