Using UV-vis Spectroscopy

UV-vis spectroscopy is not often used for structure determination, but it has it's value.

KeynoteChemistry.com

The video is available on YouTube at https://youtube/OVjlvaq3bxA

YouTube

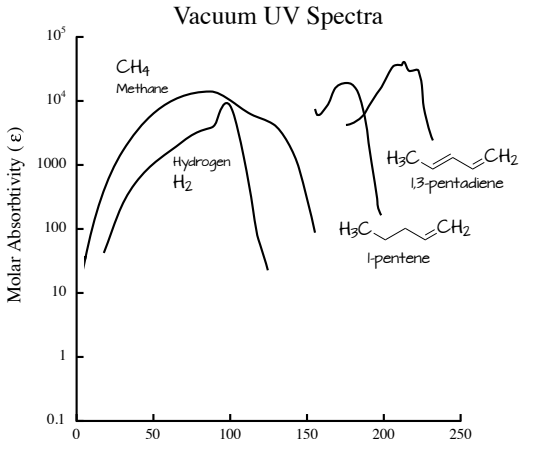
Review

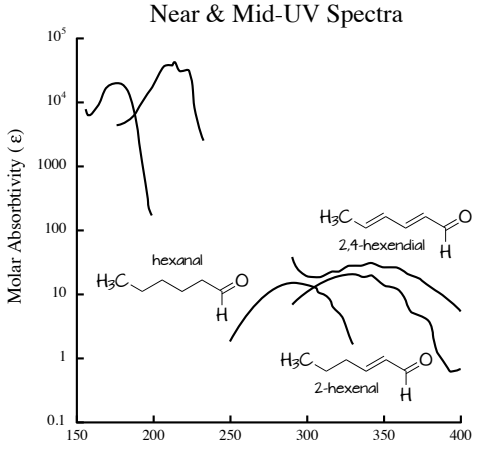
UV-vis spectra detects absorbances associated with electronic transitions.

$\sigma \rightarrow \sigma^*$ transitions are allowed but are observable only in the vacuum UV spectrum

$\pi \rightarrow \pi^*$ transitions are allowed and are observable in the mid to near UV spectrum. Most instruments can observe this region.

$n \rightarrow \pi^*$ transitions are forbidden and are observable in the near UV.





UV spectral data obtained from: "The MPI-Mainz UV/VIS spectral atlas of gaseous molecules of atmospheric interest", Keller-Rudek, H., Moortgat, G. K., Sander, R., and Sörensen, R., *Earth Syst. Sci. Data*, **2013**, *5*, 365–373, doi:10.5194/essd-5-365-2013 accessed at http://satellite.mpic.de/spectral_atlas/cross_sections/ on March 25, 2014

Part 1: So What?

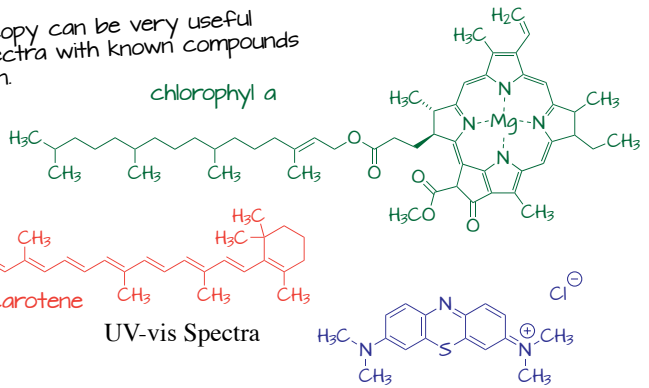
In organic chemistry UV-vis spectroscopy is rarely used to determine structure.

