Universidad Rey Juan Carlos

Different methods for the study of interactions of metal complexes with biological molecules

Dr. Santiago Gómez-Ruiz

Departamento de Química Inorgánica y Analítica,
Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos

Different methods for the study of interactions of metal complexes with biological molecules

Universidad Rey Juan Carlos

1. INTRODUCTION
2. UV-VISIBLE SPECTROSCOPY
3. NMR SPECTROSCOPY
4. CYCLOVOLTAMMETRY
5. CONCLUSIONS

1. INTRODUCTION

Universidad Rey Juan Carlos

- Study of the interactions of metal complexes with biological targets is of extreme interest in medicinal chemistry

- It is known now that platinum complexes interact with DNA, titanium complexes with proteins, gold(I) complexes interact with thioreductin reductase, gallium complexes with ribonucleotide reductase…

- This is due in part to the advances in some techniques such as UV-vis, cyclovoltammetry, fluorescence, NMR, IR…

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- It has been mainly used in the study of the interactions of metal complexes with DNA

- First is the characterization of the complex in the right medium (biological, for example Tris-HCl buffer at physiological pH = 7.4)

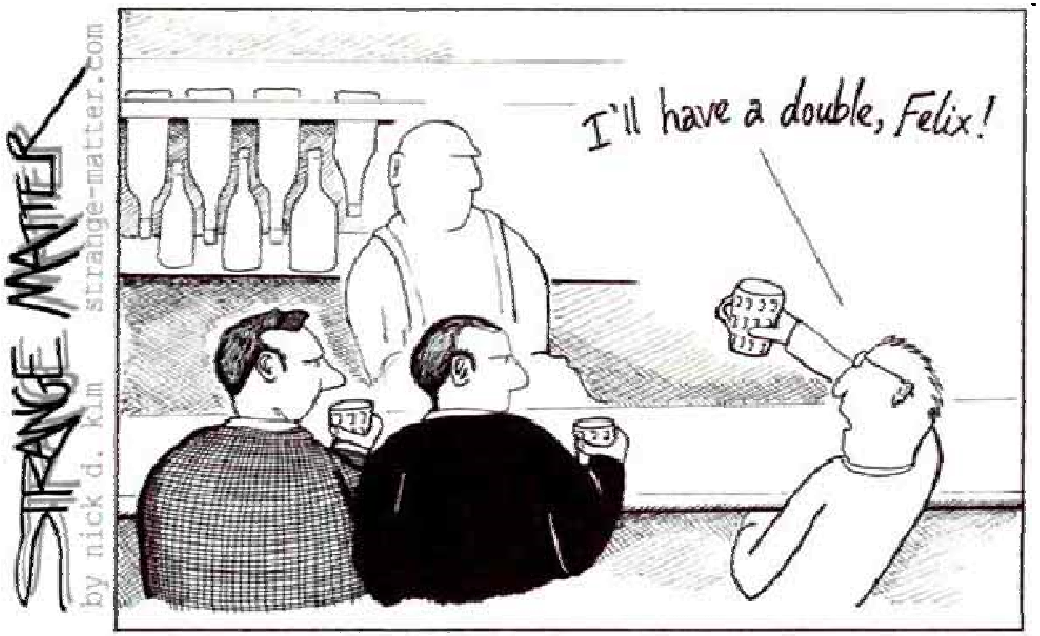
- Study of the different bands which are typical for metal complexes after hydrolisis:

    - MLCT band: observed in complexes with ligands having low-lying π^* orbitals especially aromatic ligands (2,2'-bipyridine (bipy), 1,10-phenanthroline (phen), CO, CN^- and SCN^-) found between 350-550 nm

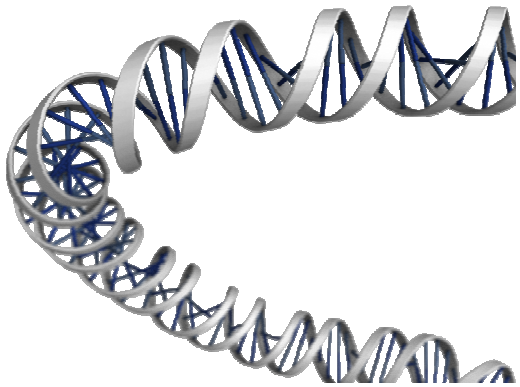
2. UV-VISIBLE SPECTROSCOPY

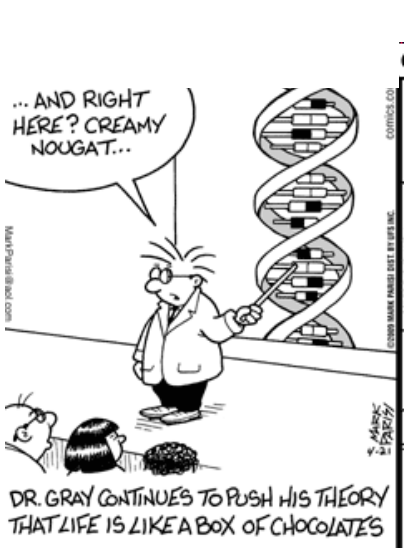
Universidad Rey Juan Carlos

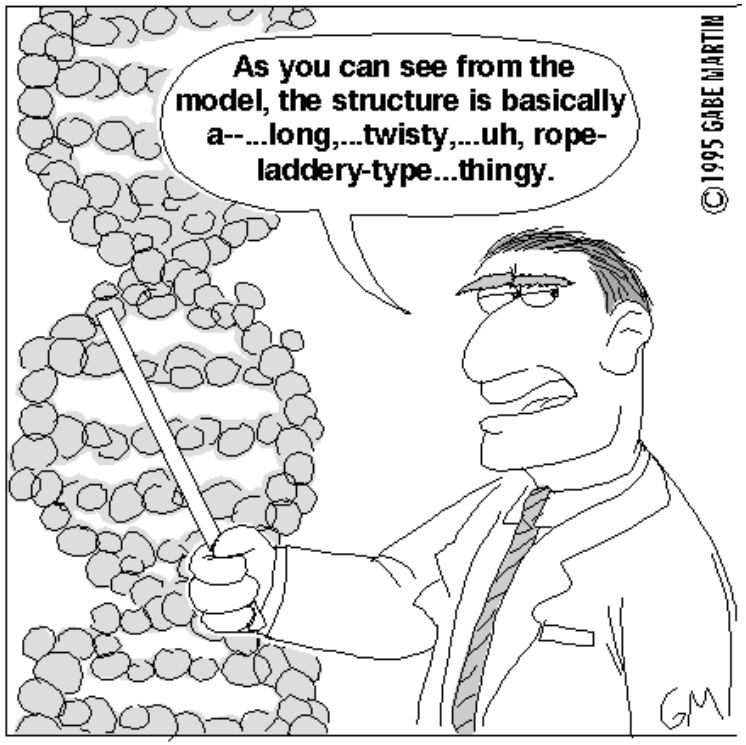
- DNA



Cambridge, 1953. Shortly before discovering the structure of DNA, Watson and Crick, depressed by their lack of progress, visit the local pub.







