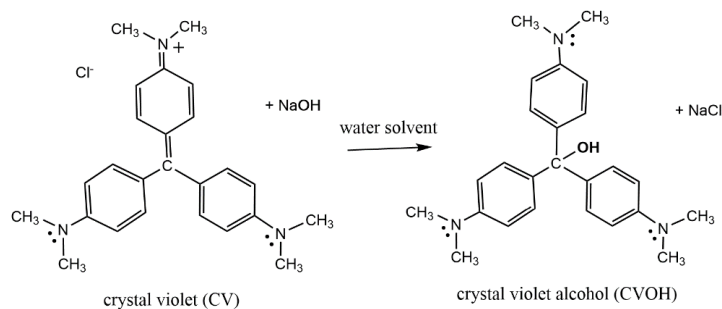


Crystal Violet and Quantum Theory

Introduction Crystal violet (CV, also known as gentian violet) is a dye used in the manufacture of paper and cloth. It is also a microbiological stain used to identify Gram positive bacteria. CV is one of a family of triarylmethane dyes, which contain various aromatic rings attached to the central carbon atom. These include malachite green, brilliant green, and victoria blue.



Under alkaline conditions (high pH), crystal violet's color slowly fades as hydroxide ion adds to the central carbon, converting CV to the colorless crystal violet alcohol (CVOH). In a previous lab we measured the rate of disappearance of CV by monitoring the decrease in absorbance at 590 nm.

In this lab we will use quantum theory calculations to study the molecular details of the bleaching reaction and, in the last part, see how CV's violet color arises from its extensive π -bonds. The main tool of computational quantum chemistry is density functional theory (DFT). This is a fast, yet accurate, method for calculating a molecule's electron distribution and energy content.

Two questions are addressed here:

- (1) How do the **molecular orbitals** of CV control its **chemical reactivity** and **color**? As we will see, one orbital in particular, the lowest energy anti-bonding orbital (or lowest unoccupied molecular orbital LUMO), plays an important role in both.
- (2) In water, hydroxide ion is surrounded by strong **hydrogen bonds**. How do these affect the CV + OH⁻ reaction?

This exercise has three parts:

- (A) Study the distribution of partial charges within the CV cation.
- (B) Calculate reaction energies and assess bonding changes during the reaction.
- (C) Uncover the molecular basis for the violet color of CV.

The **general procedure in this lab** is to (1) view interactive 3D models at the [informational website](#); (2) obtain structure files from those pages; and (3) import each structure into WebMO (a graphical interface for the Gaussian computational program) and carry out a short calculation.

The **worksheet** provides space to record your results, gives additional background information, explains issues raised by the calculations, and asks questions about the results. Fill in the worksheet as

you do the quantum calculations. If you wish to complete the worksheet at a later date, the calculation jobs can be opened at any time by logging back into WebMO.

This **handout** provides background theory and describes how to use WebMO, Gaussian, and the supplemental web pages. The **appendix** on p. 7 has more information on the following topics:

- Using WebMO
- Quantum chemistry background
- What is a "Molecular Energy" calculation?
- What is a "Molecular Orbital" calculation?
- Practical uses of molecular quantum theory
- How does WebMO work?

A. Partial charges

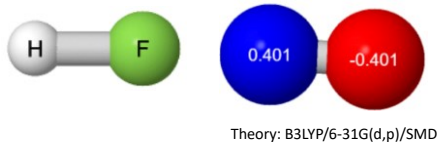
Background

In molecules containing atoms with different electronegativities, bond electrons are shared unequally, being pulled **toward** the more-electronegative atom and **away from** the less-electronegative atom. This creates a partial negative charge on the former, and a partial positive charge on the latter. These charges always add up to the total charge on the molecule or ion. The partial charges calculated by Gaussian are displayed in WebMO's View Job page under Calculated Quantities, Partial Charges. Four examples are discussed below.

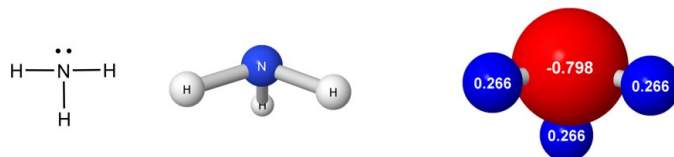
HF. In hydrogen fluoride, the partial charge on fluorine is -0.401. This indicates that on average 0.401 electrons – of the 2 that inhabit the H-F bond - shift away from H and toward F. (Sect. 6.2 in your text also discusses HF.)



