Mn-TETRATOLYLPORPHYRIN-NANO-Au COMPLEX SENSITIVE TO 4-AMINOSALICYLIC ACID

Anca Lascu[a], Diana Anghel[a], Bogdan Taranu[b], Luminita Salageanu[c]

[a] *Institute of Chemistry Timisoara of Romanian Academy, M. Viteazul Ave. 24, 300223-Timisoara, Romania,* Tel: +40256/491818; Fax: +40256/491824
[b] *National Institute for Research and Development in Electrochemistry and Condensed Matter, 1*
Plautius Andronescu Street, 300569 Timisoara, Romania
[c]*Health Insurance House Timis, Corbului Street 4, 300239-Timisoara, Romania*

Abstract

Porphyrins and metalloporphyrins provide recognition sites for amino acids both through their cationic central metal ion and due to various functional groups at the four *meso*- and the eight *β*-positions of pyrolles. Immobilized metallo-phenylporphyrins can be used as sensitive and selective sensors for different analytes as they provide two ways of interaction via the metallic center of porphyrin and between the π electrons of the macrocycles and the solute [1].

The purpose of this study was to establish an easy way to determine low concentrations of 4-aminosalycilic acid (***PAS***) using hybrid materials obtained from porphyrins, namely: Mn(III)-tetratolylporphyrin chloride ***(MnTTPCl)*** and gold nanoparticles (***n-Au***).

During the adding of 4-aminosalycilic acid to the ***MnTTPCl-nAu*** hybrid it can be observed that the intensity of the plasmonic band is continuously decreasing with the increase in 4-aminosalycilic acid concentration. Another notable feature is the bathochromic shift of the major peak to higher wavelengths from 619 nm to 622 nm. Equilibria processes are involved and accompanied by an isosbestic point around 725 nm. The dependence between the absorption intensity of the plasmonic complex and the concentration of 4-aminosalicylic acid is linear, with an excellent corelation coefficient of 99.31% in a wide range of 4-aminosalicylic acid concentrations: 2.88×10^{-5}M - 8.89×10^{-4}M.

Introduction

Aminosalicylic acid is known to perform bacteriostatic activity against *Mycobacterium tuberculosis*, being involved in suppression of reproduction of bacteria, leading finally to cell death. This compound was also used as drug against tuberculosis (***TB***) but it has debilitating side effects such as anorexia, epigastria distress, nausea so that it is currently used only in the severe cases of multi-drug resistant ***TB*** and only by targeted release [1]. The detection of PAS in urine is necessary in order to monitor and avoid the toxic doses intake of PAS, due to its side effects [2]. The detection of amino acids is affected by the changes of pH [3]. A detector for amino acids at pH 7 should have a receptor that is sensitive to both the deprotonated carboxyl group and protonated amino group.

Spectrophotometric techniques are also applied in detection of ***PAS,*** based on the changes in the color of different dyes such as p-dimethylaminobenzaldehyde (DAB), and p-dimethylaminocinnamaldehyde (DAC) [4] upon interaction with amino acids.

Another type of analyses were capillary zone electrophoresis and flow injection analysis that was used for the determination of both p-aminosalicylic acid (PAS) and its metabolite N-acetyl-p-aminosalicylic acid (N-acetyl-PAS) in urine without any sample pretreatment [5, 6].

Porphyrins and metalloporphyrins might provide recognition sites for amino acids and by two avenues of interaction: via the metallic center of porphyrin [7] and between the π electrons of the macrocycles and the solute [3].

The aim of this work was to establish an easy spectrophotometric way to determine low concentrations of 4-aminosalycilic acid using hybrid materials obtained from porphyrins, namely: Mn(III)-tetratolylporphyrin chloride (structure in Figure 1) and gold nanoparticles.

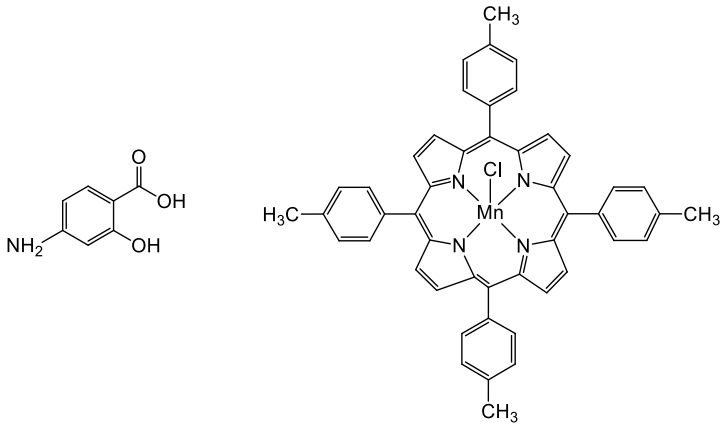

Figure 1: The Structure of ***PAS*** and ***MnTTPCl***

Materials and methods

The solvents and PAS were purchased from Merck (THF, 4-aminosalicylic acid). 5,10,15,20-tetra(4-methyl-phenyl)porphyrinato manganese (III) chloride (MnTTPCl) was prepared from the porphyrin base previously synthetized and characterized [8] using modified Adler's method [9, 10] and the gold colloid was synthesized in an environmentally friendly manner [11].