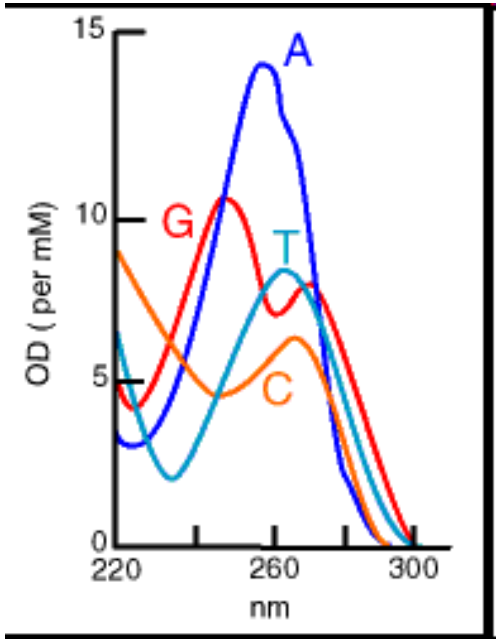
1953: The structure of the DNA molecule is first described.

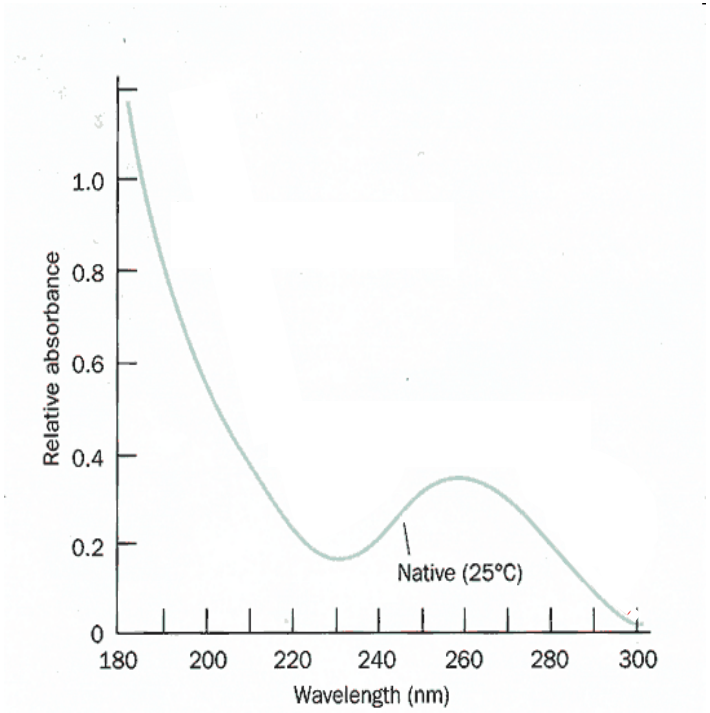
2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- UV-Visible spectroscopic properties for DNA:

  - Due to the ability of the nitrogenated bases of DNA to be protonated, UV-spectrum of DNA is sensitive to pH. Normally it is used in biological media, where the maximum absorption is at 260 nm as a mixture mainly of the signals of guanine, cytosine, thymine and adenine.



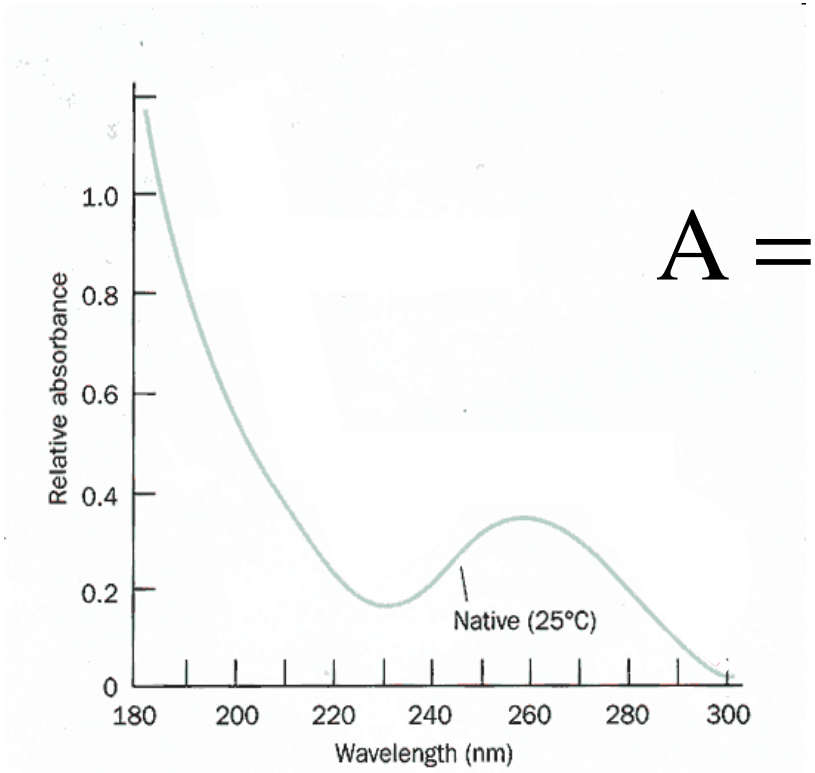


2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- UV-Visible spectroscopic properties for DNA:

  •The DNA concentration (per nucleotide) can be calculated using the extintion molar coefficient (ε) which is 6600 $M^{-1} \cdot cm^{-1}$ at 260 nm.



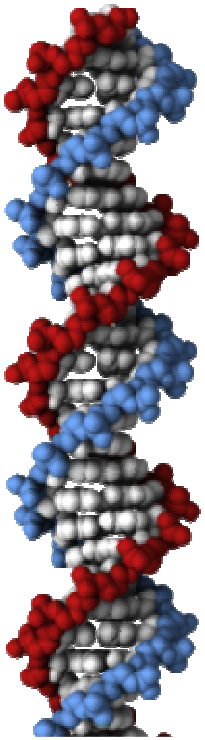
$$A = \varepsilon \times b \times c$$

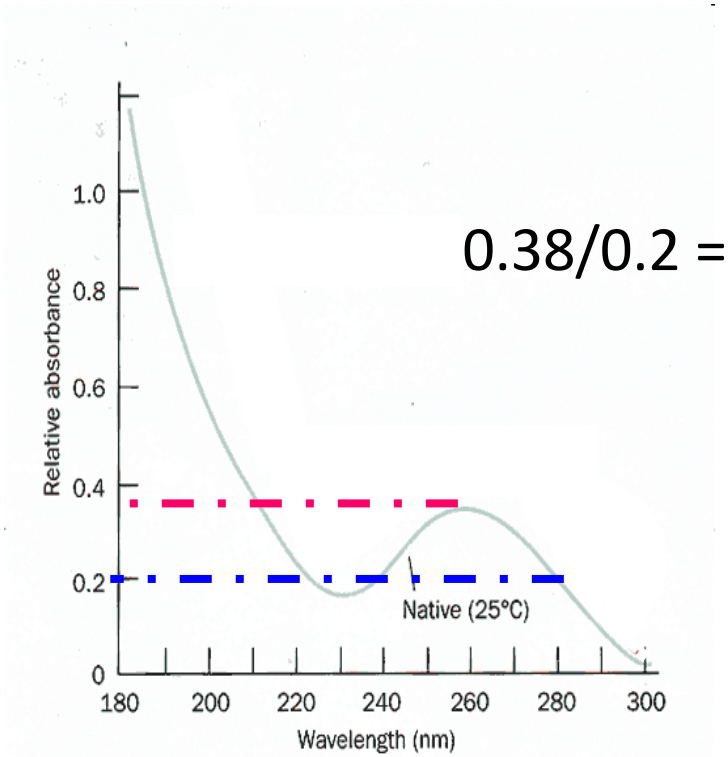
2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- UV-Visible spectroscopic properties for DNA:

