University of South Bohemia
Faculty of Science

The Interaction of Graphene Oxide with Humic Acids, a Computational Study

Bachelor's Thesis

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Annotation

This study was focused on the interactions of Graphene oxide with humic acids in aqueous solutions. Additionally, to this work, Graphene oxide and water interactions with and without the addition of ions were studied. The behavior of a fulvic acid in the presence of Graphene oxide has been investigated as well. The study was performed using computer simulations that were achieved by Molecular Dynamics simulations. These simulations provided useful data on a nanometer scale.

Declaration

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Stefan Ferlin

Acknowledgement

I am extremely grateful to Dr. Babak Minofar, PhD for helping me to get this bachelor thesis done and for being there whenever I had difficulties and did not know how to proceed. Sometimes the advice that finally helped came at ungodly hours and I appreciate very much the effort it must have taken for a supervisor to be this reliable.

Even though most of my family will not be able to read any of these words since they are in English, I would like to thank them all for the financial and emotional support through this big struggle that has been this study program.

A big thank you to Prof. RNDr. Libor Grubhoffer, Csc! Without him and his kindness and willingness to be there for his students, I do not think that I would have managed to stay in this program for so long.

1. Abstract

Humic substances are a big part of natural water source pollution. Together with heavy metals they form dangerous complexes, which affect wildlife as well as civilizations which are consuming non-filtered water. Eliminating such threats from our natural water sources would greatly affect the overall well-being of many species and signify a big step into the right direction in the science of water treatment. As of yet, the availability of graphene and its derivative, graphene oxide (GO), is quite low. However, since these substances are very promising to be cost-efficient and a good alternative to the methods used for removal of organic substances and organic pollutants, which are mostly based on activated carbon, research into this matter is quite pressing.

A computational study was conducted, researching the interaction of GO with humic acids, fulvic acids and ions in a scenario were two molecules (or just one in the case of ion interactions), dissolved in water, are free to move around and interact. The performed simulations were conducted using programs specialized for use in the Linux operating system. Super computers facilities were involved to ensure accurate calculations over the time period of 100ns at 300K.

This method produced results that confirmed to the earlier findings and showed the efficiency of GO as a filtrating agent for removal of humic acids and fulvic acids and other possible organic pollutants from water. The aromatic stacking interactions between the humic acid molecules and other weak interactions such as hydrogen bonds and Van der Waals forces, are involved in sorption of humic acids to the surface of GO and were successfully simulated. Distances between the aromatic rings and the number of hydrogens bonds were plotted in graphs over time, and snapshots of the simulations were produced. For this study, several models of humic acids and fulvic acids were used. Although not all findings were expected but these results brought new insights for further research and provides new knowledge for the graphene-research community.

Table of contents

2. Introduction

2.1. Humic acids and their environmental impact

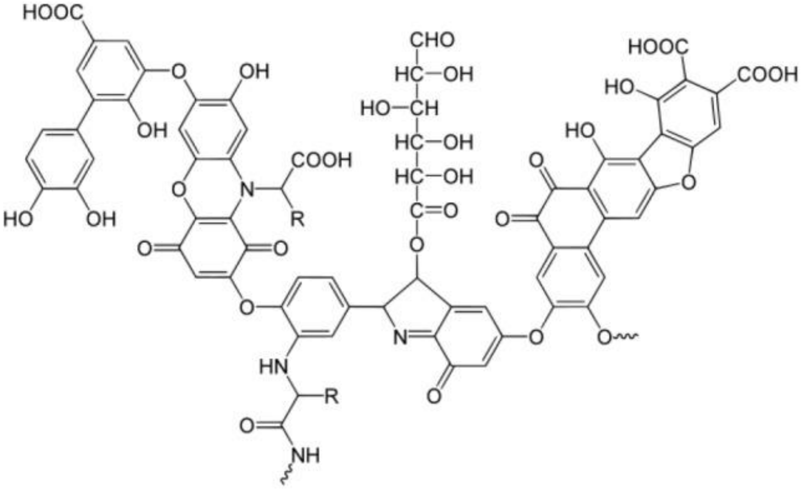


Figure 1: average formula of humic acid [2]

Together with heavy metals and suspended solids, humic acids and other dissolved organic carbon compounds (DOC, e.g. fulvic acids) are considered major pollutants in water. When dissolved organic compounds interact with heavy metals or other organic contaminants in water, they may form dangerous metal-organic complexes, which pose a major threat to the health of people consuming such water. Moreover, humic acids can interact with emerging contaminants such as pharmaceuticals, personal care products, pesticides, and hormones to make them available in aquatic ecosystems which can lead to toxic biological effects such as mutagenicity, and genotoxicity.

Also, heavy metals are known to cause health problems such as neuro-behavioural disorders, mental retardation, various types of cancers, kidney damage, and can even cause death. They may also affect aquatic life in similar negative ways [1].The interaction of heavy metal ions with humic acids may cause the bioavailability and toxicity for aquatic ecosystems.

Although plant life and many organisms oftentimes thrive in environments containing heavy metals, the absence of such particles even leading to limitation of growth, heavy metals in high quantity are never desirable. Additionally, to their health compromising nature, they can interfere with many natural processes in the environment, leading to substantial changes in the biogeochemical cycle.

These particle’s toxicity, mobility and the lacking ability to biodegrade make it very difficult to decontaminate [3]. Countries utilize a variety of methods to deal with pollutants in water, often absorbents. And while there may be some methods to get rid of heavy metals [4], these methods may get less effective when DOC are introduced.

A major percentage of naturally occurring DOC are the humic acids (HA) which make up as much as 90% of all DOC. Normally, heavy metals and other pollutants are filtered using granulated activated carbon (GAC). However, the interaction between DOC and heavy metals may cause disturbances in this method of filtration [1].

The Humic acids themselves are a major component of natural organic matter in soil, water and geological organic deposits. Oftentimes they are a major cause of the brown color on decaying plant matter, as well as the brown or black color of earth matter. As a large component of ground soil, they play an important role in the improvement of fertility of the ground, as well as the physical and chemical properties of the soil itself. In water, they affect the acidity, or alkalinity.

Overall, humic acids have an overwhelming effect on bioavailability and the cycling of elements, as well as transport and degradations of chemical compounds. These acids are complex chemical substances formed through a process of decay of plant and animal matter by biological and chemical processes (humification).

Differences in humic and fulvic acids are given, for example, in their chemical properties. If one were to extract humic acids from other humic compounds, such as fulvic acids and humin, the solution in which they are contained would be treated with strong acids (HCl, until ph1), leaving the humic acids to precipitate.

These acids are very reactive compounds and yet recalcitrant with respect to biodegradation. The average properties across humic substances are remarkably similar, even though they have diverse structures and molecular weights. The precise structure and molecular weight are dependent on the way of degradation of organic matter [5].

2.2. Activated carbon for drinking water treatment

Activated carbon for drinking water treatment has been used widely for many years. Many European countries, as well as the United States of America utilize this method. Even before this widespread usage, carbon and other absorbents in various forms were used for detoxification for pharmaceutical substances and water treatment for several centuries, even going as far as to being referenced in the bible [6].

‘Activated carbon’ is an umbrella term for a family of substances, mostly characterized by their sorptive and sometimes catalytic properties. Different ways of production of activated carbon compounds may produce a variety of end products with distinct characteristics.

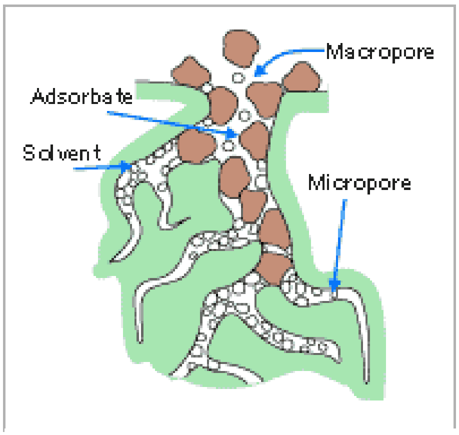


Figure 2: The porous structure of activated carbon (source: http://www.kuraray-c.co.jp/en/activecarbon/about/)

The basic structural unit of activated carbon is approximately that of pure graphite, although, since impurities may oftentimes be present, the final product is not referred to as graphite. The structure of activated carbon is quite disorganized due to random oxidation of the graphite layers and the regular array of carbon bonds in the surface disrupted during the activation process, leading to a high reactivity. This reactivity is further enhanced for the purpose of filtration by its porous nature, since activated carbon contains macropores (effective radii of 500-20,000 Å), transitional pores (40-200 Å) and micropores (18-20 Å) as can be seen in Figure 2. These are arranged in such a way, that the macropores, which are present on the surface, branch off into transitional pores, which in turn branch off into the micropores. Only a few of the micropores reach the outer surface, however at least 90% are assumed to amount to the total surface area of activated carbon substances [6].

