

"Metals and life" exam**Datum: November 8th, 2016****Tijd: 2-5 pm****Zaal: C1****Docent: Dr. Sylvestre Bonnet**

Voorzie elke blad van naam en nummer collegekaart.

Bij het tentamen is het gebruik van de syllabus of mobiele telefoon niet toegestaan. Voor elke vraag is de waardering aangegeven.

On each page write your name and the number of the college card.

It is not allowed to use the syllabus or a cell phone during the examination. For each question the rating is given.

When a justification is asked it counts at least as many points as the answer itself. The number of points per question is indicative and may be re-evaluated.

Important advice: do not lose time on questions you can't do. First answer the questions you can answer, and then spend time on the more difficult ones.

A table of essential aminoacids and a periodic classification of the elements are available at the end of the exam (page 8).

Gold-based anticancer compounds: auranofin (8 points)

Since the FDA approval of the anti-arthritis compound auranofin, which contains a gold atom, many gold-based coordination compounds have been prepared and their medicinal properties evaluated, in particular in the field of cancer research. Figure 1 shows the structure of 3 of them.

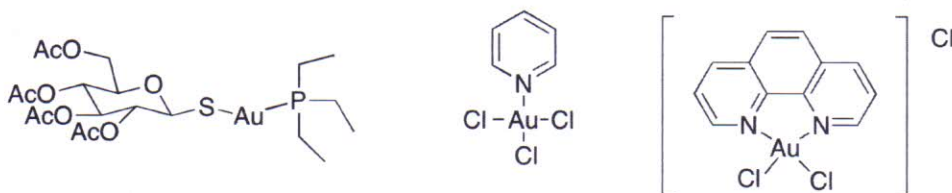


Figure 1. Structures of auranofin (left), trichloridopyridinegold (center), and Auphen (right). AcO=acetyl.

- 1) What is the oxidation state of gold in auranofin, trichloridopyridinegold, and Auphen? Explain. (0.5 point)
- 2) Early literature studies demonstrated that auranofin is stable in neutral aqueous medium in absence of thiols, but unstable in presence of thiols. Explain using qualitative chemical arguments, and compare with cisplatin. (0.5 point)
- 3) In 1987 Bryan et al studied the reaction of decomposition of auranofin in presence of strong acids such as hydrochloric acid, called reaction 1. What happens to thiolates in presence of acid? And to metal compounds in presence of chlorides? Figure 2 (left) shows the dependence of the rate constant of reaction 1 on proton concentration for two acids, HCl and HClO₄. The difference shows that protons and chlorides play a different role in this reaction. Write down equation 1. (0.25 point)
- 4) When [HCl]=A₀=0.1 M and at 25 °C the equilibrium constant of reaction 1 is K=4.0×10⁻⁴ M⁻¹. In such conditions, and under the hypothesis that the advancement of the reaction x (in

mol/L) is small compared to A_0 , what is the percentage of intact auranofin in an HCl solution initially containing $C_0=7.7 \times 10^{-5}$ M of auranofin? (0.5 point)

- 5) Why is the study of the acidic degradation of anticancer compounds relevant in cancer research? Does the chloride concentration also play a role for the fate of metallodrugs in cells? (0.25 point)

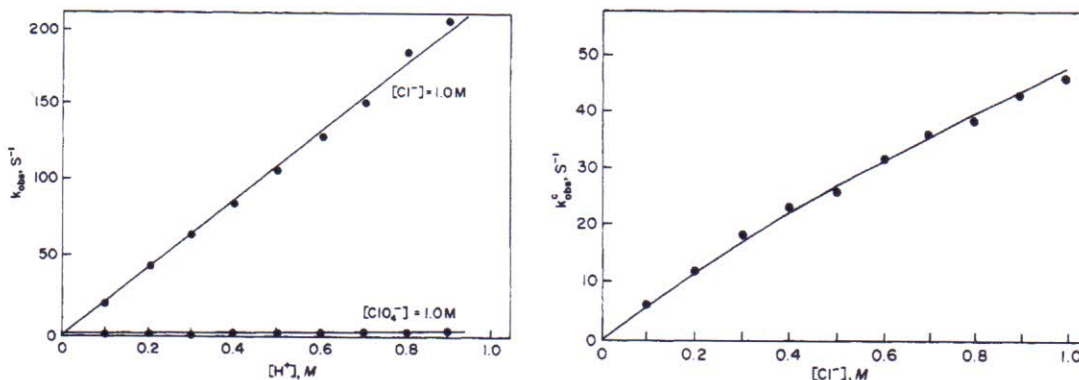


Figure 2. Evolution of the rate constant of the acid degradation of auranofin (reaction 1, see text), left: as a function of proton concentration in presence of 1.0 M chlorides and perchlorate anions; and right: as a function of chloride concentration at constant pH, keeping the ionic strength at 1.0 M using sodium perchlorate. Conditions: solvent is 50% methanol/water, $T=16.4$ °C.