Apparatus

A JASCO model V-650 spectrometer was used for the UV-vis measurements in 1 cm quartz cuvettes at room temperature. A Titan G2 80-200 TEM/STEM microscope (FEI Company, The Netherlands) was used to record the TEM and STEM images. Samples were prepared by drop-casting the nanomaterials with and without 4-aminosalicylic acid from THF-water mixtures on 200 mesh TEM copper grids coated with continuous carbon film. The images were registered at 200 kV using TEM Imaging & Analysis v. 4.7 software.

Method for obtaining of gold-porphyrin hybrid material

To 3.5 mL gold colloid solution in water ($c=4.58x10^{-4}M$) a solution of ***MnTTPCl*** in THF ($c=1.1x10^{-6}M$) was added and the mixture was stirred for five minutes. This mixture is selected because it produces the widest and most intense plamonic band.

Results and discussions

In order to detect PAS, portions of 4-aminosalicylic acid solution ($c=4.353x10^{-3}M$) in distilled water were gradually added to 3 mL of the gold-porphyrin hybrid material as follows: 20 μL for the first 16 steps, 50 μL for the next 3 steps and 100 μL for the last 3 steps. After each adding of 4-aminosalicylic acid solution the mixture was stirred and the UV-vis spectrum of the sample was recorded.

From the superposed UV-vis spectra (Figure 2) registered during the adding of 4-aminosalycilic acid to the ***MnTTPCl-nAu*** hybrid it can be observed that the intensity of the plasmonic band is steadily decreasing with the increase in 4-aminosalycilic acid concentration.

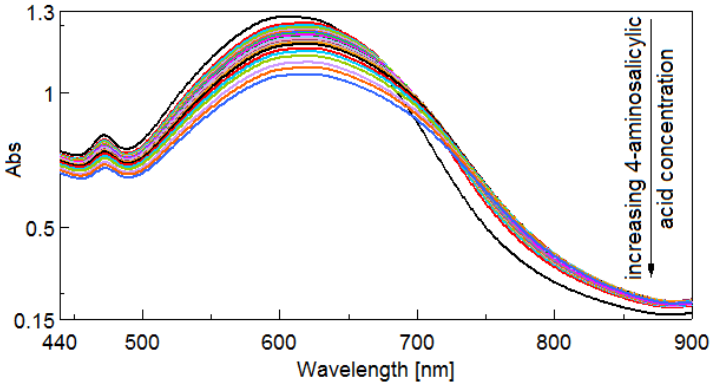

Figure 2: The overlapped UV-vis spectra registered during the adding of ***PAS*** solution to the ***MnTTPCl-nAu*** hybrid solution

Another notable feature is that the shift of the peak is moving to higher wavelengths (from $2.88x10^{-5}$M which is the lowest recorded concentration producing the peak at 619 nm to the $8.89x10^{-4}$M which is the highest PAS concentration that is located at 622 nm). An isosbestic point around 725 nm is the proof for the equilibrium process that accompanies the PAS recognition and mechanism of detection.

The dependence between the absorption intensity of the ***MnTTPCl-nAu*** hybrid and the concentration of ***PAS*** (Figure 3) is linear in a wide range of ***PAS*** concentrations: $2.88x10^{-5}$M - $8.89x10^{-4}$M characterized by an excellent corelation coefficient of 99.31% .

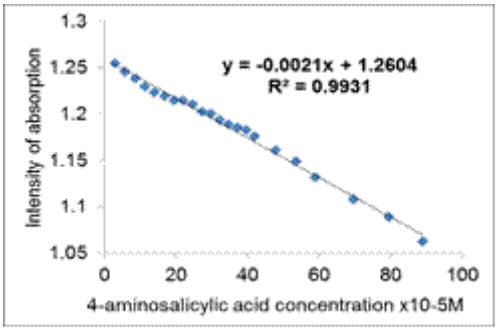

Figure 3: The dependence between the absorption intensity of the ***MnTTPCl-nAu*** hybrid and the concentration of 4-aminosalicylic acid

In order to prove the efficiency of the hybrid material as compared to the bare Mn-porphyrin alone, the experiment of adding a dilute solution of 4-aminosalycilic acid to a solution of ***MnTTPCl*** alone was performed. So, a solution of 2.5 mL ***MnTTPCl*** in THF ($c=1.1x10^{-6}$M) is treated with portions of 20 µL of a solution of 4-aminosalicylic acid in water ($c=4.353x10^{-3}$M). The mixture is stirred and the UV-vis spectra are recorded for each step.