Nowadays, activated carbon is produced by controlled thermalization of carbonaceous material, mostly wood, coal, coconut shells or peat. This can then be used either in powdered (PAC) or granular (GAC) form. When the adsorption capacity is exhausted, it can be reactivated by burning off the aggravated organic matter, however, in practice, most of the activated carbon is discarded after single use. The choice between PAC and GAC is mostly dependent on cost-effectiveness, frequency and the dose that is required for a specific application. PAC generally preferred in the case of low dosage rates and seasonal or intermittent contamination.

PAC is dosed as a slurry, leaving the adsorbent in solution and filtering it by filtration of sludge that accumulates at the end of the process. This means that the PAC can only be used when existing filtration is already present. GAC in so called fixed-bed absorbers offer therefore higher efficiency, since the effective carbon use per litres of water is lower.

Most applications of GAC utilize the adsorption capability for taste and odour control. The service life of such a GAC bed is dependent on the capacity of the carbon used and the contact time between the water and the carbon. Contact times are usually between 5-30 minutes. However, the capacity for different organic compounds varies very much, meaning that the lifetime of a GAC bed is dependent on its type of usage. The deciding variables are the solubility in water and the octanol-water partition coefficient. Chemicals with low solubilities and high log octanol-water partition coefficients are well absorbed most of the time.

Outside of odour and taste control GAC and PAC may also be used for removal of pesticides and other organic chemicals, cyanobacterial toxins and total organic carbon [7].

2.3. Graphene

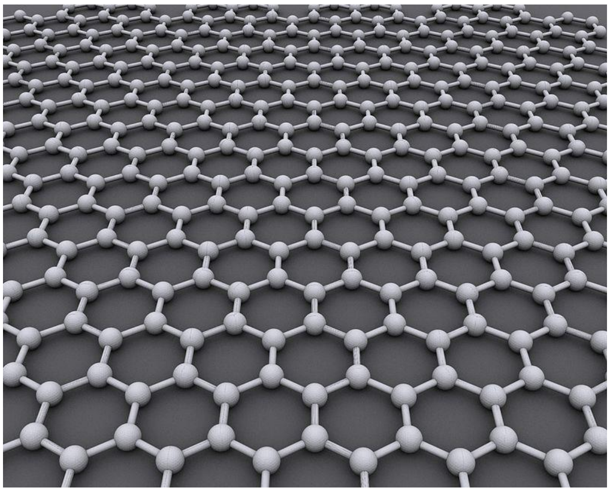


Figure 3: The structure of graphene (Source: AlexanderAlUS (Wikipedia.org 2019))

Graphene is an exciting material. It has a very large surface area, high intrinsic mobility, leading to a high electrical conductivity and a high thermal conductivity, to name only a few of its exciting properties among many other things.

The structure of graphene reminds of that of a honeycomb, the lattice of carbon atoms with a network of delocalized electrons forms a thin sheet. However, this sheet can, through microscopic corrugations, possess wave-like deformations in the structure, meaning that this structure is not always a flat surface. These so-called ripples can be induced by localized strain on the carbon lattice. Apart from this, other defects may occur like topological defects (e.g. pentagons, heptagons, or their combinations), vacancies, adatoms, cracks and impurities.

Interesting fields of use for graphene could be as large-scale transistor arrays with uniform electrical properties. Another application could be sensors that detect gases or biomolecules by detection of charge transfer and chemical response. It could also be used as a transparent conductive film because of its high electrical conductivity and optical transmittance. Environmentally interesting ideas of development even include a clean energy source by making graphene into a one-of-a-kind electrode material [9] [10].

The synthesis of graphene can be achieved in many ways. One method includes chemical vapour deposition, where a solid component is deposited out of a gaseous phase onto a suitable material. Another uses so-called microchemical exfoliation, where scotch tape is used to create a thin layer of graphene out of graphite [8]. A third method describes exfoliation of graphite in solvents, where powder of graphite is used and dispersed in different solvents with high intensity ultrasound. Methods of graphene creation are numerous [9] [10].

2.3.1. Graphene oxide

Graphene oxide (GO) is synthesized using the Brodie, Staudenmaier or Hummer’s method. Brodie and Staudenmaier use a combination of potassium chlorate ($KCLO_3$) with nitric acid (HNO_3) to oxidize graphite, while Hummer’s method involves a process of treating graphite with potassium permanganate ($KMnO_4$) and sulfuric acid (H_2SO_4).

The polar oxygen functional groups cause GO to be hydrophilic, rendering it dissolvable in water. GO can be exfoliated in many solvents, but dispersion in water is especially efficient. An effective dispersion is mostly achieved by stirring and sometimes by sonication of GO in solvents.

Theoretically, a chemical reduction, and thereby removing of the functional group is possible through reducing agents, although for our purposes this was not needed [9] [10].

GO is a very promising compound, holding relevance in opto-electronics, energy storage, solar cells, biomedical technologies and membranes for selective molecular separation. There is a growing interest in GO membranes for rapid transport of water for desalination, water purification, dehumification and filtration. Since most water-treatment options are not cost-effective, energy intensive or inefficient, GO holds the promise of an inexpensive and scalable method to guarantee waste water treatment in poorer regions of the world. One of the reasons this is not yet possible is the lack of understanding of mechanistic and molecular interactions of GO. Even details like structure are yet to be definitely determined [12].

In earlier experiments, it was found that even GO nanoparticles could interact in such a way, that a strong adsorption to matter was possible. In particular a humic acid (Suwannee River humic acid) and fulvic acids were found to bind to theses nanoparticles in a relatively strong manner (relative to surfaces coated with silica). Which would make it possible to, somewhat ironically, use humic acids to attract GO nanoparticles and remove them from waters. After filtration by humic acids, the attached GO particles could then be removed upon introduction of de-ionized water [11].

GO is considered to be amorphous and non-stoichiometric, having hydroxyls and epoxides as functional groups, with carbonyls at the edges, see figure 4. GO is known to be rippled with sheet holes of about $5nm^2$.

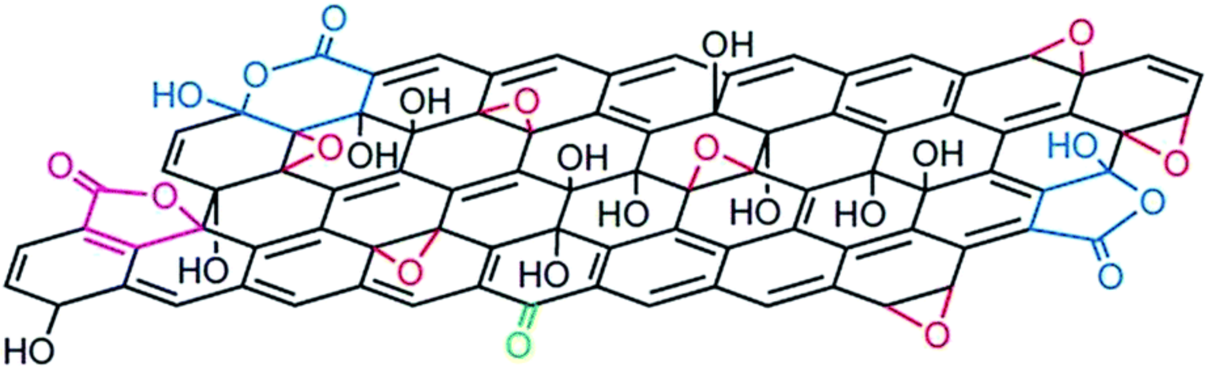


Figure 4: Structure model of Graphene oxide (Source: Royal society of Chemistry, RSC Advances) [13]

GO interactions with water are quite frequent. The functional groups build hydrogen bridges around water at almost every hydration level. It was noted that the spacing between individual GO layers increased in size after the introduction of water (from 0.8 to 1.1nm). It is important to realise, that oxidized and unoxidized, as well as polar and unipolar regions of GO will have different effects on the interactions between GO and molecules around it [12]. Devanathan, Chase-Woods, Shin and Gotthold [12], also call upon computer simulations to study GO as there is a pressing need to understand these intermolecular interactions.

