

Especiação

Especiação de complexos metálicos em solução: relevância en los sistemas biológicos

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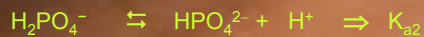
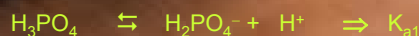


What is speciation?

Speciation of a "species" is the identification and quantification of all forms that this "species" may present in a certain medium.

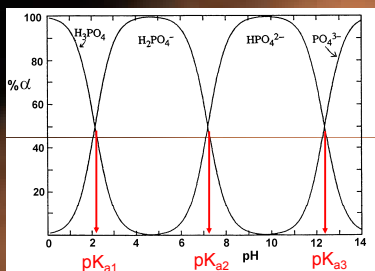
Caso do ácido fosfórico H_3PO_4

$$C_{\text{Fosf.}} = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}]$$



$$[H^+] = [H_2PO_4^-] + 2 \times [HPO_4^{2-}] + 3 \times [PO_4^{3-}] + [OH^-]$$

Especiação do ácido fosfórico (H_3PO_4) em função do pH



$$C_A = [PO_4^{3-}] \{ 1 + \beta_1^H \times [H^+] + \beta_2^H \times [H^+]^2 + \beta_3^H \times [H^+]^3 \}$$

Briefly, the term **speciation analysis** means **all analytical activities for identifying and/or measuring** the quantities of one or more individual chemical species in a sample (aqueous solution, serum, urine, etc.).

This may involve a kind of detective work.

Techniques used:
any that might give information.

In systems containing metal ions this is important because it is well known that different forms of a metal possess different **activity** and/or **toxicity** hazards.

Examples of techniques:

Potentiometric techniques

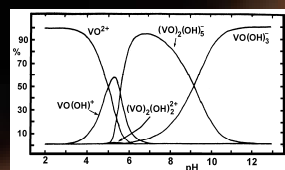
Spectroscopic techniques (UV-Vis, circular dichroism, NMR, EPR, IR, fluorescence)

Separation techniques (chromatography, electrophoresis)

Electrochemical techniques

Mass Spectrometry

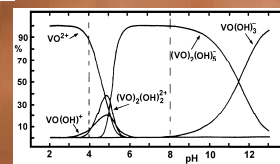
Even for the same simple system the speciation in water depends on the total metal concentration



$C_{VO} = 10^{-5} \text{ M}$

$C_{VO} = 2 \times 10^{-3} \text{ M}$

$C_{VO} / [OH^-]$
monomeric/oligomeric



The speciation of metal complexes in aqueous solution depends on:

- total metal and ligand concentration
- the possibility of formation of oligomeric species
- pH
- the presence of other ligands

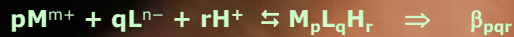
In biological fluids it may be important to know/understand the role of bio-ligands that may be present.

In systems containing metal ions this is important because it is well known that different forms of a metal possess different activity and/or toxicity hazards.

The particular biological activity observed may be due to one of the forms present (in major or minor concentration)

Potentiometric techniques

Objective: identify and determine the stability constants of species formed in solution



Fundamental to have good & reliable data

Adequate computer programs for the calculations

Miniquad: Gauss-Newton least-squares method where the sum of the squared residuals between observed and calculated analytical concentrations is minimized.

Superquad: similar but more flexible program. Errors are permitted in some input data, model choice differs, some systematic errors may be taken into account.

Hyperquad: a development of Superquad which may run in Windows.

Spectroscopic techniques

Spectroscopic techniques (UV-Vis, circular dichroism, NMR, EPR, IR, fluorescence)

Basis of method

It is advisable to have good & reliable data

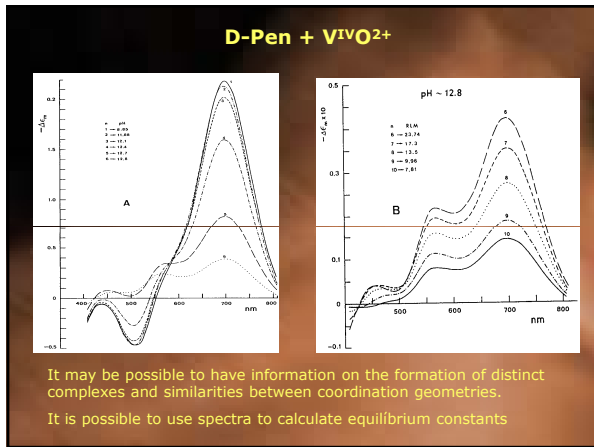
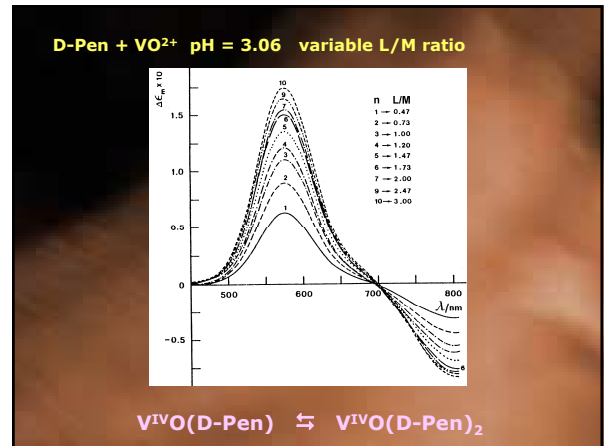
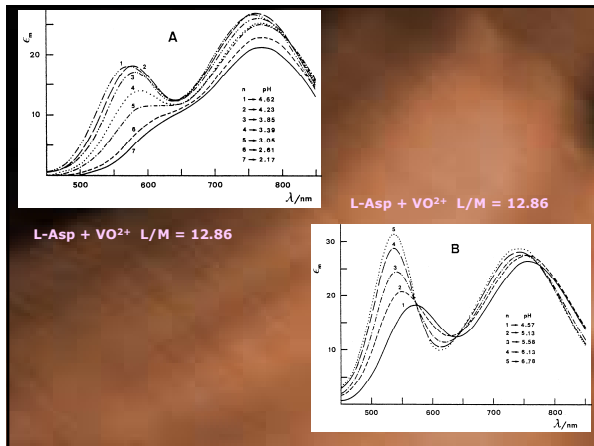
Adequate computer programs for the calculations