  •In the isolation of DNA some protein traces may be present, so that the UV–Vis absorbance at 260 and 280 nm must give a ratio of about 1.9, indicating that the DNA is sufficiently free of protein



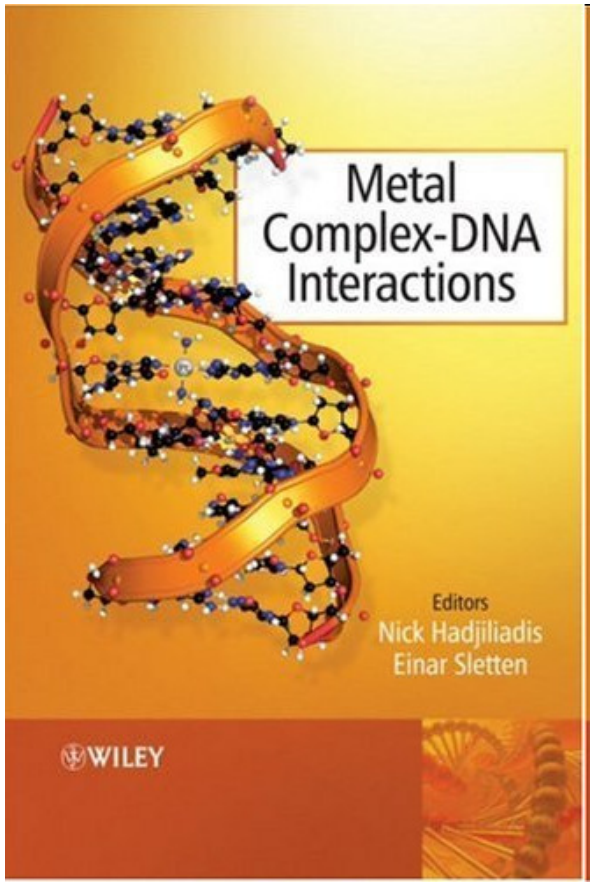


0.38/0.2 = 1.9

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

Interactions of metal complexes with DNA

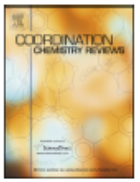


Coordination Chemistry Reviews 253 (2009) 2021–2035

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr



Review

Metal complexes as structure-selective binding agents for nucleic acids

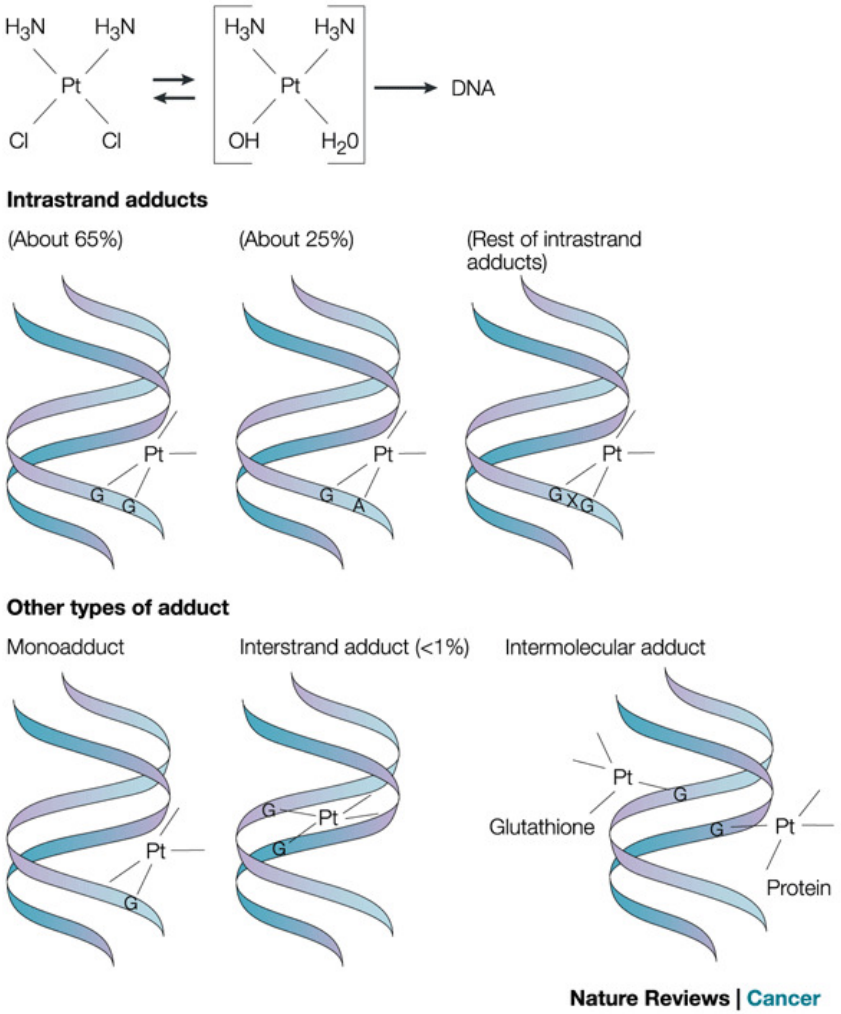
F. Richard Keene[a,*], Jayden A. Smith[a], J. Grant Collins[b,**]

[a] School of Pharmacy & Molecular Sciences, James Cook University, Townsville, Queensland 4811, Australia
[b] School of Physical, Environmental and Mathematical Sciences, University College, University of New South Wales, Australian Defence Force Academy, Canberra, A.C.T. 2600, Australia

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- Interactions of metal complexes with DNA are normally:
  - *Covalent Binding*: is typical in the aquated form of cisplatin with guanine

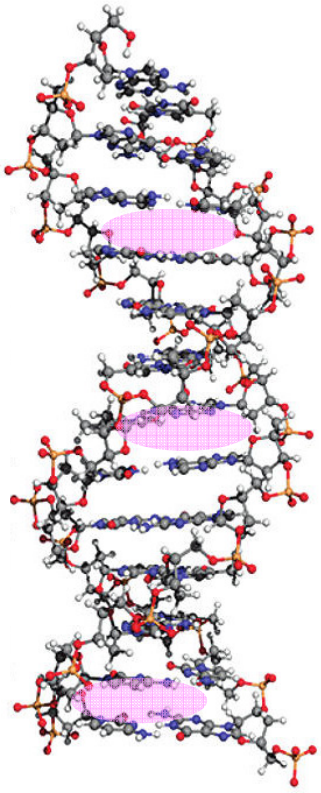


2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- Interactions of metal complexes with DNA are normally:

  - *Intercalations*: Metallointercalators bind DNA through the insertion of a planar polycyclic aromatic ligand into the π-stack between two base pairs