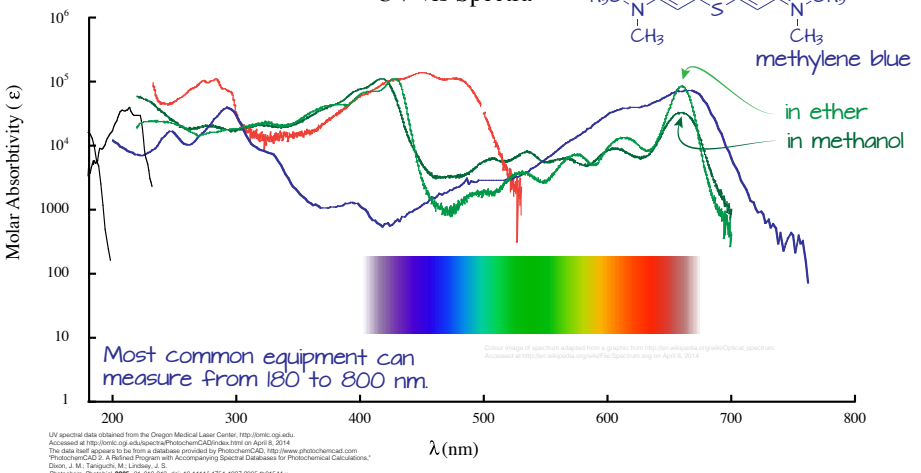
It can be useful in determining the structures of conjugated regions in a molecule if no other information is available (e.g. you can't do an NMR of the atmosphere).

See the **Woodward-Feiser rules** and other empirical tables for more information.

You can predict the frequency and extinction coefficient for many conjugated ketones, alkenes and aromatic compounds.

UV-vis spectroscopy can be very useful in comparing spectra with known compounds for identification.





UV spectral data obtained from the Oregon Medical Laser Center, http://omlc.ogi.edu. Accessed at http://omlc.ogi.edu/spectra/PhotochemCAD/index.html on April 8, 2014. The data itself appears to be from a database provided by PhotochemCAD, http://www.photochemcad.com "PhotochemCAD 2: A Refined Program with Accompanying Spectral Databases for Photochemical Calculations," Dixon, J. M.; Taniguchi, M.; Lindsey, J. S. *Photochem. Photobiol.* **2005**, *81*, 212-213, doi: 10.1111/j.1751-1097.2005.tb01544.x

Using UV-vis Spectroscopy

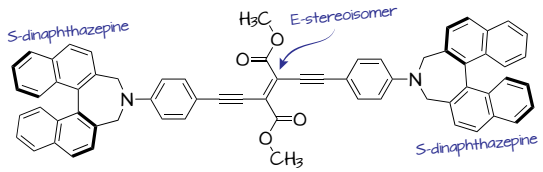
UV-vis spectroscopy is very useful to organic chemists in many ways.

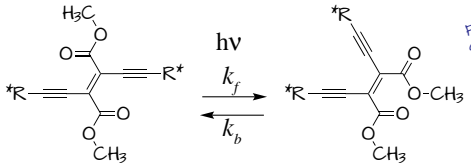
Part 2: Practical uses of UV-vis for organic chemists

UV-vis spectroscopy can be used to identify suspected structures by comparing to known spectra; it can be used to measure concentrations of known molecules; it can be used to follow reaction kinetics; and circular dichroism can be used to identify chitality or changes in conformation.

Dinaphthazepine groups spaced by a photochemically (E)/(Z)-isomerisable 1,2-diethynylethene scaffold.

This was investigated as a chiral agent for inducing reversible phase changes in liquid crystal systems. See Yi-Lin Wu et. al., "1,2-Di(phenylethynyl)ethenes with axially chiral, 2,2'-bridged 1,1'-binaphthyl substituents: potent cholesteric liquid-crystal inducers", Org. Biomol. Chem., 2012, 10, 8016-8026, DOI: 10.1039/c2ob25983d

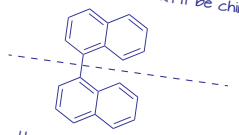




For some situations cis and trans are not good enough. E and Z can make things easier.

Do you remember how to use E and Z?

Wait a minute! a binapthyl group has a plane of symmetry. How can it be chiral?



Here is a good opportunity to review conformational stereoisomerism and haw we can use the Cahn-Ingold-Prelog (R & S) designations to describe them.

UV-vis spectrum of the E-SS stereoisomer...

