Molecular catchers for pharmacologically active substances in wastewaters, a theoretical study

P J Salazar Valencia¹, S T Pérez Merchancano¹, H Paredes² and L E Bolívar Marínez¹
¹ Universidad del Cauca, Popayán, Colombia.
² Universidad Industrial de Santander, Bucaramanga, Colombia.

E-mails: pjsalazar@unicauca.edu.co

Abstract. A basic and pressing need in the treatment of residual waste waters for urban and rural centers is the removal of pharmacological active residues from them, these residues are originated in a wide array of domestic, agricultural and industrial sources and can’t be removed in the residual waters treatment plants by conventional methods, the result is the incorporation of them into the ecosystem altering the physiology and behavior of living organisms. Among the most active pharmacological substances found in very high concentration in residual waters is paracetamol, an analgesic of very wide excessive use due to its ease of access and low cost [1]. No pharmacological substance is entirely absorbed by the human organism and therefore a wide family of molecular residues is excreted by the urinary tract. In this work we have used the AM1 (Austin Model 1), PM3 (Parametric Method 3) and ZINDO/Ci semiempirical methods, from the NDO (Neglect Differential Overlap) family [2] to study and observe the structural, electronic and optical characteristics of paracetamol while immersed in different basic and acidic aqueous environments, either alone or interacting with lignosulphonates. We have previously found that lignosulphonates, a lignin derivatives of wide industrial applications, can be engineered as a binding and floculant agent therefore showing the potential to be used as a mean to filter and eliminate molecular residues from the residual waters [3].

1. Introduction

An important problem in the water treatment for human consumption is the increasingly presence of significant amounts of pharmacologically molecular active contaminants or SFA substances, which are incorporated into the water cycle after being excreted by living beings through sewage water from domestic, industrial, and clinical origin. Among these SFA one of the most abundant corresponds to prescription free painkillers, which are widely used due to their low cost and wide availability, one of them is paracetamol, commercially known as acetaminophen.

Acetaminophen is a drug with analgesic properties, which acts by inhibiting the synthesis of prostaglandins, cellular mediators that are responsible for the onset of pain, has good antipyretic (fever control) but no substantial anti-inflammatory properties, it also has a wide spectrum of commercial presentations.

This compound comes from N-acetyl-para-aminophenol and acetyl-para-aminophenol and in Figure 1 we can see its molecular structure [4].
Figure 1. Paracetamol (acetaminophen) Structure.

Paracetamol is mainly metabolized in the liver, resulting in some cases in non-toxic products by means of glucuronidation (glucuronic acid addition) and sulfating, and in other cases generating toxic products such as NAPQI or N-acetyl-p-benzoquinone imine.

Lignosulfonates (LS), also called lignosulfonates, lignin sulfonates or lignin sulfites are essentially lignins in sulfonated forms, obtained from "sulfite liquors" which in turn are a residue of the pulp extraction process in the wood pulping industry. They can be used as additive agents, dispersants, binders, fluidificants, agglutinative, etc. Since they can be manufactured relatively easily and quickly, and that its molecular size can be manipulated to obtain fragments of very low molecular weight, they are used as transport agents in the food, cosmetics, pharmaceutical industry and in the development of drugs and molecular elements for health problems treatment [5].

The LS extraction process is known as delignification and acts by breaking the ester bonds (C-O-C) that connect the lignin constituent elements, then the electrophilic carbonations produced during the breaking of ester bonds react with bisulfite ions to produce the sulfonated at the same time incorporating functional groups to the molecular structure. Among the incorporated ions are for example, calcium, sodium, magnesium and ammonium, this process can be schematized briefly in Figure 2 [6].

In this process, the woody adhering tissue components are eliminated, resulting in a class of polyelectrolytes' polymers with high acidity due to the presence in their molecular structure groups of highly dissociated sulfonic acid, and unlike lignin when it is present in the cell walls this are water soluble, this family of molecules is collectively known with the name of lignins.