Clicking the blue icon in the Partial Charges table in WebMO displays spheres whose diameters are proportional to the partial charge value.

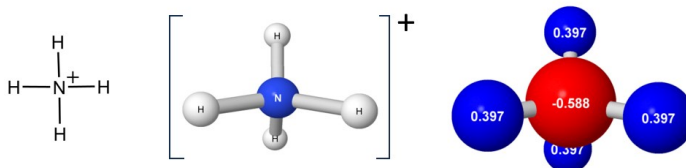


NH₃. In ammonia, the calculated partial charge on nitrogen is -0.798. Thus, in each of the bonds, 0.266 electrons shift away from H and toward N.

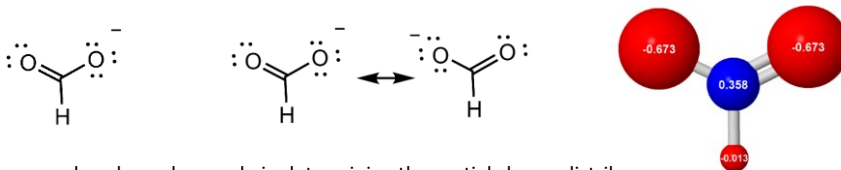


NH₄⁺ In ammonium ion, the calculated partial charge on nitrogen is -0.588. It is negative despite the “+1” formal charge that appears in the Lewis formula! In each of the four bonds, 0.147 electrons (0.588 ÷ 4) must shift toward N. The N atom is still more electronegative than H, however its negative charge is less than in NH₃. The + charge is spread out among the H atoms.

The N atom in NH₄⁺ is somewhat analogous to the N's in CV cation. That is, they are positive in the Lewis formula but have negative calculated partial charges.



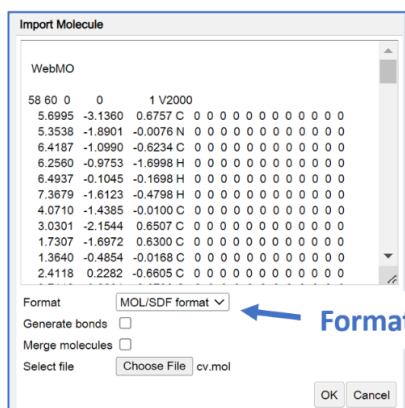
Formate ion (HCO₂⁻) Another factor that can influence the partial charges on atoms is **resonance**. For example, the Lewis formula of formate ion is sometimes written with a -1 formal charge on one O (*left*). However, the complete formula shows that it is a **resonance hybrid** with the negative charge **shared** between two oxygens (*center*).




In CV, **resonance** also plays a large role in determining the partial charge distribution.


To do:

1. First, view a 3D model of CV by opening the [informational website](#), and following the link to CV. Save the cv.mol structure file on your local computer. Note the file location.
2. Log into WebMO. Select the **New Job** menu item. Click **Create New Job**. Then **File, Import Molecule**. Verify that the Format box says "MOL/SDF format." Using the Choose File button, navigate to the folder where you saved cv.mol. Click the OK button at the bottom right. The CV ion should appear in the editor window.




[A 2-min YouTube video shows how to save a .mol file and import it into WebMO.](#)

 Now, using the **Rotate tool** (3rd from the top on the toolbar), left-click-and-drag to rotate the molecule – this is the best way to get a clear view of this non-planar ion.

 **3.** Click the Continue arrow at the bottom right corner of the workspace. Respond “OK” to the “Continue without symmetrization?” question.

On the Choose Computational Engine page, select “Gaussian” and the “First Available” server.

 Click the Continue arrow.

On the Configure Gaussian Job Options page, fill in the boxes:

Job Name -- (enter any informative job name)

For the following boxes, the correct default settings are inserted automatically. Do not change them:


Calculation - Molecular Energy

Theory -- B3LYP


Basis Set -- Standard: 6-31G(d,p)

Charge -- 1

Multiplicity - Singlet

 Clicking the Continue arrow takes you back to the WebMO Job Manager.

4. The calculation should take 2-3 minutes. When the job is **complete**, click the job name.

 Now scroll down to the Partial Charges table and click the blue magnifying glass icon (next to the word Charge). This places a sphere on each atom with a label showing the partial charge value. **Record partial charges** for the atoms indicated in the worksheet.