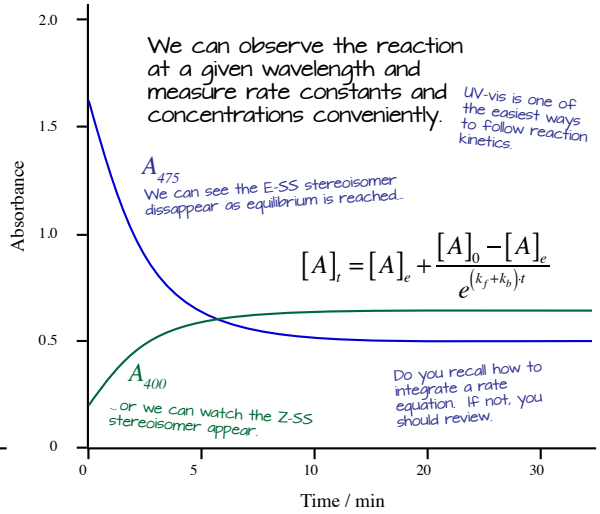
... and watch as it converts to the Z-SS stereoisomer when irradiated at 475 nm.

An isosbestic point at 419 nm.

Adapted from an image in "1,2-Di(phenylethynyl)ethenes with axially chiral, 2,2'-bridged 1,1'-binaphthyl substituents: potent cholesteric liquid-crystal inducers", Wu, L.-Y.; Ferroni, F. Pieraccini, S. Schweizer, W. B.; Frank, B. B.; Spadab, G. P.; Diederich, F. Org. Biomol. Chem., 2012, 10, 8016-8026, doi: 10.1039/c2ob25983d

We can observe the reaction at a given wavelength and measure rate constants and concentrations conveniently.

UV-vis is one of the easiest ways to follow reaction kinetics.



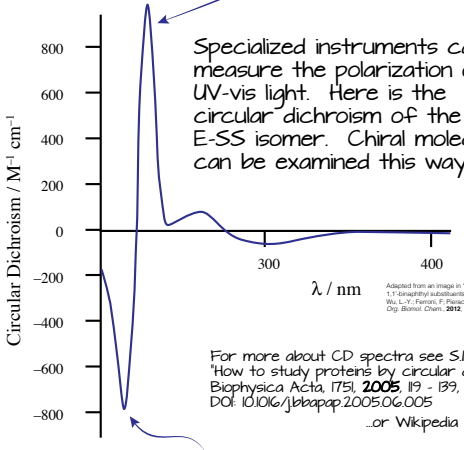
A_{475} We can see the E-SS stereoisomer dissappear as equilibrium is reached...

$$[A]_t = [A]_e + \frac{[A]_0 - [A]_e}{e^{(k_f + k_b)t}}$$

A_{400} ...or we can watch the Z-SS stereoisomer appear.

Do you recall how to integrate a rate equation. If not, you should review.

This plot is the difference in extinction coefficient between left- and right-circularly polarized light.



Specialized instruments can measure the polarization of UV-vis light. Here is the circular dichroism of the E-SS isomer. Chiral molecules can be examined this way.

Adapted from an image in "1,2-Di(phenylethynyl)ethenes with axially chiral, 2,2'-bridged 1,1'-binaphthyl substituents: potent cholesteric liquid-crystal inducers", Wu, L.-Y.; Ferroni, F. Pieraccini, S. Schweizer, W. B.; Frank, B. B.; Spadab, G. P.; Diederich, F. Org. Biomol. Chem., 2012, 10, 8016-8026, doi: 10.1039/c2ob25983d

For more about CD spectra see S.M. Kelly, T.J. Jess, N.C. Price, "How to study proteins by circular dichroism", Biochimica et Biophysica Acta, 1751, 2005, 119 - 139, DOI: 10.1016/j.bbapap.2005.06.005

...or Wikipedia

Note that it is a small effect. $\varepsilon_{22B} = 2.5 \times 10^5$ but $\Delta\varepsilon_{22B} = -7.7 \times 10^2$.