It has been empirically observed, that LS possess capabilities that make them behave in a very effectively way as free radical hunters and antioxidants [7]. The properties of the LS depend, as in any molecular system, from its structure and the characteristics of the individual components, sharing with lignin the basic precursors form and bond mechanisms [8]. By observing the molecular structure we can see a series of small aggregates of 2 or 3 precursor units linked through Carbon Oxygen bonds, among this there is a high proportion of the β-O-4 linking mechanism, that connects the aromatic parts with the carbon chains, in this chains other substituent groups adhere, which in the case of the LS are sulfonates (functional groups containing SO₃⁻ ions); other functional groups adhere to either sulfonates, available positions in the carbon chain or simply appear in molecular or ionic form near the structure.
The macromolecule grows when it polymerizes randomly until an amorphous and three-dimensional biopolymer is formed, which, like lignin, is difficult to study because of their size and to the randomness of its formation. Previous studies have shown that in the case of lignins in general, the properties of the basic units or precursors can easily be extrapolated, and be used to describe certain characteristics and behavior of the macrostructure [9]. In this work it was established that the lignosulphonates or sulfonated lignin, residues from the paper pulp extraction process that can be used as binders and flocculating agents of wide industrial use, adhere and encapsulate to the analgesics, paracetamol in particular, fulfilling the function of filter and frame grabbers of molecular pharmacologically active waste in sewage water, for it we use AM1 (Austin Model 1) semi-empirical methods, PM3 (Parametric method 3) and ZINDO/CI, (HyperChem 5 from the NDO family (Neglect Differential Overlap)) that characterizes the compounds in structural, electronic and optical way in different aqueous environment.

2. Methodology

The geometric structure of the compounds with which we worked are known from the literature, the paracetamol molecule is combined with small fragments of up to three (3) LS basic units containing functional groups of Ammonium, Sulfur, Calcium and Sodium in aqueous environments, acidic and basics to study the interaction among molecular systems through specific characteristics such as atomic distances, heat or enthalpy of formation, simulations of electrostatic potentials, etc. The studies were conducted using the molecular modeling program Hyperchem 5 [10] using semiempirical methods AM1 and PM3 from the NDO (Neglect of Differential Overlap) family for calculating the structural and electronic properties and in the case of the optical properties it was used the semiempirical method ZINDO/CI.). A convergence gradient criterion type RMS with $10^{-3}$ limit was used in all calculations obtained. Electronic and optical properties were calculated in Single Point mode with 6-occupied orbitals and 6 unemployed criterion seeking energy stability of the structure. Paracetamol was structurally characterized using the mentioned methods in several environments (vacuum, acidic and basic aqueous). Then we proceeded to combine the paracetamol molecule with the LS and determine again the molecular systems characteristics consequently formed in each of the environments. For modeling of different environments in which the structures are embedded a periodic box was designed, with 50 water molecules in random positions to make up the environment, and then in it, it submerges the paracetamol and the lignosulphonate considered.

3. Results and discussion

Table 1 shows the values of the heat of formation enthalpy of paracetamol obtained by geometries optimizations using the three main methods of NDDO approach, the best value is provided by the PM3 method, with a difference of about 10kcal/mol respect to the AM1 and MNDO methods. The heat of formation enthalpy indicates how much energy is needed to create the structure, and as more negative the value is, greater will be the probability that the structure exists and be stable, these results are presented in their neutral state and in vacuum.

| Method | Heat of Formation |
|--------|------------------|
| MDNO   | -58.01           |
| AM1    | -56.97           |
| PM3    | -67.12           |
Since PM3 offers the best results it was decided to use as a basis for the development of the other optimizations geometries in this work. A representation of the geometrical structure obtained from these calculations can be seen in Figure 3.

The graphical representations of the geometries obtained for the studied molecular systems in this paper are two-dimensional projections of three-dimensional structures, for example the geometry obtained shows a seemingly flat molecule of about 6.5Å in length by 2.42Å wide, with a small side chain leaving the plane with a twist angle of -27° and a molecular mass of 151.15Da (atomic mass units). Figure 3 shows this configuration.

![Figure 3. Molecular structure of Paracetamol obtained by optimizing the geometry with the semiempirical method PM3.](image)

To determine the preferential charge state of the structure Heat of Formation is determined for each one of its charging conditions in vacuum and water. The results shown that paracetamol in the vacuum has a more stable structure in its negative charge state (-1) with -77.71kcal/mol indicating that it has a tendency to behave as an electron acceptor. Geometry optimizations with other environments (Water) are performed, in neutral charge state and ionized with one proton and one electron, to determine if the molecule behaves as an acceptor or electron donor, the results are reported in Table 2, where the first thing you notice is that there is a significant decrease in the value of the Heat of Formation when passing from the vacuum to the aqueous environment which shows a great affinity of paracetamol with water; another important aspect is that in vacuum, paracetamol behaves as an electron acceptor, while in water is neither donor nor acceptor, and at least for this structure the bond mechanism by free radical is discarded.

| Charge State | Heat of formation in kcal/mol for paracetamol for vacuum and water in various charging conditions, results obtained with the method PM3. |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|              | Vacuum | Water   |                                                                                   |
| +1           | 117.73 | -1147.02|                                                                                   |
| 0            | -67.12 | -1409.72|                                                                                   |
| -1           | -77.71 | -1401.16|                                                                                   |

Graphical representations of the electrostatic potential, also known as maps of potential energy, allow us to illustrate the distributions of molecular charge in two and three dimensions, these allow us
to visualize how they molecular charge is distributed and how electrically charged molecules regions behave, knowing this allow us to determine how molecules interact with each other.