2.4. Molecular interactions

2.4.1. Van der Waals forces

In 1873, van der Waals first used the discovered deviations from the ideal gas law to formulate the van der Waals equation of state. This equation takes into account the interactions between molecules. He argued that, since molecular interactions would take away from the force exerted onto a container, the outward pressure would decrease.

This equation's importance was underlined by the fact that a prediction of gas to liquid transition and of a critical point for pure substances was possible. Although the equation might have been superseded already, its success led to interactive and repulsive forces of molecules to be referred to as van der Waals forces.

What distinguishes van der Waals forces from other intermolecular interactions (such as hydrogen bonds), is their relative strength. Van der Waals forces are comparatively weak (typical single bond dissociation energy: 150-500 kJ/mol, interactions between small molecules: 1-25 kJ/mol). [14]

2.4.2. Types of forces

The first level of classification that one can divide intermolecular forces in is long range and short range forces. Long range forces vary as inverse powers of distance r^{-n} and short range forces decrease with distance exponentially (exp $(-\alpha r)$).

We divide into three types of long-range forces: electrostatic, induction and dispersion.
-Electrostatic interactions refer to coloumbic forces, which are caused by static charge distributions between two molecules. They can be either repulsive or attractive and are pairwise additive.
-Induction interactions are caused by distortions in charge distribution by an electric field applied by a number of other molecules present . These are always attractive and non-additive.

-Dispersion interactions are always present and always attractive. They are pairwise additive and arise from spontaneous fluctuations of electron distributions across the interacting molecules.

The two most important short-range forces are refered to as exchange and repulsion. Oftentimes, for simplification, they are combined into one and called exchange-repulsion. These are both non-additive and have opposing sings. Repulsion dominates strongly at short distances. Overlap of electron-dense molecules can cause short range penetration, charge transfer and damping effects [14].

2.4.3. Aromatic ring interactions

Aromatic interactions are of great importance to this work. The ring interactions are responsible for the distance that is kept between the two ineracting molecules.

In the case of the benzene dimer, many different studies have already been conducted on the exact structure of such bondings (Figure 5). Attempts to extract this information date back to the 1960's with crystal spectroscopy. This was one of the earliest attempted discoveries in the field of physical chemistry since it became active in the study of gas phase interactions and gas clusters and van der Waals forces. This mistery has been therefore attempted to be solved by many research groups in many different ways.

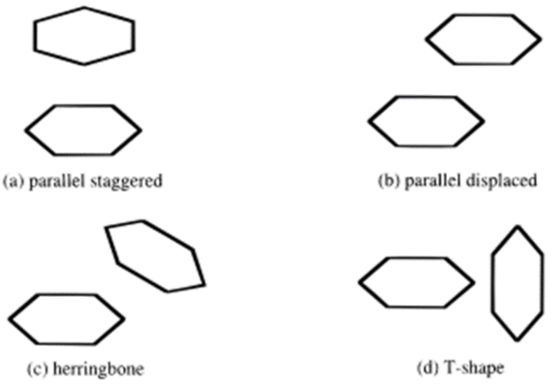


Figure 5: The four proposed models for benzene dimers: (a) parallel staggered, (b) parallel displaces, (c) herringbone and (d) T-shape. (Source: [15])

Just like in the case of this study, theoretical calculations have been employed, as one of many variants. These calculations of benzene dimers offered many different solutions to the same problem. In the case of the research team of van der Waal, a discovery was made that even slight changes in atomic charge can alter these shapes in a rather major way. Even though this might look like the discoveries are all over the place, one may still extract some useful details out of all the information. We can assume, that the interaction potential between the two is quite flat and that many local minima exist for the dimer. Only small barriers ultimately decide which confirmation is chosen. Additionaly, since discoveries point to these interactions being non-rigid, this could mean that the dimer could change confirmations over a period of time [15].

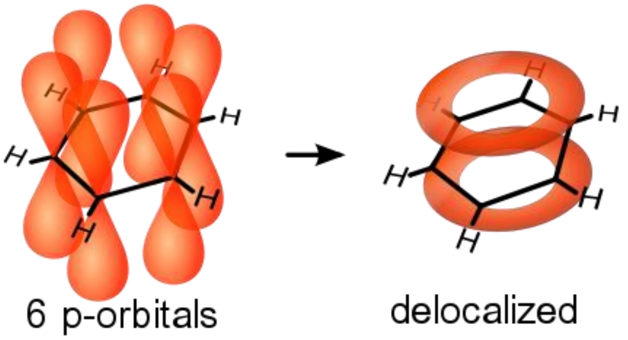


Figure 6: Orbitals responsible for the stacking interactions (Source Proteopedia, Wikicommons)

The underlying mechanism here is the π-Stacking, which is created by the π-orbitals around the aromatic ring structure. The electrons in their orbitals are free to interact with electrons from another ring structure and therefore create an attraction that can be seen as a varient of sorbtion. This kind of sorbtion is strong enough to guarantee a stable attraction between two molecules. This was tested by experimentation with GO and TBBPA (4,4-isopropylidenebis(2,6-dibromopheno l)). It was found that with increasing pH, meaning the functional groups of GO being protonated, and therefore one of the only viable interactions being the π-Stacking between the aromatic rings, the sorbtion still worked very well [16].

The stacking itself is due to quadrupole/quadrupole interactions due to the local C-H dipoles and London dispersion forces [17],[18].

2.5. Molecular dynamics (MD)

2.5.1. Principal of molecular dynamics

The principle of molecular dynamics allows simulation of physical phenomena around molecules. This is done by the application of Newton’s equations of motion, and the calculation of potential energies, interatomic potentials or molecular mechanic force fields.

In MD, particles move at constant velocity between perfectly elastic collisions. Although many experimental and statistical physics procedures exist that allow for detailed descriptions or even microscopic analysis, specific aspects of systems remain to be analyzed via simulation only. One could imagine computer simulations to act as a bridge between the data sets that were acquired regarding the structures of atomic models and the physical experimentation that was carried out on much larger scale [19].

2.5.2. Requirements and calculations for MD simulations

Building a fitting model of a molecule can prove to be one of the hardest parts of experimentation. Limitations could go as far as not having an exact molecule structure at all. Additionally, choosing a correct representation state proves to be another important aspect of molecular simulations. One could go with a detailed model (atomistic model with proper bonds), or a rather simplistic one (spheres and springs as for making bonds between atoms). Most of the times, relatively simple methods prove to be realistic enough.

To make complex analysis possible, the simulations are performed using numerical methods. These mathematical models and simulations lead to an accumulation of errors in the long-run, since they are not designed for numerical integrations over a longer period of time. To counter this, a minimization can be utilized, which ensures proper selection of parameters and algorithms. However, this does not eliminate problems entirely.

MD simulations require definitions of a potential function, these describe the terms by which the particles interact. These quantum approaches deliver useful information on structural and conformational changes but chemical reactions cannot be reproduced since electronic motion is neglected, while mostly only nucleic motion is considered.

MD relies on equations of kinetic and potential energy. For every particle N, there are 3N $(q_1, q_2, \dots, q_{3N})$ coordinates and 3N $(p_1, p_2, \dots, p_{3N})$ conjugate momenta, represented in a Hamiltonian function:

$$
\begin{gathered}
H(q,p) = K(p) + V(q) \\
q = (q_1, q_2, \dots \dots \dots q_N) \\
p = (p_1, p_2, \dots \dots \dots p_N) \\
K = kinetic\ energy \\
V = potential\ energy
\end{gathered}
$$

The kinetic energy is the sum of kinetic energies of all the particle numbers, depending on various components of their momenta, while the potential energy is the sum of various potentials of the systems. External fields on individual atoms and pair potentials are the most important parameters and factored into this calculation to give the following equation which is at the heart of all of MD:

$$\frac{d^2\vec{r}}{dt^2} = -\nabla V(\vec{r})$$

A major challenge in the field of molecular dynamics poses the molecular potential V(r) which can be divided to inter and intra-molecular potentials. The accuracy and validity heavily depend on it.