Answer questions on the worksheet regarding the Lewis formula of CV and the nature of the central carbon atom.

B. The OH⁻ reactant and the CV-OH⁻ transition state

Background

With few exceptions, all chemical reactions require the reactants to surmount an energy barrier, or activation barrier, on the way to the product. For this to happen, individual reactant molecules must gain extra kinetic energy from thermal motions within the solution or gas phase. This is the reason that reactions speed up at higher temperatures. At higher temperatures, molecules move faster, and therefore a higher proportion can gain enough energy to overcome the reaction barrier.

What is the barrier for the reaction of CV with OH⁻? If you take the saying that “opposites attract” literally, it is surprising that there is *any* barrier for combination of a cation and an anion. However, in water the reaction is slow, so therefore the reactants must be encountering a substantial energy barrier. It turns out this is due to OH⁻ ion being hydrogen-bonded to surrounding water molecules. A certain amount of energy is required to “free up” OH⁻ from the solvent before (or as) it bonds to CV. OH⁻ accepts 3 to 4 H-bonds on average in water (Fig. 1). When OH⁻ reacts with CV, some of these must be loosened or broken. The O atom in CVOH has a much-reduced negative charge compared to OH⁻, which weakens the remaining H-bonds.

Fig. 2 shows the energy changes for this chemical reaction. Initially, the energy goes up as H-bonds are weakened or broken. The energy of the system reaches a maximum at the transition state, then decreases as the C-O bond forms in the stable product.

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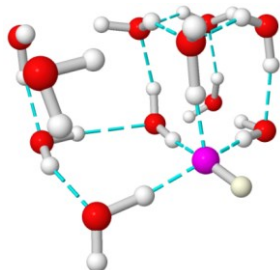


Figure 1. Computer model of an 11-water–OH[−] ion cluster. OH[−] is colored fuchsia. Hydrogen bonds are cyan.

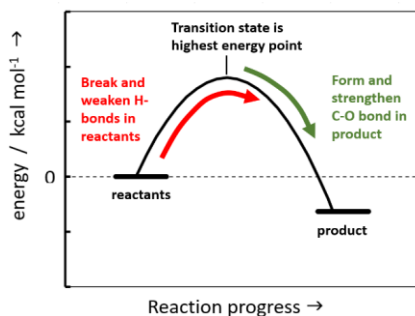


Figure 2. Energy changes during CV + OH[−] reaction.

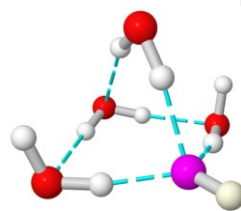


Figure 3. Computer model of a 4-water–OH[−] ion cluster.

What are the reactants? Clearly, **CV** is one. The other reactant must be a **water cluster** containing OH[−]. The cluster in Fig. 1 is too large for a detailed quantum calculation, so we will use a 4-water-OH[−] cluster as a model (Fig. 3). When the multiple H-bonds in this model are weakened in the transition state, a realistic reaction barrier is created.

[Actually, several kinds of OH[−] water clusters exist in aqueous NaOH. They react with CV with slightly different geometries, energies, and reaction progress curves. Nevertheless, the sum of these curves still looks like the one in Fig. 2.]

To do:

5. Go to the [informational website](#) and import the oh-4wat.mol, cv-oh-ts-4wat.mol, and cvoh-4wat.mol files. Log into WebMO. Import each .mol file and carry out three Molecular Energy calculations, entering an informative job title and accepting the defaults for the other settings. (The “defaults” are the ones automatically inserted by WebMO for standard calculations.)

When each calculation is **complete**, open the job. On the View Job page scroll down to the Overview box and record the RB3LYP energy. The energy units are Hartrees, where 1 Hartree = 627.5095 kcal/mol. Also record and analyze several H-bond distances indicated in the worksheet table.

C. The color of Crystal Violet

Background

In Chapter 3 of your text, and in the Chem 105 spectroscopy lab last semester, you learned that electrons in single atoms inhabit atomic orbitals (AOs), which have defined energies and shapes. If extra energy is added, say by heating, electrons jump up from filled orbitals into higher-energy, unoccupied orbitals. When they fall back down, they emit photons, creating an emission spectrum. The frequency of

the photon ν is related to the energy of the orbital gap by $\Delta E = h\nu$. Similarly, atoms can absorb photons of those same frequencies from a light beam to create an absorption spectrum.