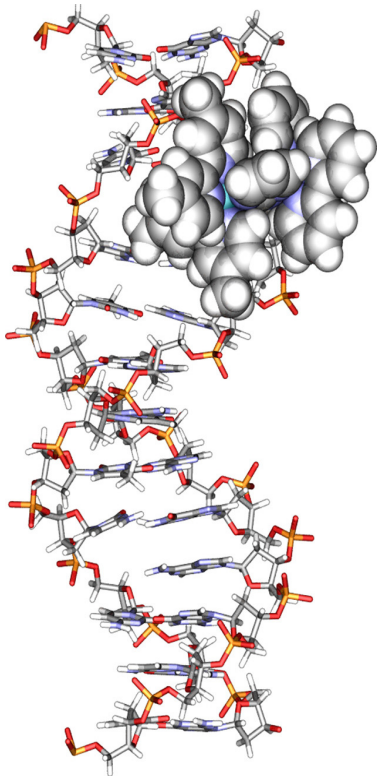


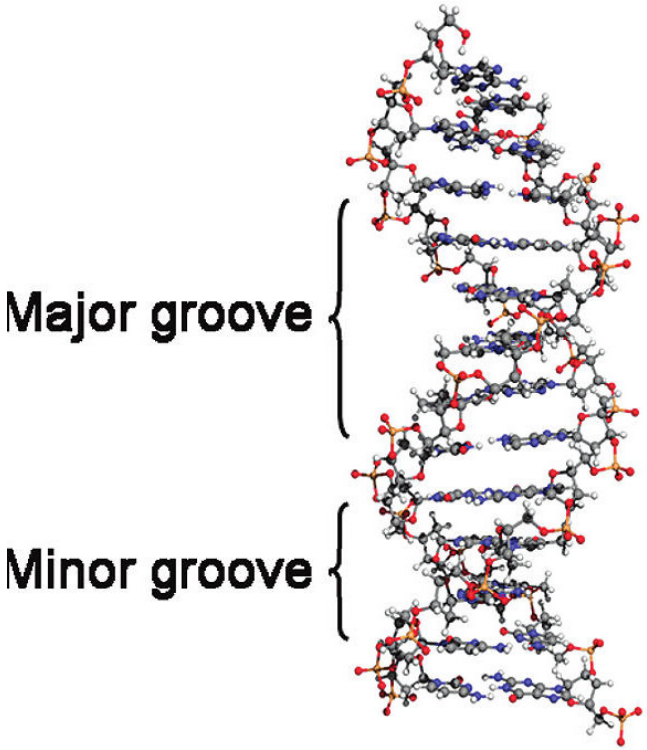
2. UV-VISIBLE SPECTROSCOPY

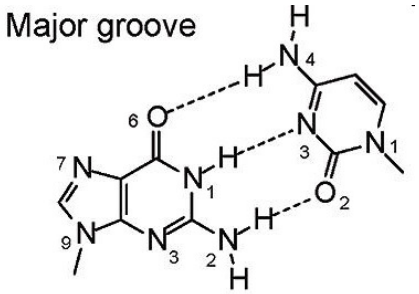
Universidad Rey Juan Carlos

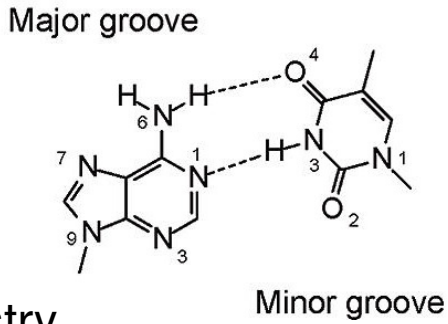
- Interactions of metal complexes with DNA are normally:

  - *Groove binding:* many mononuclear inert metal complexes associate in the DNA minor groove and show a preference for AT-rich sequences. Copper and some other complexes may also bind at major groove in GG-rich sequences





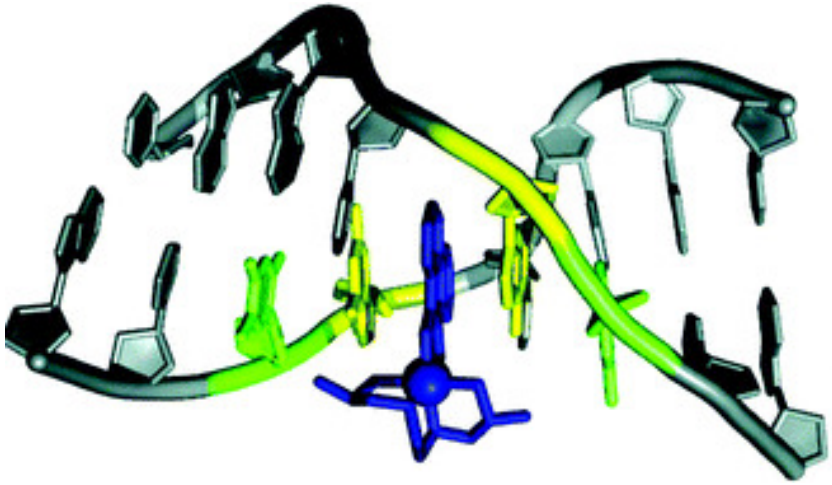




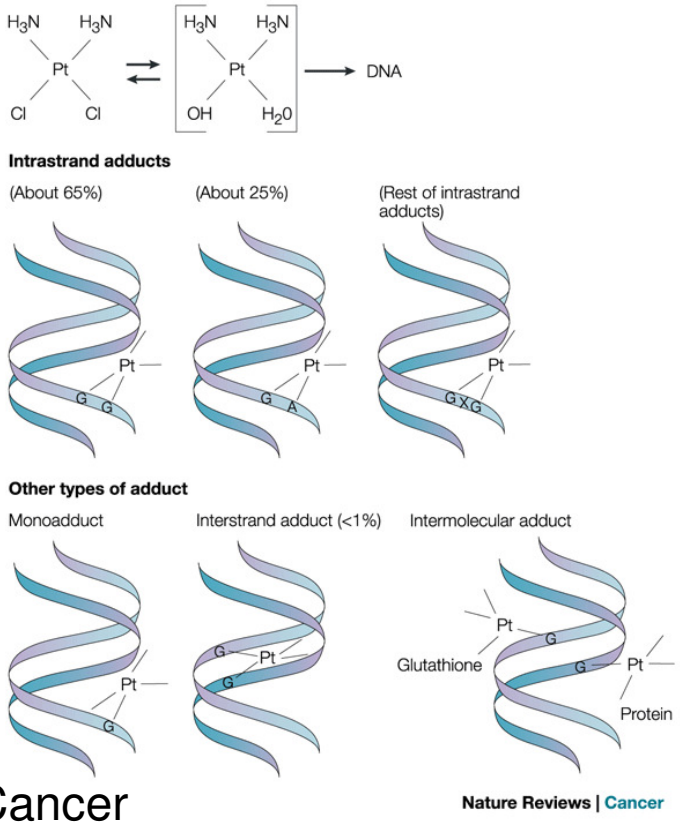
2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

• Metal complexes may bind to DNA in different modes on the basis of their structure, charge in the medium and type of ligands and this is translated in different changes in the absorbance intensity and maximum of the observed peaks.