- 6) In an article from 1985, Snyder et al proposed the first kinetic study of the cell uptake and intracellular distribution of auranofin. This study was based on auranofin analogues radiolabelled within the triethylphosphine (Et_3P)[3H], the gold [^{195}Au], or the tetraacetylthioglucose (TATG)[^{14}C] moieties of the molecule. In one word, the cells (RAW264.7 macrophages) were grown and suspended in 900 μL medium, 100 μL of the solution of radiolabelled auranofin was added and incubated in well-defined conditions of temperature and concentration; the cells were then centrifuged at 4 °C, and separated from the supernatant. The resulting cell pellet was placed in scintillation detectors, and the relative radioactivity was measured. The results for the radioactivity of the cell pellet are shown in Figure 3. Is auranofin taken up as the intact molecule, without its TATG fragment, without its phosphine ligand, or without any ligand, ie, as a naked gold ion? Justify. (0.5 point)

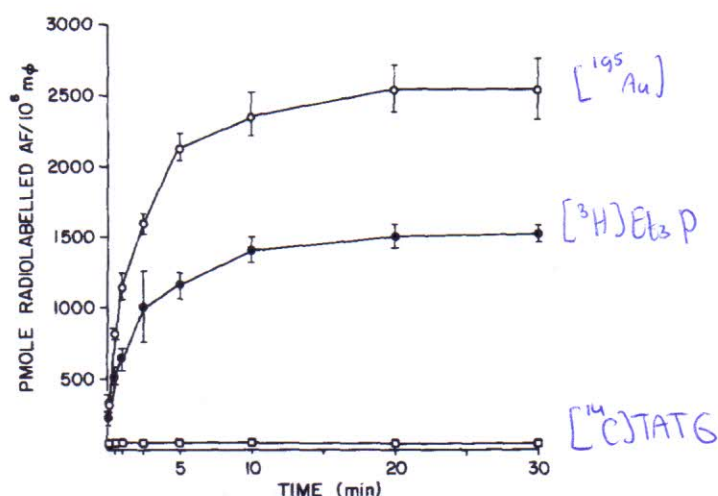


Figure 3. Cell association of auranofin radiolabeled in three sites as a function of time. Datasets: [¹⁹⁵Au] (empty circles), [³H]Et₃P (full circles); and [¹⁴C]TATG (empty squares).

- 7) Human serum albumin (HSA) consists of 585 amino acids and is the most abundant protein in plasma (~40 mg/mL). Its bovine version, BSA, is one of the main proteins in fetal calf serum (FCS), a fluid traditionally added to cell cultures to study the effect of serum proteins *in vitro*. *In vivo* the roles of serum albumin include maintenance of osmotic pressure, transport of endogenous (e.g., fatty acids, bilirubin, steroids), and transport of exogenous (drugs) substances in the blood circulation. There are three homologous domains in serum albumin with at least five binding sites for small molecules including small organic drugs, fatty acids, and metals. One of these sites is Cys34; Cys34 is one of the 35 conserved cysteine residues in serum albumin. Thirty-four of these are involved in 17 disulfide bonds. The free thiol of Cys34 binds a range of drugs and metal ions but is also thought to be involved in disulfide linkages with endogenous thiols such as cysteine, homocysteine, and glutathione. Snyder et al studied the effect of FCS addition on the uptake of auranofin (Figure 4). Interpret the data: what is the probably interaction of albumin with auranofin and which consequence does this interaction have on cellular uptake? (0.5 point)

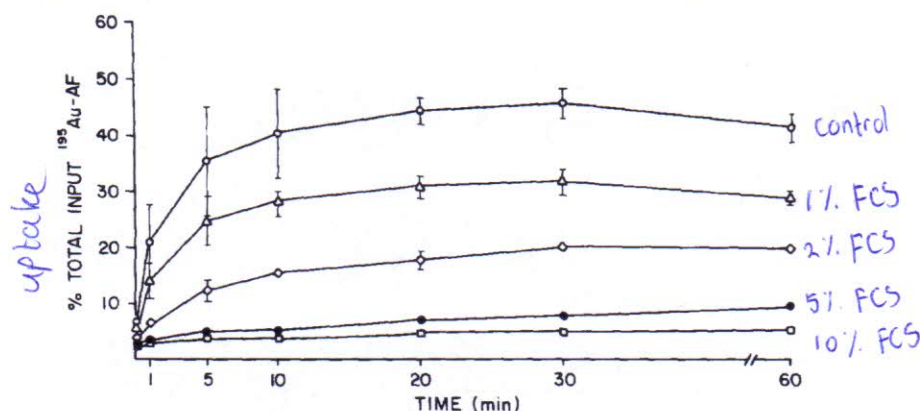


Figure 4. Effects of fetal calf serum on the cell association of 5 μM [¹⁹⁵Au] auranofin. Symbols: control (empty circles); 1% FCS (empty triangles); 2% FCS (empty diamonds); 5% FCS (full circles); 10% FCS (empty squares).

- 8) After incubating cells *in vitro* in presence of auranofin, carefully selected detergents allow for separating different fractions of the cells that contain either the nuclei, the cytosol, or the membranes. Quantification of the drug in each fraction can then be realized, here by radioactivity measurement (of [¹⁹⁵Au], see Figure 5). According to these data would metal-DNA interaction studies make any sense for auranofin? Justify. (0.25 point)