The same principle applies to molecules, but in this case electrons inhabit molecular orbitals (MOs) rather than AOs. Electrons occupy the lowest energy MOs, the bonding MOs, leaving a set of unoccupied MOs at higher energy, the anti-bonding MOs. An unoccupied MO, especially the lowest energy one, can accept electrons jumping up from below. The **lowest energy anti-bonding MO** is the **LUMO**, or lowest unoccupied molecular orbital.

The **highest energy bonding MO** is the **HOMO**, or highest occupied molecular orbital. The energy gap ΔE between LUMO and HOMO determines the color of a molecule or ion. If the gap is narrow, a photon in the **visible** wavelength range can excite an electron from HOMO to LUMO. If the gap is wide, a higher energy photon in the **ultraviolet** wavelength range is required. Fig. 4 shows these two scenarios, where the **blue arrow** identifies the HOMO-LUMO gap.

Molecules like CV with many alternating double bonds typically have narrow HOMO-LUMO gaps. Molecules with fewer (or no) alternating double bonds have wide gaps and are colorless (octane, ethanol, N_2 , etc.). Most of the colors we see in the world around us are due to the absorption of visible photons by plant pigments or commercial dyes. Coloration is thus a subtractive process where our eyes are detecting the photons that pass through or bounce off.

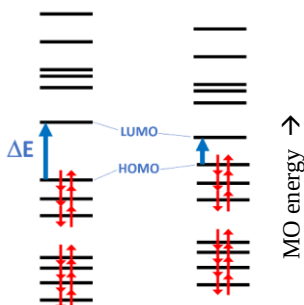


Figure 4. MO diagrams for an **ultraviolet**-absorbing molecule (left), and a **visible**-absorbing molecule (right). Each black line denotes the energy of a molecular orbital. Red arrows represent electrons inhabiting each MO.

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Fig. 5 shows the transmission spectrum of a solution of crystal violet analyzed in a spectrophotometer. The minimum transmission— and therefore the maximum absorption - occurs at 590 nm (λ_{max}). Thus, crystal violet removes green, yellow, and orange photons from white light, allowing the violet, blue and dark red photons to pass through. The result is violet.

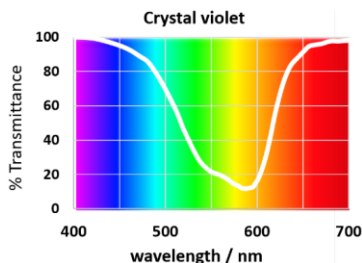


Figure 5. Transmission spectrum of crystal violet (white line). The background illustrates the color of light at the indicated wavelengths.

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To do:

6. For CV, open the Molecular Energy job done previously. Click the “New Job Using This Geometry” button near the bottom of the workspace. Continue. Select Gaussian and First Available server. Continue. Enter an informative job name; under the Calculation drop-down menu, now select **Molecular Orbitals**; and keep the same theory, basis, charge, and multiplicity settings. That is: B3LYP, 6-31G(d,p), 1, and Singlet. The calculation should take about 2 min.

We assume here that the electronic excitation responsible for the λ_{max} absorption peak is due primarily to promotion of an electron from HOMO to LUMO.

7. When the job is complete, click the job name. Go down to the Molecular Orbitals table. Notice that the table lists 100 filled orbitals in order of increasing energy, with each orbital containing 2 electrons. (CV contains 200 electrons, which is apparent from the molecular formula $\text{C}_{25}\text{H}_{30}\text{N}_3^+$.) Visualize the HOMO (#100) by clicking the blue magnifying glass icon. This is a π orbital. Like an atomic p-orbital, it has lobes above and below the plane of the atoms.



If the MO lobes appear tiny, or huge, adjust their size by clicking the Preferences tool in the left margin. On the Isosurfaces tab, set “MO isosurface value” = 0.035.

Now visualize the LUMO (#101), which is a π^* orbital. Use the Molecular Orbitals table as above or the Orbitals applet that now appears on the left side of the workspace. WebMO uses a green-yellow color scheme to represent unfilled MOs (and red-blue for filled MOs). MO display colors are arbitrary and have no physical reality.

Record the energies of both MOs (in Hartrees) from the WebMO Molecular Orbitals table.