Using UV-vis Spectroscopy

UV-vis in action in research

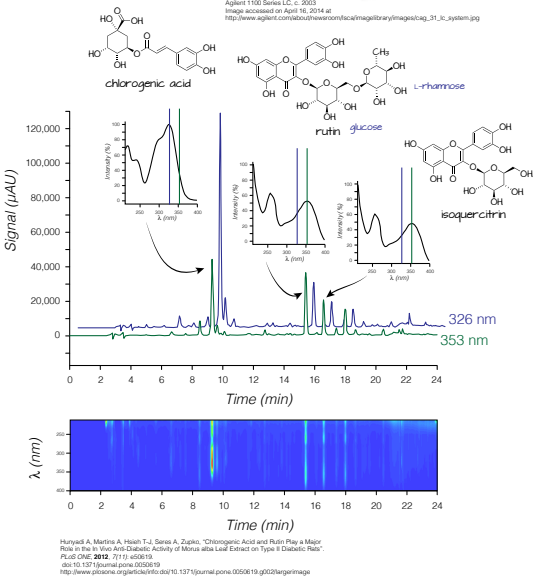
Part 3: Examples of modern uses of UV-vis spectroscopy

UV-vis spectroscopy is an important technique for detecting products in HPLC analysis and for following structural changes in biomolecules.. And we haven't mentioned fluorescence, a related subject that is also heavily used in research.

An example of LC analysis

Here is an example of an analysis of antioxidants in a biological extract performed using an HPLC with a dioide-array UV-vis spectrometer as a detector.

Agilent 1100 Series LC, c. 2003
Image accessed on April 16, 2014 at http://www.agilent.com/about/newsroom/lsca/imagelibrary/images/cag_31_lc_system.jpg



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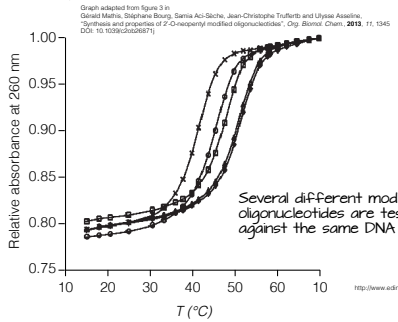
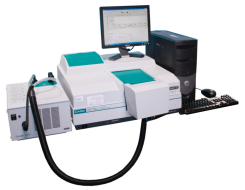
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0050619.g002/largerimage

Some examples of following thermal denaturations

Thermal denaturation plot for DNA duplexes. Followed by UV absorbance at 260 nm.



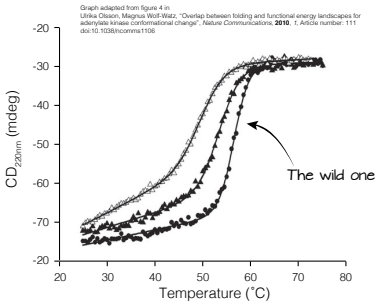


Cary 100 Bio with temperature control accessory
Image accessed on April 16, 2014 at http://lkp-co.com/upload/user/images/cary%20100.png

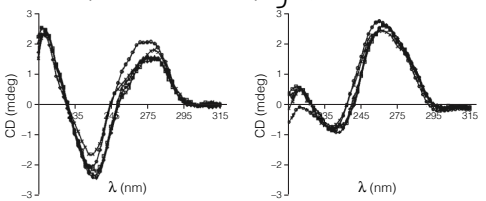
Edinburgh Photonics LP920 laser flash photolysis spectrometer
image accessed on April 16, 2014 at http://www.edinburghphotonics.com/files/file/brochures/LP920%20Brochure.pdf

Jasco 1500 CD spectrometer
http://www.jasco.de/en/content/J-815-High-T/--nm.13--nc.413/High-Throughput-CD-System.html

Thermal denaturation of adenylate kinase as followed by circular dichroism at 220 nm. A 'wild type' and two mutants are shown here.



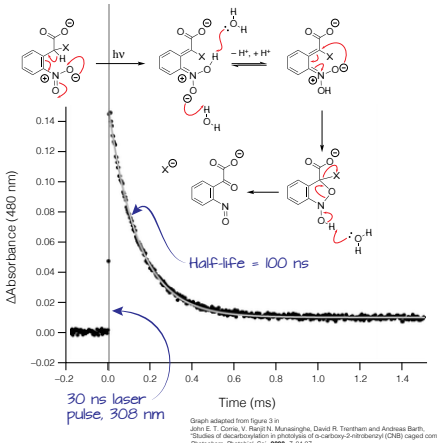
Circular dichroism of the modified oligonucleotides with DNA (left) and RNA (right).