Hyperquad & PSEQUAD

PSEQUAD: least-squares method where the sum of the squared residuals between observed and calculated spectroscopic measure is minimized.

$$U = \sum (A_{\text{calculated}} - A_{\text{measured}})^2$$

Attention to the results and their meaning.
Normally numbers may be obtained, but ...

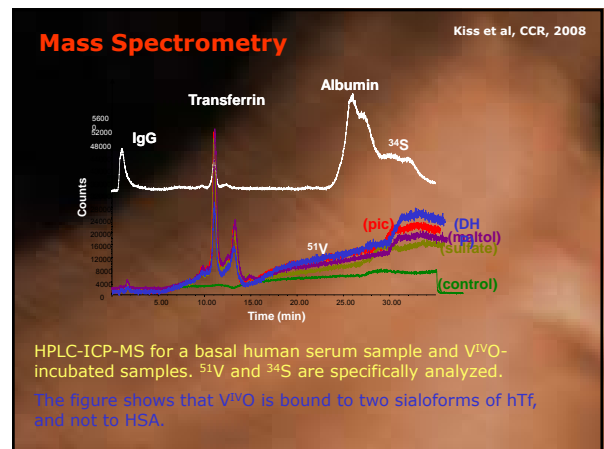
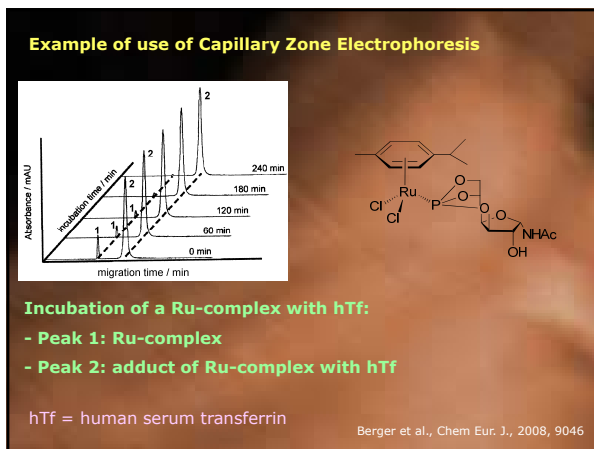


Separation techniques:

- chromatography
- **Electrophoresis**
- ultrafiltration

Chromatographic and electrophoretic techniques may be very useful to identify species present in biological media, but:

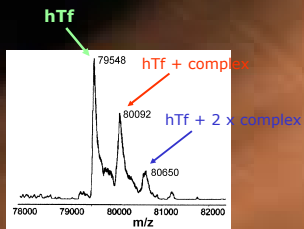
- Stability of species present
- Lack of standards



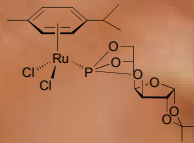
Upon incubation of the Ru-complex up to 8-fold with hTf only the 1:1 and 2:1 complexes were detected (without one Cl⁻).

Berger et al., Chem Eur. J., 2008

MS

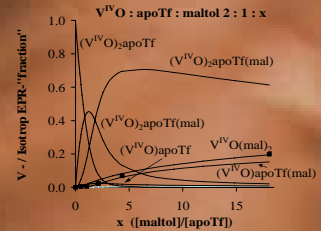
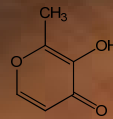


Incubation of the Ru-complex with hTf



It is extremely relevant to understand the particular speciation of a particular complex in a biological system

The particular biological activity observed for a particular compound may be due to one of the forms present (in major or minor concentration).



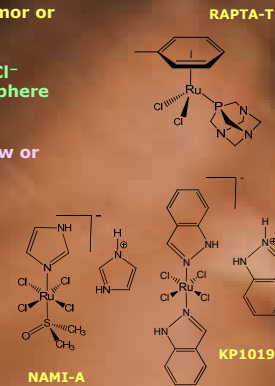
Ruthenium complexes have been extensively studied as anti-tumor or anti-metastatic agents

Often these complexes have Cl⁻ anions in their coordination sphere

In aqueous solution they may undergo hydrolysis with a slow or fast kinetics.

Knowing that biological media may contain differing [Cl⁻]:

- Blood Plasma 104 mM
- Cytoplasm ca. 23 mM
- Cell nucleus ca. 4 mM



The binding of complexes to serum proteins may be important for their therapeutic effect. To know/understand the form of transport in blood and into cells of therapeutic complexes is very relevant.

The evaluation of the biological activity in the presence and absence of serum albumin or serum transferrin has not been much studied, but when done has shown influence of the protein binding.

In vivo KP1019 was found to be bound to HSA and hTf

holo-hTf loaded with KP1019 was found to be 80-fold more effective in inhibiting SW707 human carcinoma cancer cells than KP1019 alone.

Understanding / studying the speciation of metal complexes is a relevant information.

A critical evaluation of implications of speciation of complexes in biological systems is fundamental

Doing speciation studies is partly a philosophy of making science. The effort spent should depend on the relevance of the topic