© Royal Society of Chemistry



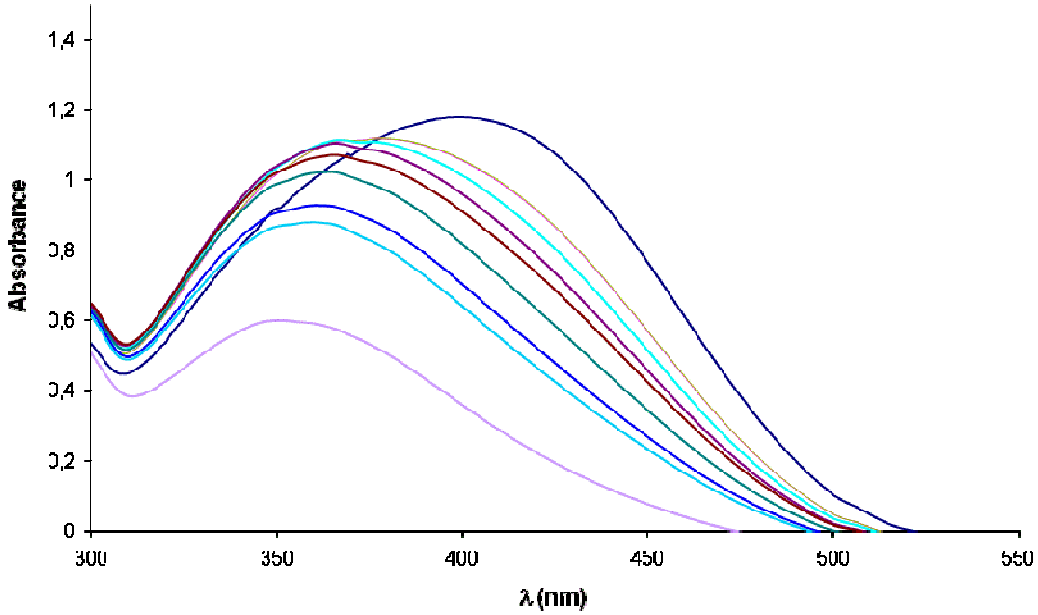
© Nature Reviews Cancer

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

• Changes of the absorbance of MLCT bands of the complexes are indicative of reaction

Example: Titanium complex

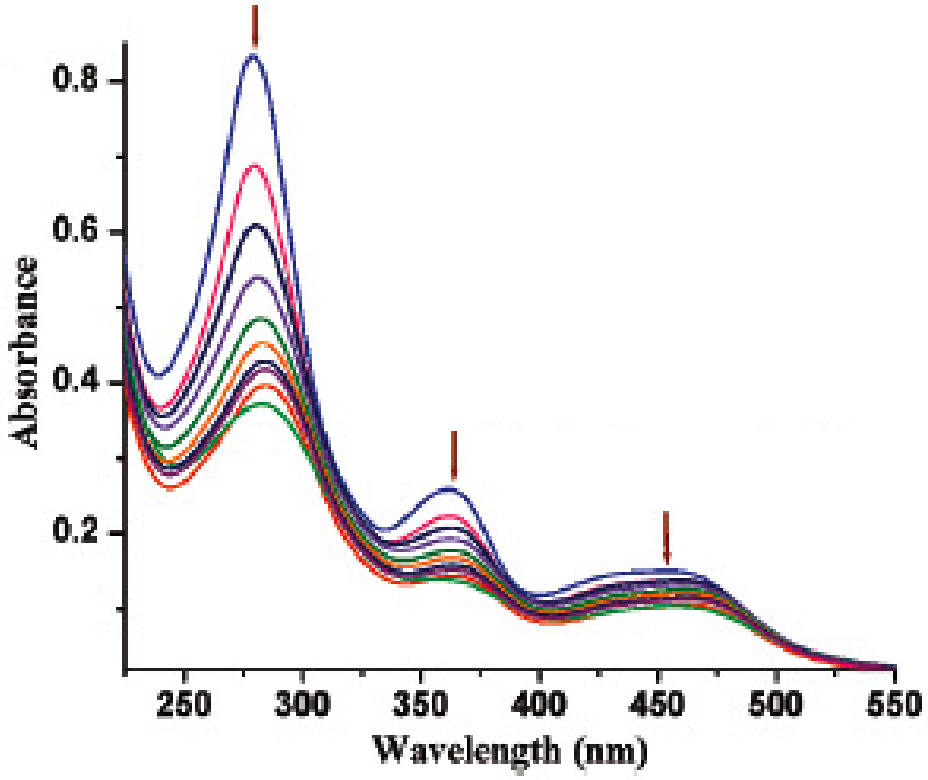


•Changes of the absorbance of MLCT bands of the complexes upon increasing concentrations of DNA are indicative of interaction with DNA.

•Hypochromism and red shift of the MLCT band are associated with the binding of the complex to the helix because of the interaction between the aromatic chromophore of the complex and the base pairs of the DNA.

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos



• In order to compare the binding strengths of the complexes, the intrinsic binding constant, K_b, can be determined using the following equation

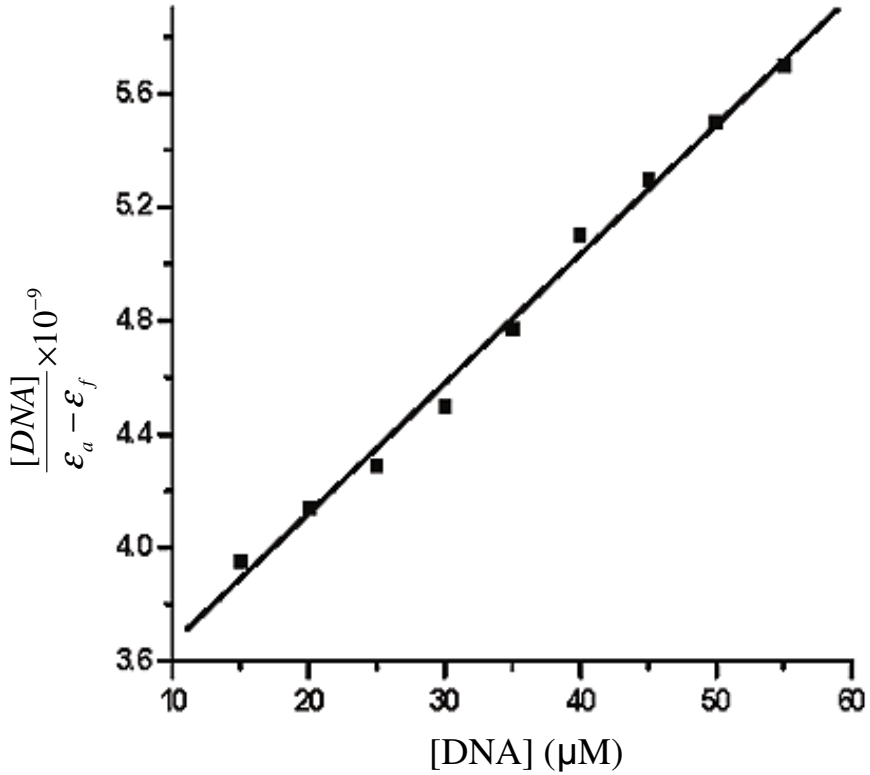
$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_0 - \varepsilon_f} + \frac{1}{K_b(\varepsilon_0 - \varepsilon_f)}$$

• [DNA] is the concentration of DNA in base pairs, ε_a the extinction coefficient observed for the MLCT absorption band at the given DNA concentration, ε_f is the extinction coefficient of the complex free in solution, and ε_0 correspond to is the extinction coefficient of the complex when fully bound to DNA.

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_0 - \varepsilon_f} + \frac{1}{K_b(\varepsilon_0 - \varepsilon_f)}$$



- [DNA] is the concentration of DNA in base pairs
- ε_a the extinction coefficient observed for the MLCT absorption band at the given DNA concentration
- ε_f is the extinction coefficient of the complex free in solution (A_{obs} / [complex])
- ε_0 is the extinction coefficient of the complex when fully bound to DNA.

A plot of [DNA]/[ε_a - ε_f] versus [DNA] gives a slope 1/[ε_a - ε_f] and Y intercept equal to 1/K_b [ε_0 - ε_f], respectively. The intrinsic binding constant K_b is the ratio of the slope to the intercept .

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- Classical intercalators such as ruthenium complexes with phenantroline ligands have K_b of about $4\text{-}6 \cdot 10^6\ M^{-1}$

- Significant differences in DNA binding affinity of different complexes could be a result of differences in the conjugation of the ligands.