This inter and intra-molecular interaction can be modelled using force fields. Such energy calculations are given by:

$$E_{total} = E_{vdW} + E_{elec} + E_{bond} + E_{angle} + E_{torsion}$$

Which describes the total energy, given by the sum of the van der Waals-, electrostatic-, bond stretching-, angle bending- and torsion energy components. Van der Waals interactions are dependent on the strength of the interaction and the range. Electrostatic interactions can be computed using Coloumb's law. The bond-stretching is described using harmonic potentials, as well as the angle bending potential energy. Torsion is lastly calculated as a function describing the rotation around bonds and the constrains this entails [19].

2.5.3. A schematic rundown of a thought process regarding a successful MD simulation

The main idea behind a simulation is to generate various snapshots of a system to calculate the thermodynamic properties. A schematic rundown of a simulation thought process regarding the integration scheme might look similar to the following list:

1. Newton's Equations should be time reversible (a simulation should be stable, independent of the direction of time (positive or negative sign))
2. Since Hamiltonian dynamics operate under the idea, that the volume and space are constant, our simulation should preserve this property
3. The simulation should be fast and require little memory
4. It should be able to produce real and classical trajectories as accurately as possible
5. It should be easy to code
6. It should permit large time steps
7. It should be stable (energy should be conserved in long term) [19]

2.6. Findings of previous research

As mentioned in the previous paragraph, similar research to this thesis' experiments has already been conducted. In the research paper by Zhan, Tang and Li (2013), it was discovered that the sorption could be attributed to π-Stacking, as well as non-polar attractive forces. The sorption was quite effective with sorption capacities of 70-94% after reaching equilibrium. Even when the TBBPA concentration was very high, the GO molecules could adapt to bind more TBBPA-molecules at once. Additionally, it seemed like GO had a faster sorption rate than activated carbon, which leads to the assumption that GO might one day become an alternative to water filtration. However, it was also noted that the rate of desorption was also a very important parameter at 298K. This desorption rate was temperature dependent and although TBBPA was assumed to not be as susceptible to desorption, the rate was significant enough to play an important role.

It was also discovered, that the sorption works even in regards to nano particles of GO and that humic acids could be used as substances for filtration [11].

Since ions were introduced in the simulations that were run, findings of ion and GO interaction are also important for this work. In a recent paper, the interaction of $CaCl_2$ and NaCl was studied and it was discovered, that the calcium ions could impact hydrogen bridges, since they can interact with the functional groups of the surface of GO, reducing its measurable negative charge. Sodium ions had a slight charge-reduction effect on GO too. The stability of GO under certain pH-ranges was also touched upon, which concluded that GO is rather unstable under a pH of 3 and negatively charged from pH range 2-11. However, when salts were introduced, this negative charge subsided and an aggregation of less charged GO molecules was more prevalent. When humic acids were introduced, these aggregations became less, since the acids interacted with the GO, while also lowering the overall amount of free ions, since those could bind to the humic substances too [20].

3. Aims

The aim of this study is to take a closer look at the interactions of GO with various models of humic acids and organic material derivatives. This was done in a computational way to get a close-up view of the underlying interactions to study the interactions with the ability to stop on interesting frames and taking an analytical look.

Humic acids were chosen as a subject of interest as they pose a threat to the health and well-being of animals and eco-systems, as well as posing a challenge in the case of water treatment. Since GO is a relatively new, although already intensely studied material, the computational study holds a lot of importance in today's research, since it might help with important innovations.

Understanding the importance of these fields of research, this thesis' aims to study the interactions and confirm previous findings computationally. With specialized programs, it was aspired to take several molecules that occur naturally in our environment and confirm their assumed attraction to GO.

Since these attractions are primarily due to the molecule's polarity and/or non-polarity, as well as their aromatic ring structures, the findings may be of interest even to researches outside of the field of environmental chemistry. These interactions have been studied for decades and are still cause to many mysteries in the field of molecular interaction.

4. Materials and Methods

4.1. Software

All the commands and software that were used were run by the UNIX operating system, a derivative of LINUX.

The programs of most importance listed are:

- VMD, or 'Visual Molecular Dynamics' – used to graphically represent the molecules, while also acting as a tool to visually assess if the distance between the two attracting molecules was sufficient to test the attractive forces.
- GROMACS – software that allows to perform molecular dynamics simulations. It is part of Molecular Dynamics (MD), which is a computer simulation method for analysis of physical interactions of atoms and molecules.
- PACKMOL – software used to create a point of origin in MD and makes it possible to create confined spaces.
- 'Midnight Commander' or 'MC' – used as a file managing tool, to transport needed data.
- Putty – a program used to connect to the servers that were managing the simulations from remote computers.
- xLaunch and xMing – tools that make it possible to visualize programs on windows devices that are connected to a Linux-system remotely.
- xmgrace – a program that creates plots out of calculated data.

4.2. The simulation process

4.2.1. Preparations for the simulation

The simulation process was started with two types of files, where one contained the topology and the structure of one molecule and the other one topology and structure of the other.

As there were two molecules, namely GO and humic acid models, both these files were '.pdb' (PDB, Protein Data Bank file)-files and therefore contained information about the atom types, their position in coordinates, classifications of those atoms, an assigned number and what kind of model they represent. Using the vi-command, which allows to view source data of a file, one can look into a '.pdb'-file. A typical line in such a file, with the help of the vi-command, might read as following:

`HETATM`	`1`	`N`	`PHE`	`1,077 2,033 3,440`
(atom)	(Assigned number)	(Nitrogen)	(Residue of Aminoacid)	(Coordinates [x,y,z])

These files were copied into the present working directory (pwd) through the ‘scp’-command, which, when typed together with right IP-Adress and file path, copied the required data from another device or address to the needed folders.

Here, theoretically, several files could be combined as input files and used for running the MD simulations and with this, several files could be produced by GROMACS as output files. The ‘.xtc’-type files, which were the trajectory files storing information on calculated positions, accelerations and movements in general, and ‘.top’ files which were topography files, containing all information about molecules, such as type of atoms, charge of atoms, bond data, angle and other needed information for used potentials.

4.2.2. Combination of graphical models and their arrangement in space

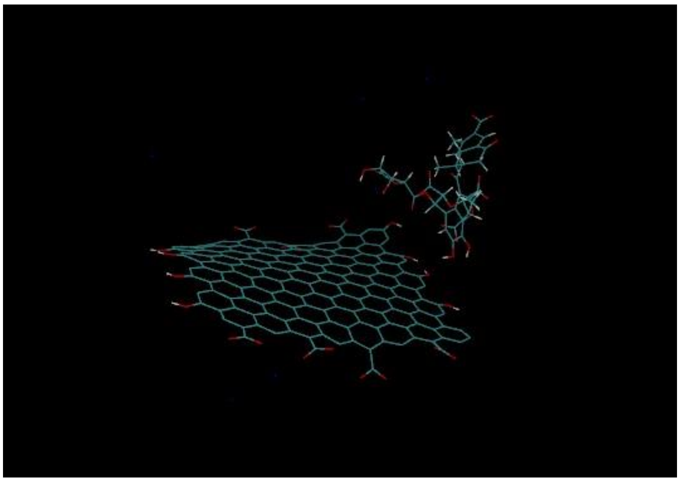
Most of the time, an old model of GO (which in our case was saved under the name ‘GEO’, in most cases) was used. To use it again, after a simulation was successfully run, one could use the ‘grep’ command, which takes one specific file out of a combination of many which were stored under the ‘.pdb’-file ending. For this, the code looked like the following:

```
grep GEO (filename.pdb)
```

The combination of GO and the humic acid itself was done using the ‘cat’ command, this command could save two existing files under a completely new filename. A possibility here was to create ‘.gro’ files which serve as another way of concatenating data. This combination was without taking into account any complex interactions however. The command in question was:

```
Cat (filename1) (filename2) > newfilename
```

Using VMD, the two models could be moved from one another to ensure the distance between them was satisfactory. Oftentimes, coordinates were overlapping causing the two models to interfere. This had to be fixed and the models had to be separated, to ensure that a truthful measurement about the attraction-interaction could be made.



underside) with a Humic acid (or in this case, rather a fulvic acid) on top.