Graph adapted from figure 4 in Gérald Mathis, Stéphane Bourg, Samia Aci-Sèche, Jean-Christophe Truffert and Ulysse Asseline, "Synthesis and properties of 2'-O-neopentyl modified oligonucleotides", *Org. Biomol. Chem.*, **2013**, *11*, 1345 DOI: 10.1039/c2ob26871j

Following fast reaction kinetics

Some reactions are over in microseconds or less. How do we follow them?



Graph adapted from figure 3 in John E. T. Corrie, V. Ranjit N. Munasinghe, David R. Trentham and Andreas Barth, "Studies of decarboxylation in photolysis of α-carboxy-2-nitrobenzyl (CNB) caged compounds", *Photochem. Photobiol. Sci.*, **2008**, *7*, 84-97 DOI: 10.1039/B711398F

Can you understand the reaction mechanism above? Can you propose which is the RDS after for the breakdown of the photolysis product?

Now would be a good time to review some reaction mechanisms from o-chem. Just examine one a day.

References and Notes

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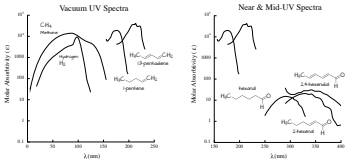

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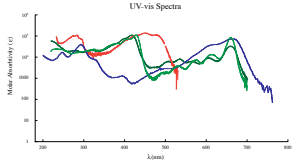

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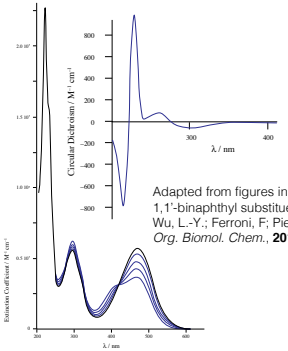
Colour image of spectrum adapted from a graphic from http://en.wikipedia.org/wiki/Optical_spectrum.
Accessed at http://en.wikipedia.org/wiki/File:Spectrum.svg on April 8, 2014



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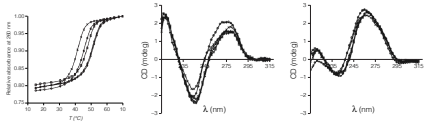

Graphs adapted from figures 3 & 4 in
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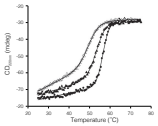
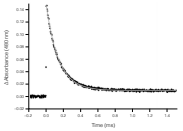


Graph adapted from figure 4 in
Ulrika Olsson, Magnus Wolf-Watz, “Overlap between folding and functional energy landscapes for adenylate kinase conformational change”, *Nature Communications*, **2010**, *1*, Article number: 111
doi:10.1038/ncomms1106



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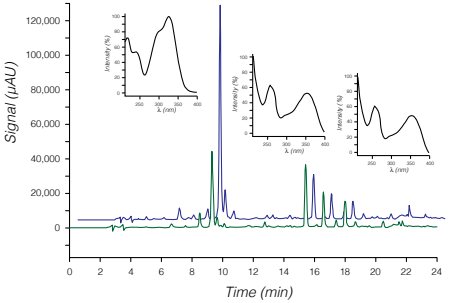

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Edinburgh Photonics LP920 laser flash photolysis spectrometer
image accessed on April 16, 2014 at
http://www.edinburghphotonics.com/files/file/brochures/LP920%20Brochure.pdf

Agilent 1100 Series LC, c. 2003
Image accessed on April 16, 2014 at
http://www.agilent.com/about/newsroom/lsca/imagelibrary/images/cag_31_lc_system.jpg

Jasco 1500 CD spectrometer
http://www.jasco.de/en/content/J-815-High-T/~nm.13~nc.413/High-Throughput-CD-System.html

The video is available on YouTube at
https://youtube/OVJlvaq3bxA

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