- K_b of the order $10^3\ M^{-1}$ may be indicative of interaction with DNA by groove binding

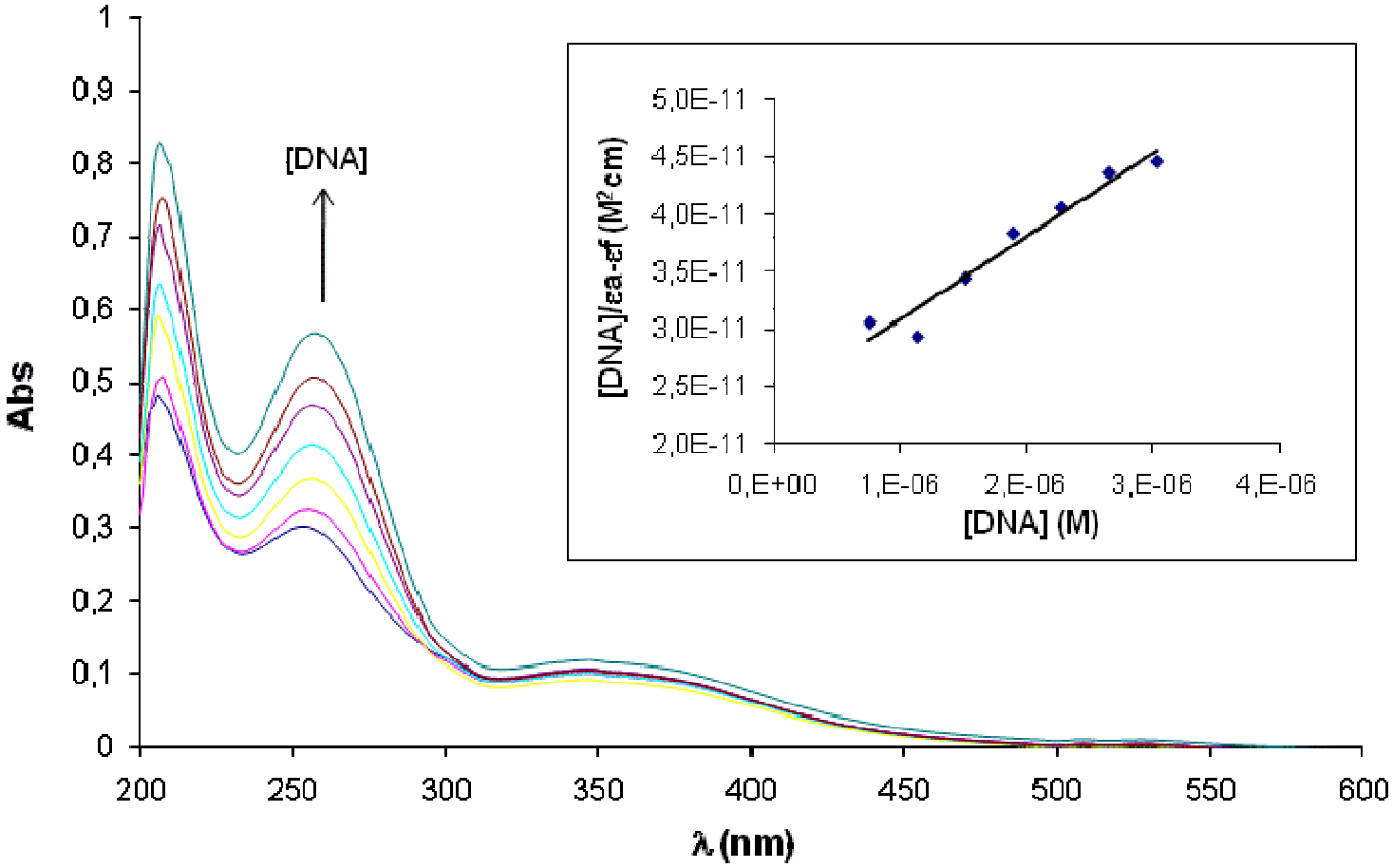
- K_b of the order $10^4\ M^{-1}$ may be indicative of interaction with DNA through a mode that involves a stacking interaction of the aromatic chromophore and the base pairs of DNA.

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

Example: Titanium complex

$K_b = 3.09 \times 10^5\ M^{-1}$



2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- For complexes without a MLCT band, the DNA absorption at 260 nm needs to be examined to obtain the information about the possible interaction.

- A typical behaviour of classical electrostatic interactions is the hyperchromism and blue shift of the absorption bands of the complexes and DNA (in many cases they may be located at similar wavelengths)

- In addition, hydrophobic associations of aromatic rings of the complex (if any) with the hydrophobic interior of DNA may also be possible when observation of hyperchromism and blue shift

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- There is the possibility of other non-linear fittings, which are very rare and not typical for the simple interactions of metal complexes with DNA.

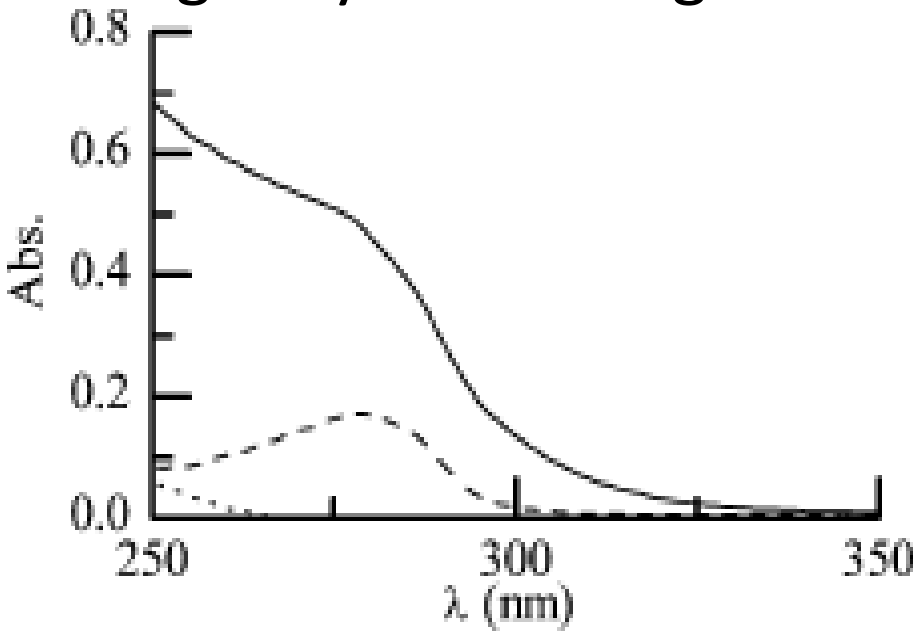
- When severe overlap of the bands occurs, this may lead to the non-fitting of the data, being only the qualitative analysis possible.