4.2.3. Setting space- and environmental conditions

To set important parameters for the calculations, a virtual box was created that defined the space in which this molecular interaction could happen. This was done with:

```
editconf -f (.pdb) -c (=center) -box 666 (measurement) -o (name)
```

Then, water molecules were added with:

```
genbox -cs (coordinates of solvents) -cp (.gro) -o (name.gro) -p
(.top)
```

It was important to check, if the number of atoms on the models were still matching to the previous models. If they were not, it was an indicator for deprotonated acids, which was solved by the addition of ions. Before the addition of ions, a minimisation of the system was done with the help of the 'grompp_465'-command. The grompp-command will be explained in 4.2.4..

The file that is created is a '.tpr' file, it is a binary input file that includes all combined data. After this the command for the addition of ions is started and reads as following:

```
genion -s (.tpr) -o (name) -p (.top) -np (position of ions)
```

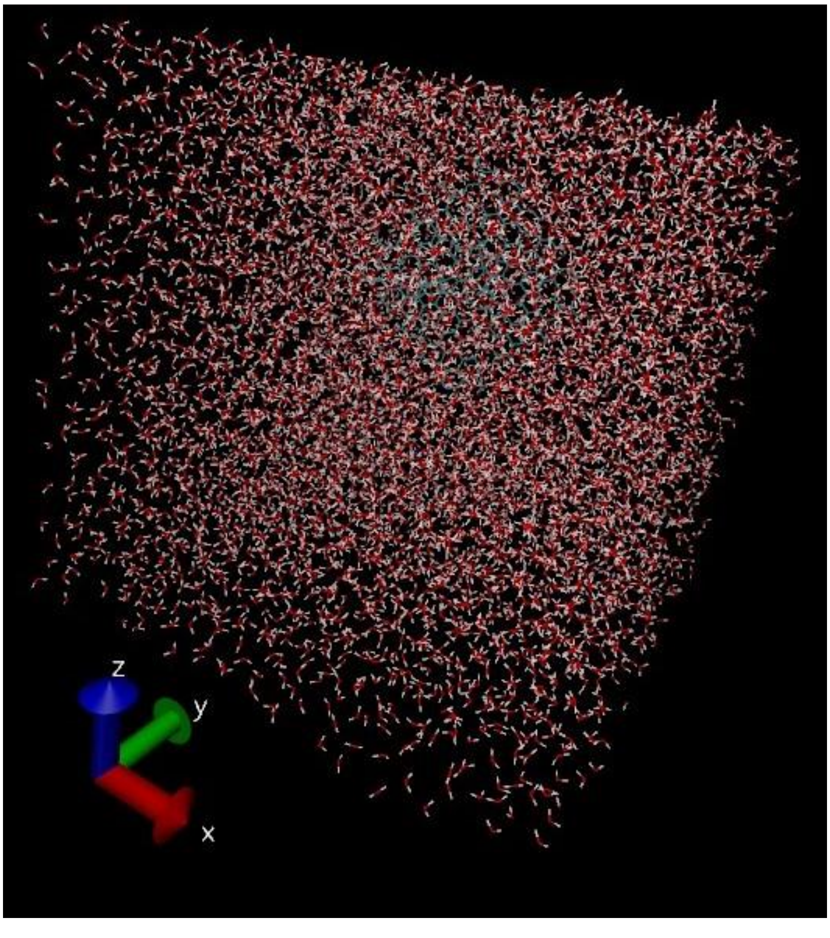


Figure 8: The system after addition of GO, HA, the ions and water molecules.

4.2.4. Minimisation, grompp and mdrun

After the genion-command, another minimisation is needed. Now, what this means is, that files get combined and minized to a low energy level. To combine the files in such a way that that the molecules can interact, one had to perform a rather complex step. In the first such one, after the genbox-command, all available data were combined with:

```
grompp -f mini_pbc.mdp -p (insert .top-file) -o (new filename)  -c
(coordinates in the form of .gro-files or .pdb-files)
```

This minimisation causes an energy optimization in the system, which combines all of the files that were used in the command into one single input file.

Secondly, a different type of minimisation is used, since volume, temperature and number of atoms can now be assumed to be constant. The box filled with the atoms and all their interactions are now minimised to an energy-efficient level again.

```
mdrun -deffnm (.tpr file, but the .tpr is deleted) -v
```

4.2.5. Setting time- and temperature conditions

For temperature and time parameters, the following command chain was used:

```
gromp -f nvt_dposres_300K.mdp -p (.top) -o (name) -c (most recent
.gro-file)


mdrun -deffnm (.tpr, but without ‘.tpr’) -v


grompp -f npt_dposres.mdp -p (.top) -o (filename with suffix _npt)
-c (.gro)


mdrun -deffnm (.tpr, without ‘.tpr’) -v


grompp -f md2_cpu.mdp -p (.top) -o (filename with suffix _100ns) -c
(.gro)
```

In the command chain above, the temperature (300K) and the duration of the interaction (100ns) were set, applied and minimised. This was done as one of the last few steps to the simulation preparation.

4.2.6. Calculations at the supercomputers

The resulting file after all conditions are set and minimised, which is a ‘.tpr’-file, is send to the supercomputers, which were operated in the National Grid Infrastructure (MetaCentrum). This was done with the ‘scp’-command.

Then with the ‘ssh’-command and usage of script the files were submitted to queuing. The executed command for running the simulations can be as following.:

```
mdrun -deffnm (.tpr-file without ‘.tpr’)
```

These calculations ran for several hours, sometimes even days. If a calculation randomly stopped, it was restarted again.

4.2.7. Acquisition of output files

The animations were imported to the original computer and viewed by typing:

```
editconf -f (.tpr) -o (newfilename.gro)
```

This converts the ‘.tpr’-file into a ‘.gro’-file. This file can then be opened and visualized with VMD and the calculated ‘.xtc’-file can be load to make the molecules move and show their interactions. The resulting simulation could then be viewed to assess interactions between the two molecules visually.

4.2.8. Calculations to obtain valuable data from the simulations

First, a ‘.pdb’-file was created using the ‘.tpr’ file created by the mdrun command, which was achieved by the editconf-command, like in the step described beforehand. This ‘.pdb’-file was then edited, with the help of the vi-command, in such a way, that everything except the data for the humic acid was deleted. This allowed us to view the molecule and select all the carbons in ring formations, that are participating in the aromatic interactions. With the following command:

```
make_ndx -f (.tpr) -o (newfilename.ndx)
```

these carbons were then sorted into their own groups, which the program could then use to analyse the distance between them and the GO-molecule. This calculation was then performed by the command:

```
g_dist -f (.xtc) -s (.tpr) -n (.ndx) -o (newfilename)
```

For analysis of hydrogen bonds between the functional groups of GO and the water around it, another analysis could be performed, the command being:

```
g_hbond -f (.xtc) -s (.tpr) -num (newfilename.xvg)
```

Obtained data could then be viewed by the xmgrace software, which read the ‘.xvg’-file which was the output of the two last commands above. This program then created a graph plotting the distance over time and the number of bonds over time.

4.2.9. Creation of snapshots

Snapshots were created using VMD, with the '.xtc'-files loaded into the molecule models to enable movement and interactions. At certain points that showed desired interactions, one could take a screenshot. It was possible to render the H_2O-molecules invisible and change the way of representation of the molecules such that it would highlight developments of importance to our study.

4.3. Interpretation of results

The graphs and snapshots that were created could be interpreted in the following ways:

1. Distance between the aromatic rings: This gives information on the nature of the sorption. If the distance stays the same, or in a stable range over time, we might assume that the interaction between the rings is quite stable. Because of steric hindrance, this distance might not correlate to the values obtained for benzene dimers (under 3.2Å for short range interactions, over 3.2Å for long range interactions [21]) but still indicate pi-stacking of two aromatic rings.
2. Hydrogen bonds: The bonds can be counted and analysed as a confirmation of the weak hydrogen bonds. When bonds between water and the GO form, they have enough space to do so. If humic acids are introduced and stack onto GO, they might represent a steric hindrance and therefore a decrease in hydrogen bonding.
3. Snapshots: They give visual confirmation of the assumed interactions. One can both measure the distance with VMD in certain parts of the simulation, as well as study the interaction freely just by eyesight.