In the case of paracetamol, it can be seen that in the neutral charge state the more reactant sites are those in which nitrogen and oxygen atoms located, when the molecule is ionized, that is, in the state -1 the most reactive regions become larger and tend to cover the aromatic ring all around.

However, the state where the molecule has more reactivity is the corresponding to the ionization +1, it means, in the presence of a proton; in this case the entire molecule is reactive, the Heat of Formation values for this state showed that the molecular system at the charge state can exist in the aqueous environment (Figure 4). The best reactivity, observed through the graphical representations of electrostatic potential, shows that the more reactive places correspond to the nitrogen and oxygen atoms, and when ionize an electron, it means in the state -1 the most reactive regions become larger and tend to cover the surroundings of the aromatic ring.

![Electrostatic Potential](image)

**Figure 4.** Graphical representation of electrostatic potential, for paracetamol in various states of ionization. Results obtained from the application of ZINDO / CI method.

From previous studies it is known the acceptor behavior of the electron possessing the lignosulfonates [7]. The optimization results of paracetamol in ammonium environment attached from one to three lignosulfonates units in vacuum and water are presented in Table 3.

| Paracetamol + LS with: | Charge | 1 Unit | 2 Units | 3 Units |
|-----------------------|--------|--------|---------|---------|
| Ammonium in vacuum    | +1     | -108.79| -305.67 | -502.14 |
|                       | 0      | -287.27| -484.87 | -683.88 |
|                       | -1     | -332.97| -532.37 | -739.16 |
|                       | +1     | -2431.56| -2751.04| -3066.47|
| Ammonium in water     | 0      | -2598.47| -2925.17| -3238.89|
|                       | -1     | -2655.64| -2972.61| -3298.66|

The data for in the Table 3 shows the values of the Heat of Formation for new molecular system, this value show the affinity of paracetamol with the environment considered in this work (Ammonium) and lignosulphonates growth, allowing us to visualize the molecules stability. These results, in vacuum and aqueous environments exhibit a high affinity for the paracetamol molecule with aqueous environments.
In these new molecular systems, the LS were incorporated as they grow, and the macromolecules properties can be inferred with those corresponding to small polymeric precursors and fragments.

In the Figure 5 we present the graphical representations of the geometry molecular of the studied systems show that the alignment phenomenon actually occurs, just that it is not as significant as had been observed for natural lignin, the combination with ammonium does not presents the alignment phenomenon, but in the aqueous environment the LS closed around of paracetamol entrapping inside.

![Figure 5](image)

**Figure 5.** Graphic representation of the molecular interactions between paracetamol and monomeric LS fragments studied in water. (a) In vacuum, (b) in aqueous ammonium.

### 4. Conclusions

The results from the calculations carried out with the paracetamol, on the environments considered show that they have a great compatibility with those that contain water, particularly if molecules of an acid are present. The paracetamol also behaves as an electron acceptor showing a great tendency to interact with other molecular systems by the free electron mechanism and also showing a hint of the reasons for it’s great pain relief action.

The electric and optical properties calculated also have shown us that paracetamol has a variety of regions with high electronegativity, and that the size of this regions is related to the environment were the molecule is set up, with acid environments being the most reactive ones, it is also found the LS studied also share the same electric and optical characteristics.

By studying the interaction between the paracetamol and LS molecules (LS with ammonium) we have found that, as expected, there is an interaction with the potential of becoming a "molecular capture" mechanism. We have seen that in the molecular structure of the resulting systems the LS "embraces" the paracetamol with the heat of formation values getting smaller as the geometry of the systems are optimized. Besides the addition of the aqueous medium assists in the process by lowering both, heat of formation and distance between molecules with an aligning of the aromatic parts and the interlacing of the carbon chains.

All this characteristic show us that the LS have a great potential to be used as aids in the removal of paracetamol molecules from the residual waters. The great variety of LS existing in the market, it’s low prices combined with the fact that they are very environmentally friendly gives this alternative a great deal of attractiveness.

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