- An alternative to the binding behaviour with DNA is the use of fluorescence emission (for the complexes with fluorophore-emitting ligands)

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

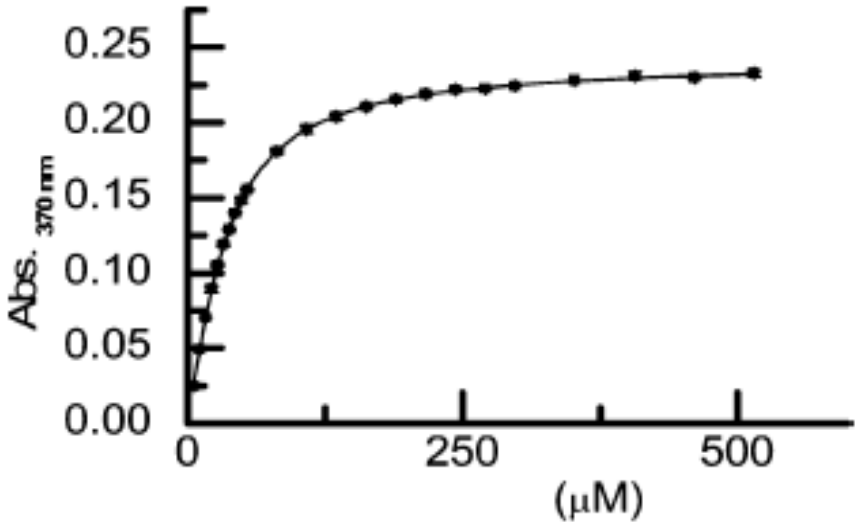
- Qualitative studies may also be carried out for the study of protein interactions or monitoring reactions with other biologically interesting molecules



——————— Free Cp_2TiCl_2

– – – – – – – – – – HSA + Cp_2TiCl_2

. . . . . Free HSA

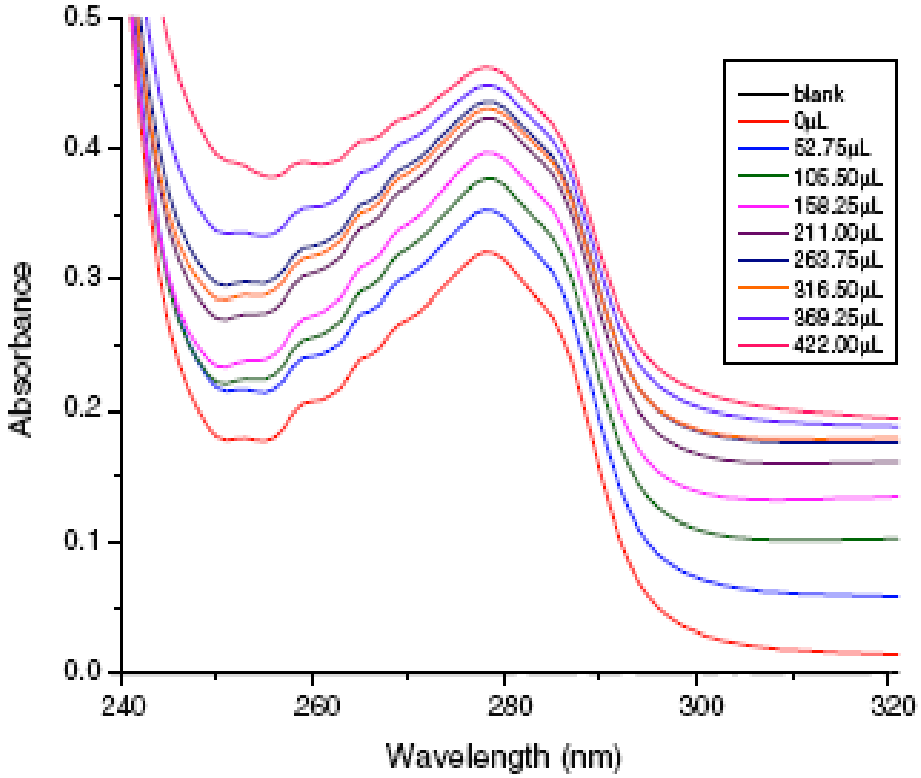


Change in the absorbance of the MLCT band of a titanium complex upon increasing concentrations of an aminoacid

2. UV-VISIBLE SPECTROSCOPY

Universidad Rey Juan Carlos

- Qualitative studies may also be carried out for the study of protein interactions or monitoring reactions with other biologically interesting molecules



3. NMR SPECTROSCOPY

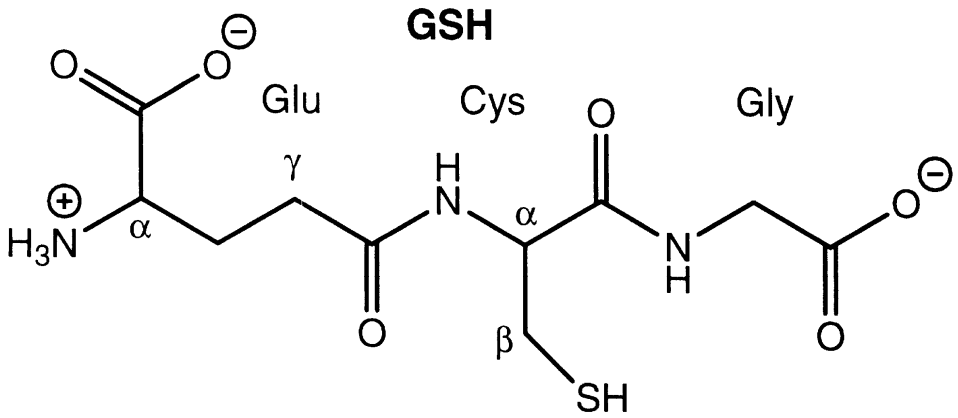
Universidad Rey Juan Carlos

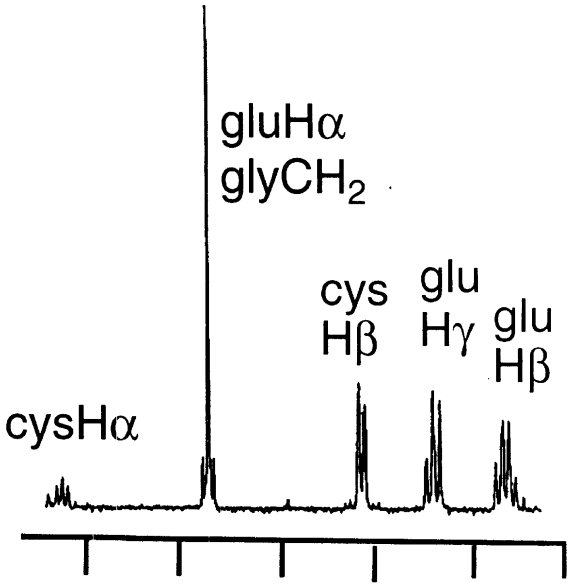
- NMR methods are widely used for the characterization of metal complexes, however, there are also studies for the interaction of metal complexes with interesting molecules.
- One of the most intriguing molecules for biochemists is Glutathione (γ-*L*-glutamyl-*L*-cysteinylglycine, GSH) is an important intracellular tripeptide that serves for a variety of fundamental functions including reacting with cytotoxic electrophilic drugs and reacting with DNA damaging different species such as peroxides.
- The formation of GSH metal complexes must be considered in any mechanism involving metal-based drugs containing labile ligands.

3. NMR SPECTROSCOPY

Universidad Rey Juan Carlos

- NMR methods are very useful for the monitoring of the interactions of glutathione with metallocene derivatives due to the typical signals of glutathione





3. NMR SPECTROSCOPY

Universidad Rey Juan Carlos

Addition of GSH to Cp_2MoCl_2 resulted in an upfield shift of the Cys Hβ resonance of GSH to become coincident with the Glu Hγ resonance, the appearance of a new Gly singlet and two new Cys Hα resonances. The absence of the Cys Hβ of free GSH is indicative of interaction of all free GSH with Cp_2MoCl_2 being all signals assigned to complex or complexes formed.

Many possibilities, other characterization techniques should be used.