   Such snapshots may look like the following picture and help to see if a run was successful:

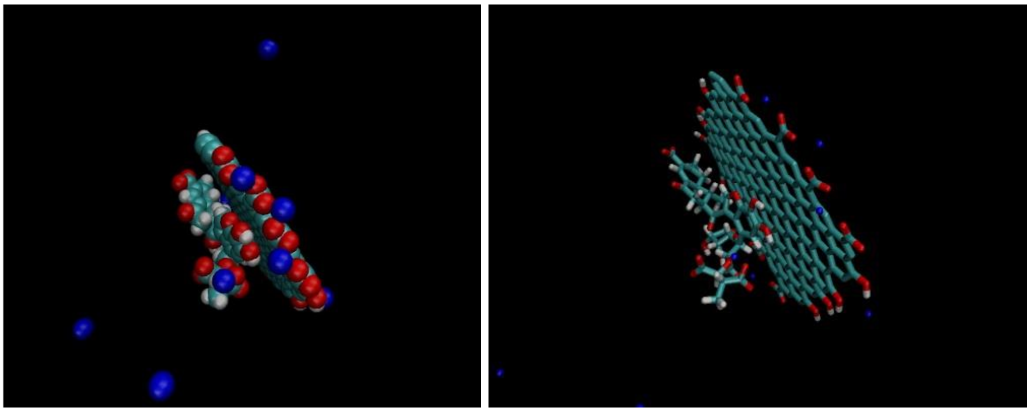


Figure 9a and 9b: Steelink ion HA with ions in solution, suspended in water (which was made invisible for this snapshot). Figure 9a shows the atoms in 'VdW'-representation (short for Van der Waals) and Figure 9b shows the same structure in a slightly different angle with 'licorice'-type representation.

In this example, the interaction becomes quite apparent, since the two molecules are separated by an almost even layer of emptiness.

5. Results

To get meaningful results, the distance between the aromatic ring carbons of the humic substances and the GO-molecule were calculated and visualized with ‘xmgrace’.

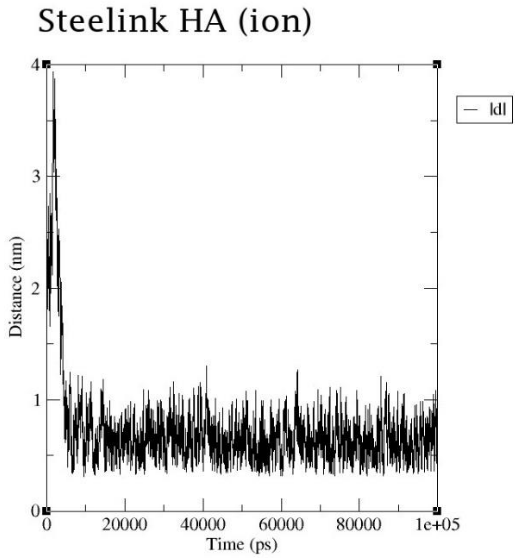


Figure 10: An example of relatively stable distance between GO and one aromatic ring of ionized Steelink HA.

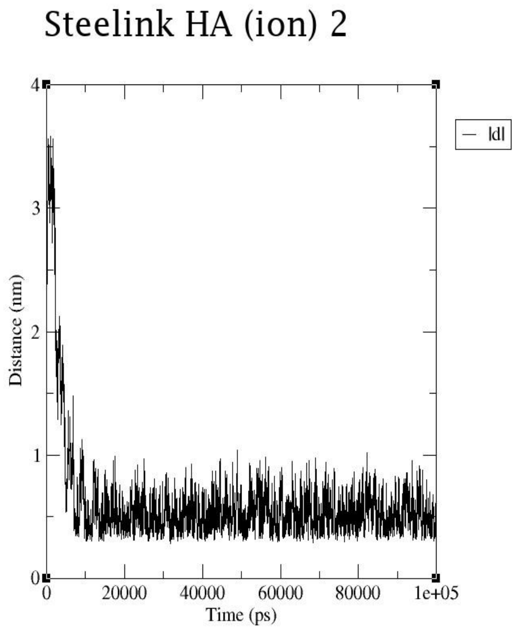


Figure 11: Another calculation, performed at the same molecule as in Figure 10. However, another aromatic ring structure of the HA was used as a reference point.

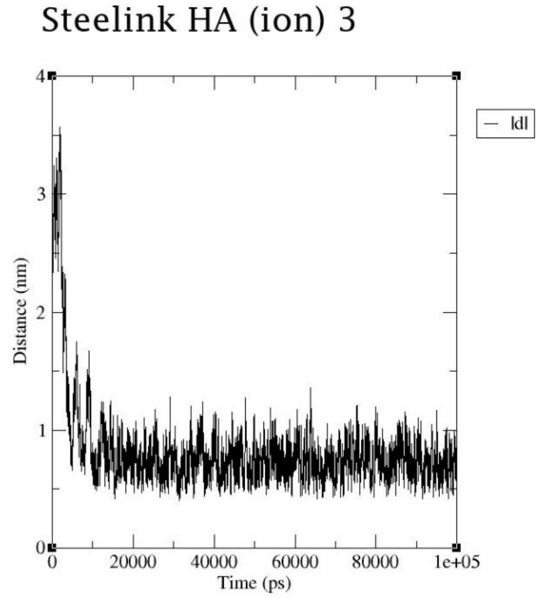


Figure 12: The third aromatic ring that was singled out as a reference point in the same way as in Figure 10 and 11.

All of these graphs show the distance between two identical structures over time, only differentiated through the focus on a particular aromatic ring. All 3 rings are very likely show the interaction of aromatic rings of humic acid molecule with the surface of GO-molecule. However, the second graph shows the most stable and close distance of the three. This would indicate that this second aromatic ring is the closest and the conformation that is taken when this ring is close, between the two molecules, is the most stable version of the three. The distance which seems to lie between about 0.4-1nm is very indicative of an aromatic stacking interaction, which confirms our assumptions of an interactive outcome. The ionized form of this structure seems to have minimal effect on the non-polar interactions.

Such an outcome of a long distance at the beginning and an almost immediate approach thereafter was suspected to occur with most of the calculations and in most cases, it mirrored the graphs above.

For the sake of simplicity, only these three calculations, which are seen as relevant for this particular topic, are shown in this section. The other ones can be found in the appendix without any additional calculations which were deemed of no interest. This means most of the aromatic groups without major interactions (e.g similar to Figure 10 and 12), were excluded while the ones which indicated the most stable interaction where chosen as representation for the whole molecule.

It may be important to mention, that for this study, many different models of humic acids were used, these have very particular names and were therefore chosen as means to differentiate in the graphs. Additional calculations of interest were the following:

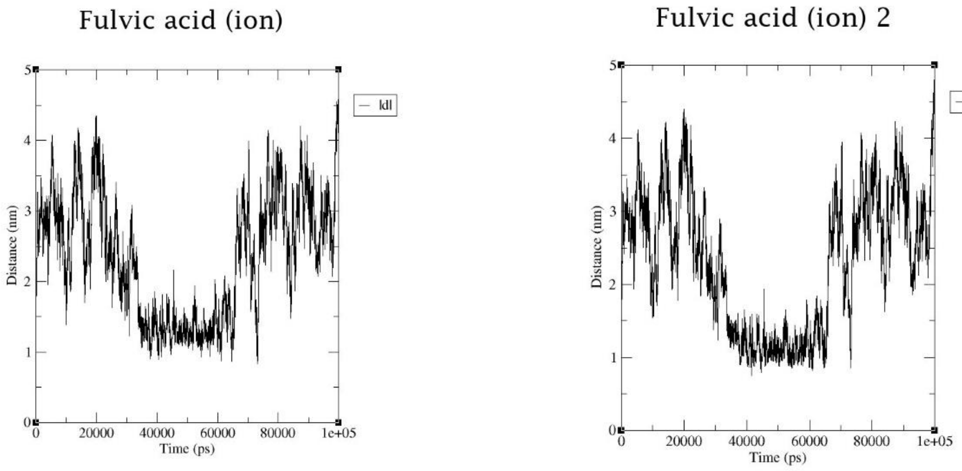


Figure 13a and 13b: The Fulvic acid (ionized) molecule, again in a graph with distance plotted against time in regard to the distance between the acid and GO.