8. For CVOH, go to the [informational website](#) and save the cvoh.mol file. Log into WebMO and import the cvoh.mol file. Using Gaussian, carry out a **Molecular Orbitals** calculation. Analyze the HOMO and LUMO as you did for CV. For CVOH, the HOMO is #105 and the LUMO is #106. Answer the questions and do the calculation on the worksheet.

Appendix

Using WebMO. First, obtain the password for the Chem106 WebMO group from your TA or instructor. The WebMO login page is at <https://antec12.cns.uaf.edu/~frank/cgi-bin/webmo/login.cgi>. Enter your UA username and password. Choose the Chem106 Group and enter the group password in the box. Click Login. This procedure is required only for the **first login**; subsequent logins take you directly into WebMO after you enter your UA username and password.

See a YouTube video (no audio or subtitles) showing how to log into a WebMO group <https://youtu.be/OgbKXchC55o>. For more help, click Help at the bottom of the blue left-hand panel in WebMO. The help page has links to Overview and QuickStart Tutorial pages.

Quantum chemistry background. Quantum chemistry programs like Gaussian calculate the energy of a molecule using a combination of theory and basis set. A **theory** refers to how, mathematically, an approximate solution is obtained for the Schrödinger Equation $H\Psi=E\Psi$. B3LYP is one of several density functional theory (DFT) methods developed in the last 20 years. The letters stand for Becke, Lee, Yang, and Parr, the developers of the method. All DFT methods use the basic principle of

molecular orbital (MO) theory, namely that pairs of electrons inhabit regions of space around the nuclei (the Ψ MOs), each having a characteristic energy (E).

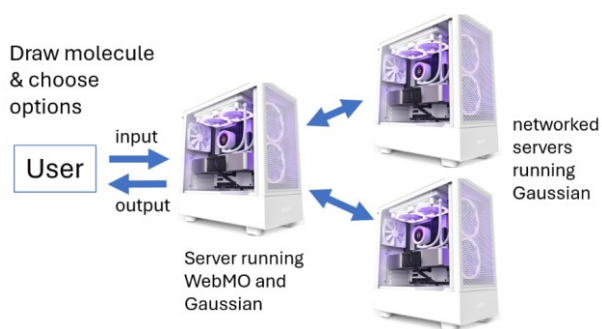
Molecular orbitals are constructed by combining atomic orbital functions (AOs) from all the atoms in the molecule. This is the same basic process as described for the diatomic molecules in your textbook. The set of AOs is called the **basis set**. The basis set used in this experiment, 6-31G(d,p), tells what kind (s, p, or d), and how many AOs are used on each type of atom (C, N, O, or H). For crystal violet cation CV, there are 570 functions in the 6-31G(d,p) basis set. The larger the basis set, the more accurate the calculation – and the longer the required computing time.

What is a “Molecular Energy” calculation? For a B3LYP/6-31G(d,p) calculation, Gaussian assigns atomic orbitals to each atom for a total of 570; constructs an equal number of molecular orbitals (MOs) by linear combination of atomic orbitals; and adds electrons (2 per MO = 200 electrons) to the 100 lowest-energy MOs. It then calculates several properties including the total energy, the partial charge on each atom, and the dipole moment.

What is a “Molecular Orbital” calculation? This is like a Molecular Energy calculation. Both calculations require computing 570 MO functions, however in this case the coefficients that describe the MOs are saved, and these are used to print the table of MO energies, and display MO 3D shapes.

Of what practical use are molecular orbitals and molecular quantum theory? These methods are widely used in materials science, atmospheric science, molecular biology, chemistry research, and others. To get a sense of this trend, use “dft” as a google search term, plus “battery design”, “tropospheric chemistry”, “covid enzyme”, or “green chemistry”. The top hits mainly are technical research articles; however, the introductory paragraphs of these papers are worth reading because they often explain the background science in terms that general chemistry students can relate to.

How does WebMO work? WebMO provides a web page that is a graphical interface for Gaussian; it uses the graphic functions in the Chrome or Firefox browser to display molecules. The WebMO server (antec12 at UAF) runs Gaussian and stores user Gaussian calculations in a database. Gaussian calculations can also be assigned to networked server computers (merlin and trx40 at UAF). Calculations on the latter go about twice as fast as antec12.



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Useful links:

[WebMO, Inc homepage](#)

[Gaussian computational program](#)

[UAF Department of Chemistry and Biochemistry](#)