4. CYCLIC VOLTAMMETRY

Universidad Rey Juan Carlos

• Among the available chemical and biochemical techniques, growing interest is being taken in electrochemical investigations of the interactions between drugs and biomolecules.

• There are many metal complexes which may have oxidation or reduction processes which can be clearly determined by cyclovoltammetry. (Pt(IV/II); Ti(IV/III); Sn(IV/II); Ru(III/II)…).

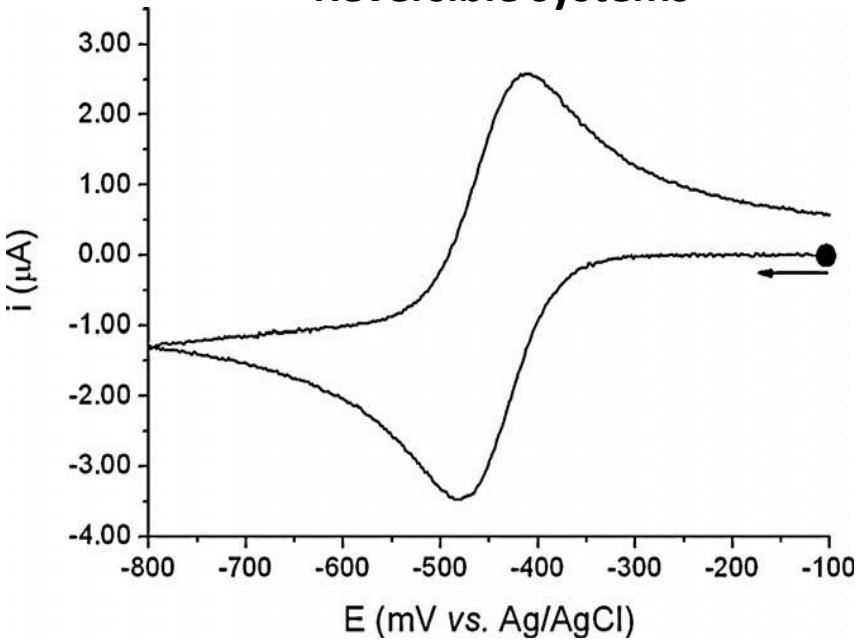
• For example, voltammetric techniques can be used for simple and qualitative studies of interaction of Cp_2TiCl_2 derivatives with human serum albumin (HSA) and DNA.

•The electrochemical test are based on the variation of the Ti(IV)/Ti(III) reduction peak before and after the addition of the above biomolecules in solution.

4. CYCLIC VOLTAMMETRY

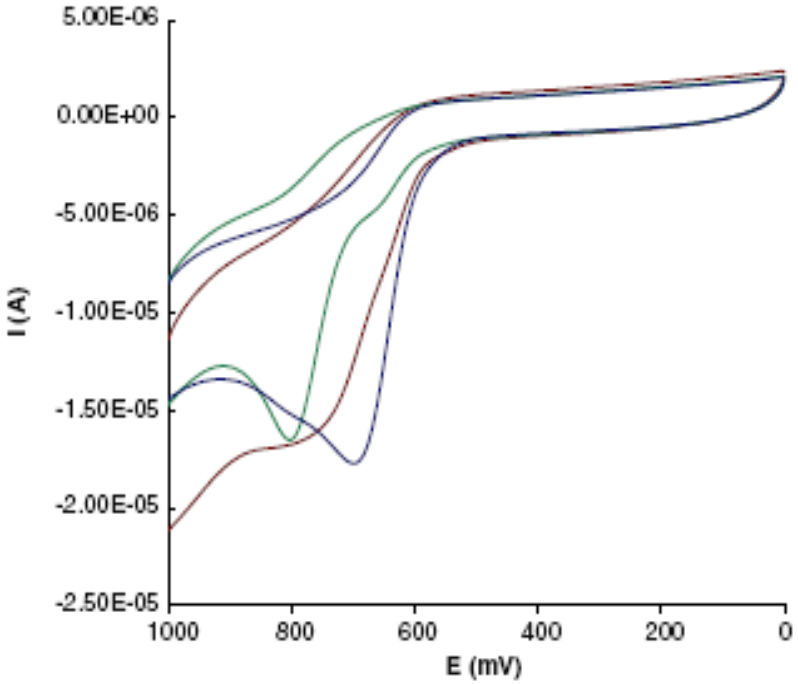
Universidad Rey Juan Carlos

Reversible systems



CV of a 1.0 mM solution of Cp_2TiCl_2 in 10% DMSO/water containing 0.10 M NaCl, pH 6.00, scan rate 200 mV/s

Quasi or non-reversible systems



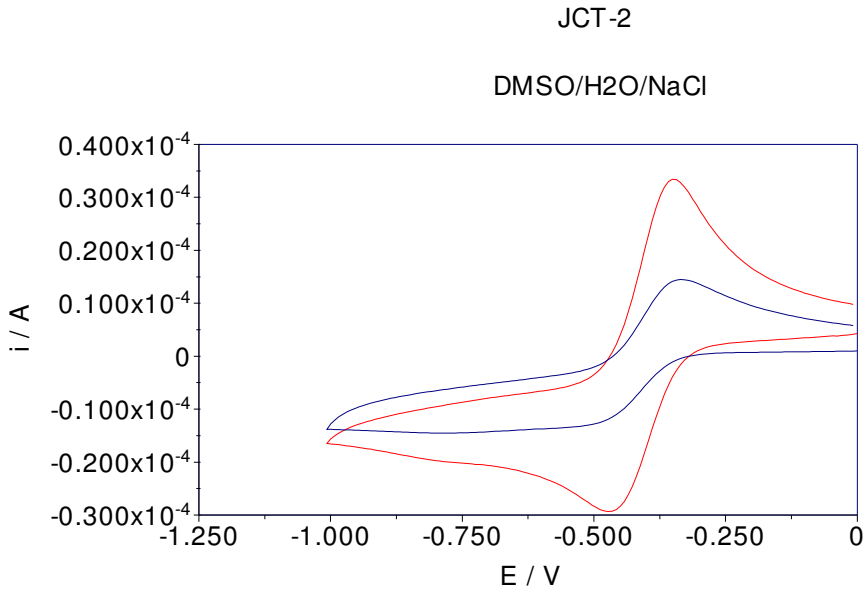
CV of 0.5 mM solutions of different molybdocene in (Tris)/10 mM NaCl, pH 7.4. at a scan rate of 100 mV/s

4. CYCLIC VOLTAMMETRY

Universidad Rey Juan Carlos

- These studies are being extended to a wide variety of small biomolecules such as guanine, guanosine and other nucleotides

- Interesting behaviour is expected for other small molecules such as glutathione (no reports up to now)



CV of 10 mM solution of JCT-2 in 10% DMSO/water containing 0.1 M NaCl, pH 7,3 at a GC working electrode (red) without guanine (blue) in the presence of 10 mM guanine

5. CONCLUSIONS

Universidad Rey Juan Carlos

- A brief review of some methods for the study of the interaction of biomolecules with metal complexes has been commented

- UV-visible is used for the study of the interactions mainly with DNA (calculation of K_b) and also for qualitative information of the interactions with proteins and small molecules

- NMR is used for the study of the binding behaviour of some small molecules such as glutathione (in several specialized groups also for the study of proteins and even DNA)

- Cyclovoltammetric methods are becoming very interesting for medicinal chemists, due to the qualitative and quantitative informations about binding behaviour of metal complexes (with redox properties) and small and macrobiomolecules