In the calculations of ionic form of fulvic acid which were done additionally out of interest, a very particular instance occurred, where aromatic interaction did not seem to occur. This was very visible when viewing the trajectory of the simulation. Since the two rings are intertwined (similar to naphthalene), the graphs look very similar. Following are the pictures taken during this interaction of the simulation:

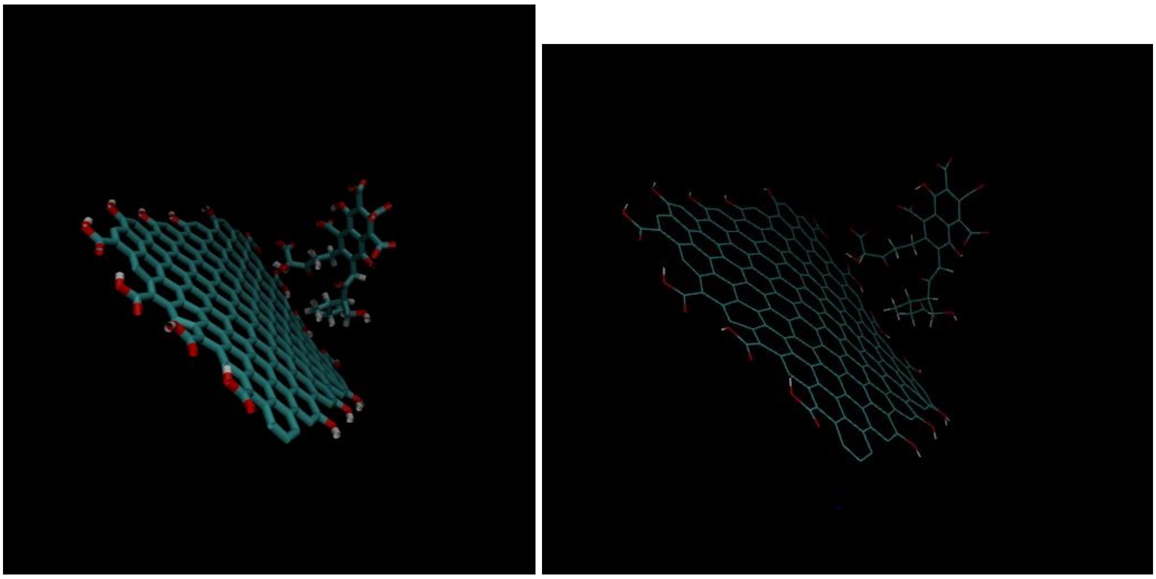


Figure 14a and 14b: The fulvic acid seems to prefer interaction with its functional groups, rather then with the aromatic ring structures.

It seems that the aromatic interaction is not the dominant force and that the fulvic acid is rather binding to the GO through by the means of its functional groups. This occurrence, where aromatic interaction does not seem to be strong enough, can be found in other calculations too. These are included in the appendix.

As one of the last things that were of interest are hydrogen bonds. Polar interactions that take place between the functional groups of the acids or the GO and that may act as another indication of aromatic bonding. A good example for this is the fulvic acid:

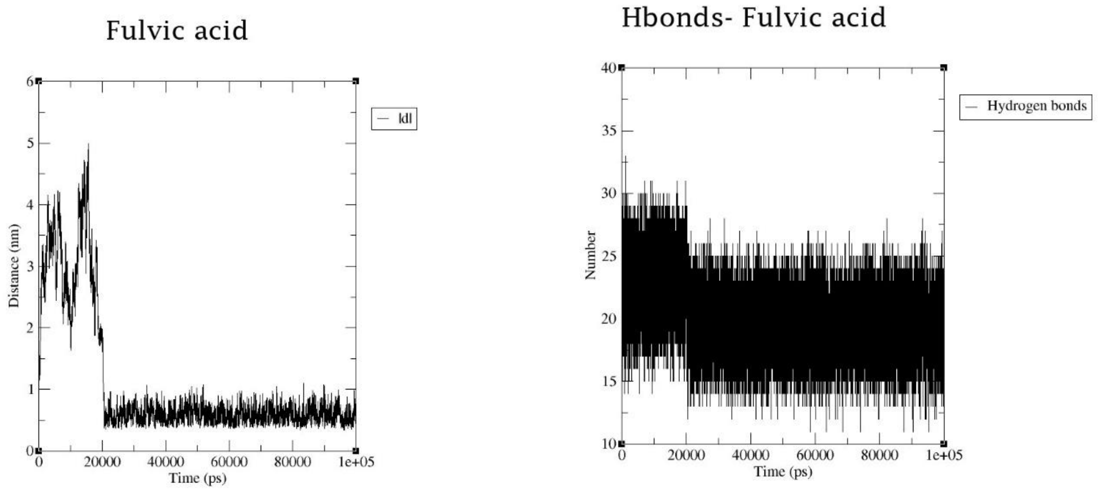


Figure 15a and 15b: Fulvic acid as a graph plotting distance over time and as graph plotting hydrogen bonds with water over time

Here we compare the interactions, measured by the distance with the hydrogen bonds that were formed throughout the calculations. The hydrogen bonds served as an indication, if through aromatic interaction, a steric hindrance came to fruition, which then hindered the development of more hydrogen interactions. Such a clear difference, as in fulvic acid could not be observed at all times however.

Lastly, these hydrogen bonds calculations were used to take a look at the role of ions in solution around GO. These two graphs show the results:

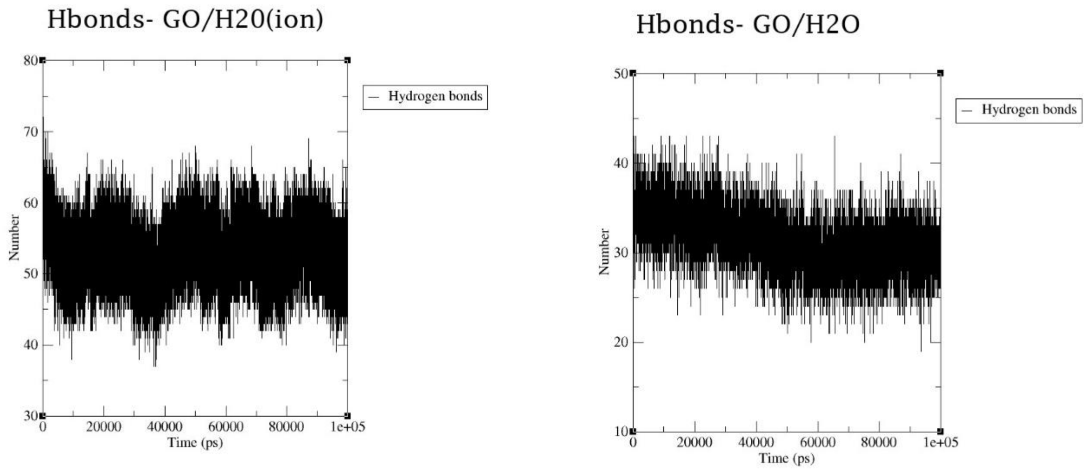


Figure 16a and 16b: The difference in hydrogen bonds between the GO in solution with ions (Na+) and without the inclusion of them

This outcome seems to indicate that the hydrogen bonds are more prevalent when Na+-ions are present.

6. Discussion

Most of the results are in accordance to the expectations. They confirm the aromatic interactions mentioned in the works of Shetty et al. (1996), R.G. Huber (2014) and S. Sun and E.R. Bernstein (1996).

The ones that are not in accordance to assumed outcomes could be attributed to steric hindrance. Steric hindrance could cause the lowest energy point to be in a confirmation that does not allow for direct aromatic interaction, however even so, all of the interactions pictured show clear signs of attraction in the medium of water.

Using hydrogen bonds as indicators for non-polar interactions proved to be quite useful, although indication is the only thing that a researcher should extract from it. This method by itself is not enough as means of prove.

The findings of GO in water with and without ions were quite surprising, since M. Bayati and M. Fidalgo De Cortalezzi (2019) showed that calcium and sodium ions tend to hinder hydrogen bonding of water and GO, by interaction with the functional groups. This may be a something to look into in the near future.

As mentioned in Devanathan et al.(2016), the computational studies of molecular interactions are very important and necessary. Although they may show no major deviations from predicted models, these findings may still be of importance as they provide researches with clarity and additional data for further experimentation. It is assumed, that since these interactions are so clearly visible and proven, GO would be eligible to act as a substituent to activated carbon in the future. Its properties, which range from wide availability through optimized processes, to in-expensive manufacturing and the ability to attract the substances studied in this thesis are outstanding. It is strongly recommended to conduct further research in this area.