As can be observed in Figure 4, the intensity of the Soret band of the porphyrin is behaving unevenly as the concentration of 4-aminosalycilic acid is continuously increasing. A slow shift of the Soret band intensity toward lower wavelengths is also obvious (λ=476 nm for the lowest 4-aminosalicylic concentration ($3.45x10^{-5}$M) and λ=469 nm for highest 4-aminosalicylic concentration ($4.1x10^{-4}$M)). An increase of intensity of the Va and VI absorption bands that are chararcteristic for manganese porphyrins can be observed (Figure 4) as the concentration in 4-aminosalicylic acid is increasing, but the dependence is not linear. This data proves that the porphyrin alone is not able to provide a good detection for ***PAS.***

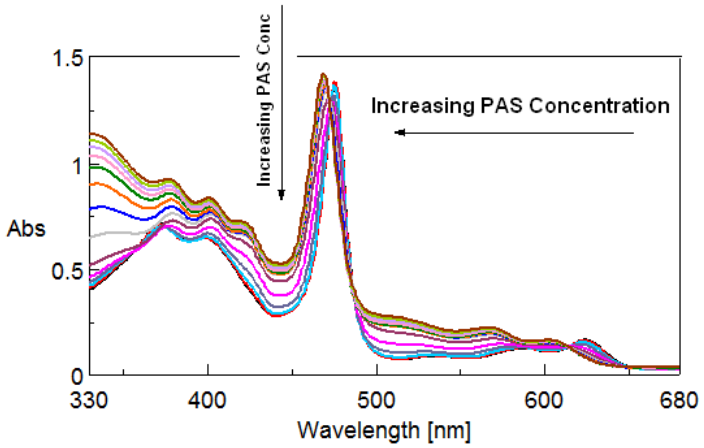

Figure 4: Superposed UV-vis spectra from adding 4-aminosalycilic acid solution to ***MnTTPCl*** solution in THF.

The proposed mechanism by which PAS chelates to Mn-porphyrin is based on the affinity of Mn to oxygen atoms of PAS, as illustrated in Figure 5, together with TEM images of ***MnTTPCl-nAu*** hybrid and of ***MnTTPCl-nAu*** hybrid after PAS detection.

From the chemistry point of view, PAS structure possesses a carboxyl group, along with hydroxyl group, providing an ideal chelating moiety for manganese, that is also capable to form Mn(V)=O bonds. This hypothesis is certified by the appearance in the UV-vis spectrum of the secondary Soret band (VI) located around 420 nm that is typical for porphyrin-Mn(V)=O species and also by the significant blue shift of the Q bands.

TEM images of ***MnTTPCl-nAu*** hybrid treated with PAS show globular assemblies of micelar type having different diameters, a completely different organization than in the ***MnTTPCl-nAu*** hybrid where they are cloudly organized. This type of spherical determined geometry is another proof for the proposed coordination.

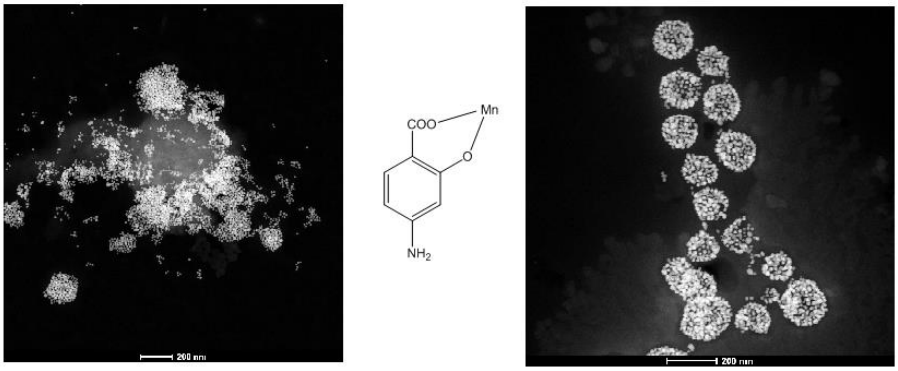

Figure 5: TEM images of ***MnTTPCl-nAu*** hybrid, type of PAS Coordination to Mn (V) and of ***MnTTPCl-nAu*** hybrid after PAS detection.

Although our experimental results imply a chelating action Mn(V) to PAS, the conclusive evidence might come only from *in vivo* urinary analysis of Mn following PAS treatment. Such a study may pave the way for future development of PAS detection in order to improve therapy using ***PAS*** [12].

Conclusions

A comparison between solely ***MnTTPCl*** and its ***MnTTPCl-nAu*** hybrid regarding the ability to recognize and detect PAS was performed.

MnTTPCl-nAu hybrid can detect 4-aminosalicylic acid with great accuracy in the range of concentrations from 2.88×10^{-5}M to 8.89×10^{-4}M, that are relevant for analysis in medical trials of patients treated with PAS, following a linear dependence between the intensity of the bands in UV-vis spectra and the PAS concentration. ***MnTTPCl*** alone cannot be used for detection, its optical behavior being unlinear in the presence of increased PAS concentrations, but is able to recognize PAS molecules.

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