7. Conclusion

Since every molecule, that interacted with GO, showed signs of attraction, even though the distance would suggest the sorption to not be the most optimal, the findings are very much in accordance to assumptions. The attraction that occurred was expected and indicates that GO might be used as a material for binding humic and fulvic acid molecules for different applications. It was shown that the sorption of humic acids to the surface of GO occurs either due to the interaction of aromatic rings of humic acid molecules with the surface of GO-molecules, or due to hydrogen bonds of functional groups of GO with humic acid molecules. Moreover, the MD simulations showed that the distance between the surface of GO and hydrophobic parts of humic acids tend to lie between 0.4-1nm which is an indication of an aromatic stacking interaction. Lastly, the ionized forms of structures seem to have minimal effect on the non-polar interactions in the systems containing humic acids and GO.

8. References

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Appendix

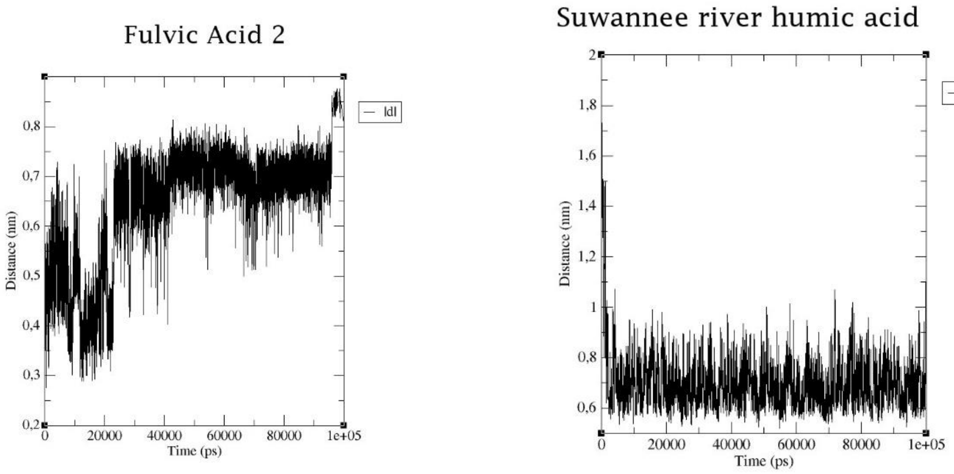

Figure 17 and 18: Fulvic acid and Suwannee river HA, calculation results

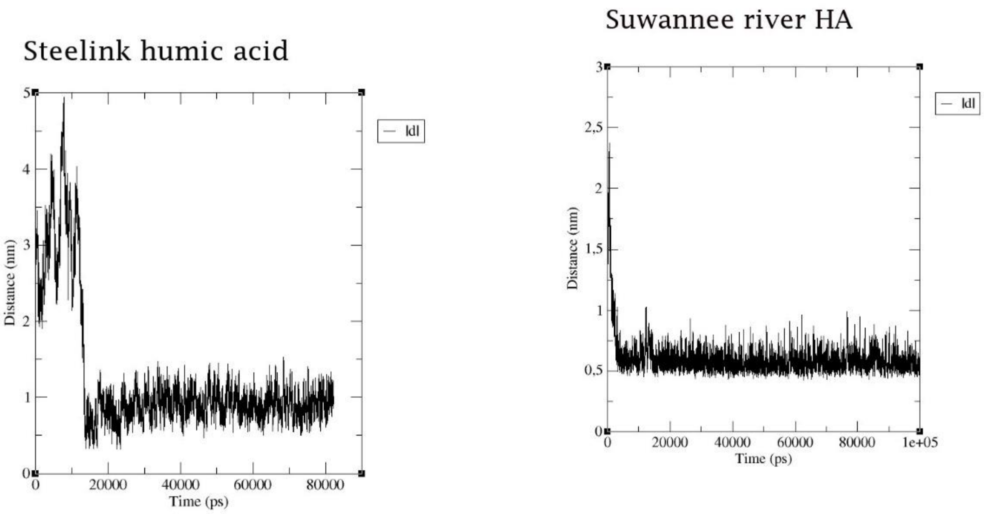

Figure 19 and 20: Steelink HA and another (structurally different) version of Suwannee river HA, calculation results

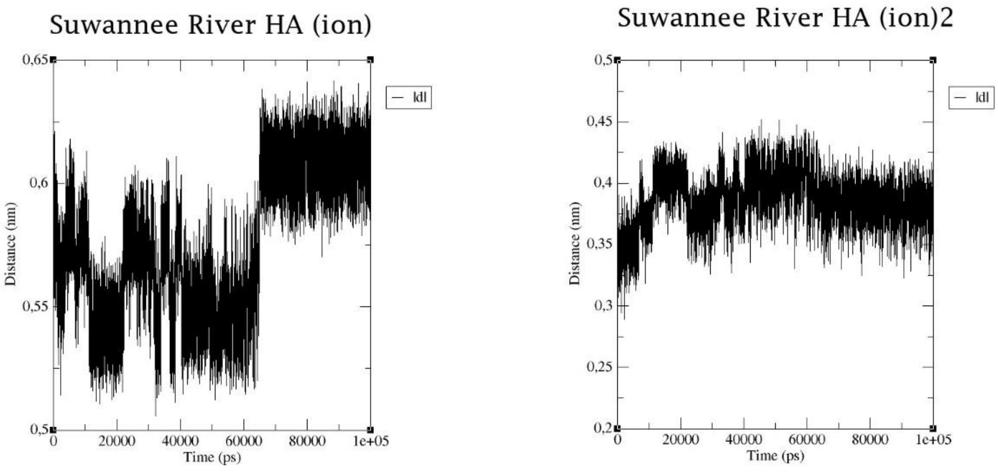

Figure 21 and 22: ionized Suwannee river HA calculations. These needed a little longer to find the right conformation it seems.

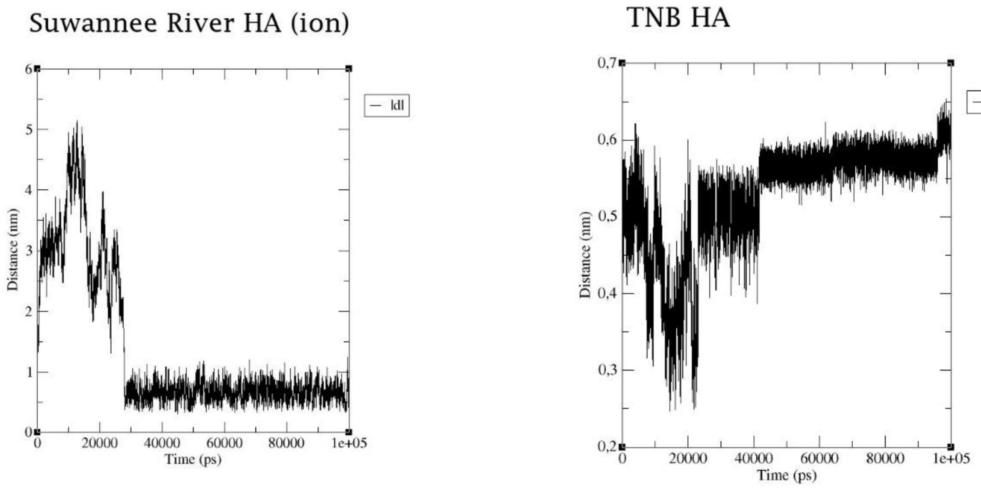

Figure 23 and 24: Again, the other version of Suwannee river HA, but ionized and Temple-North-eastern-Birmingham HA, calculation results

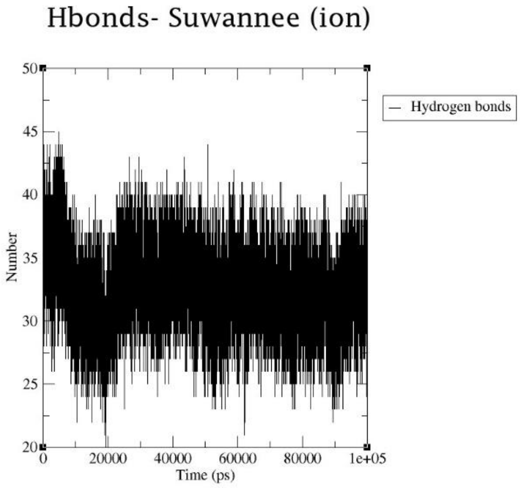

Figure 25: Hydrogen bonds of Suwannee HA (ionized)