Talanta 186 (2018) 36–43

Contents lists available at ScienceDirect

Talanta

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

Alpha fetoprotein assessment by using a nano optical sensor thin film binuclear Pt-2-aminobenzimidazole-Bipyridine for early diagnosis of liver cancer

M.S. Attia[a,*], A.O. Youssef[a], Ziya A. Khan[b], M.N. Abou-Omar[c]

[a] Chemistry Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt
[b] Chemistry Department, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabia
[c] Department of Chemistry, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt

ARTICLE INFO

Keywords:
Alpha fetoprotein (AFP)
Nano binuclear Pt(abi)(bpy)
Luminescence
Optical sensor
Liver cancer

ABSTRACT

A simple, precise and sensitive method in which, a nano optical sensor binuclear Pt-2-aminobenzimidazole-bipyridine, Pt(abi)(bpy) complex doped in sol gel is used for the early diagnosis of liver cancer. The idea depends on the assessment of the concentration of alpha fetoprotein (AFP) in the serum samples of different liver patients. The nano binuclear Pt(abi)(bpy) has strong emission spectrum upon excitation at 380 nm in distilled water. The assessment of alpha fetoprotein (AFP) depends on the quenching of the emission spectrum of the optical sensor at 610 nm in water by the alpha fetoprotein (AFP). The calibration plot was achieved over the concentration 5.0 – 350 U L^{-1} with a correlation coefficient of 0.997 and a detection limit of 3.0 U L^{-1}. The method was used satisfactorily for the diagnosis of liver cancer in a number of serum samples collected from various patients and health state; healthy ($\leq$ 30 U L^{-1}), Virus B (42.2–69.5 U L^{-1}), Virus C (75.7–98.4 U L^{-1}), Cirrhosis (112–147 U L^{-1}) and HCC (185.2–349.6 U L^{-1}). Furthermore, the assessment of the alpha-fetoprotein by the proposed method increases its sensitivity (92.88%) and specificity (91.41%) for early diagnosis of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the commonly encountered malignant neoplasms in the world. Almost 80% of HCC cases are due to underlying chronic hepatitis B and C virus infection. In patients with chronic liver diseases the early detection of HCC is very important in controlling this disease. Liver cirrhosis is a precancerous condition that in many cases can develop into HCC. Therefore, cirrhotic patients with cirrhosis are usually screened for HCC during their follow-up procedure [1–5] and the tumor markers provide good potential screening tools for the early diagnosis of tumors [6,7]. The primary tumor marker for HCC is a single polypeptide chain glycoprotein namely α -fetoprotein (AFP) [8]. In some cases at the low level of α–fetoprotein the combination between serum AFP levels and ultrasonography sometimes misses HCC [9]. Therefore, improving the sensitivity towards measuring the serum AFP by effective and low cost optical sensor remains an active area of research [9]. Different spectrometric methods have been reported for the determination of the α -fetoprotein (AFP) in serum [10,11]. These methods are limited by their long incubation time (30–60 min) for the sample and reagent blanks. α -fetoprotein (AFP) was also determined by spectrofluorimetric methods [12,13], electrochemical immunosensor [14]. Some of these methods are unselective, require careful experimental conditions, considerably time consuming and not compatible to detect the alpha fetoprotein (AFP) at the early stage of diseases. Luminescent optical sensors transition metal complexes have more advantages over the present ones; (1) the optical sensor has high stability and durability, (2) the sensor can provide constant signal response for 2 years which is a 24-fold better stability compared to the life time warranted for the chromatographic and colorimetric methods [9–14], (3) sensor is stable over all measurements which prevent the source of error in the measurement process and it gives a low standard deviation values. Square-planar platinum(II) complexes have attracted great attention in the past two decades because of their interesting spectroscopic properties and potential applications in optoelectronic devices [15–17], chemosensors [18,19], photocatalysis [20–22] and nonlinear optical materials [23–26]. The square-planar Pt(II) coordination of these complexes reduces the D_{2d} distortion that is likely to result in radiationless decay process [27,28], which enhances the emission of these complexes. In addition, the planar structure of cyclometalated Pt(II) complexes enables their association via π–π or dz^2–dz^2 interactions of metal ions and ligands to alter the nature and energy of HOMO and LUMO of the

* Corresponding author.
E-mail addresses: mohamed_sam@yahoo.com, mohd_mostafa@sci.asu.edu.eg (M.S. Attia).

https://doi.org/10.1016/j.talanta.2018.04.043
Received 20 March 2018; Received in revised form 11 April 2018; Accepted 12 April 2018
Available online 13 April 2018
0039-9140/

complex reflected in their optical and electrochemical properties [29,30]. When Pt − Pt bond is formed due to the overlap of the dz^2–dz^2 metal ions orbitals, the σ* orbital becomes the complex HOMO, explaining red luminescence and possibility to undergo two electron oxidation into Pt(III) complexes [30–33]. The present work is focused on simply doping the low cost luminescent complex binuclear Pt(abi)(bpy) into the sol prior to its gelation for assessment of α-fetoprotein (AFP) concentration. This method overcomes the difficulties caused by the previous methods [9–14] and most likely will be able to control the quality of the final marketed product in both qualitative and quantitative approaches to assure the identity of the diseases.

2. Experimental

2.1. Apparatus

All fluorescence measurements were recorded with a Meslo-PN (222–263000) Thermo Scientific Lumina fluorescence Spectrometer in the range (190–900 nm). The absorption spectra are recorded with Thremo UV–Visible double beam spectrophotometer. All pH measurements are made with a pHs-janway3040 ion analyzer. FTIR measurements (KBr pellets) were carried out on a Unicam-Mattson 1000 FT-IR spectrometer. NMR measurements were performed on a Spectrospin-Bruker 300 MHz spectrometer. Samples were dissolved in $(CD_3)_2SO$ and TMS was used as an internal reference. Elemental analyses were performed on Perkin-Elmer 2400 CHN elemental analyzer. Mass spectrometry measurements of the solid complexes (70 eV, EI) were carried out on a Finnigan MAT SSQ 7000 spectrometer, available at the National center for research, NRC, Dokki, Cairo, Egypt. The TEM image of the nano optical sensor binuclear Pt(abi)(bpy) complex doped in sol-gel matrix was measured (24 ± 0.2 nm) by using the JEOL JEM-1230 available at NRC, Dokki, Cairo, Egypt.

2.2. Materials and reagents

Acetylsalicylic, amethopterin, ascorbic, atropine, caffeine, CEA, PSA, CA-125, hCG, hLH, hTSH, hPRL were purchased from (Sigma-Aldrich). Alpha fetoprotein was purchased from Sigma. Deionized water and pure grade solvents (Aldrich) were used for the preparation of solutions. 2-aminobenzimidazole, bipyridine and $[PtCl_4]^{2-}$ were purchased from sigma. The Pt complex was prepared and characterized according to the previous work by the research group of M. S. Attia at Ain Shams University [34], (Supplementary data). Tetraethoxysilane (TEOS) buffer solution of pH 5.0 was prepared by mixing of 100 mL 0.1 mol L^{-1} potassium hydrogen phthalate with 45.2 mL of 0.1 mol L^{-1} NaOH and completed the volume to 200 mL by distilled water. The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ = 380/610 nm. Stock and working solutions were stored at 0 – 4 °C when not in use. In all experiments, clean and sterilized volumetric flasks (10 mL) were used. Stock solutions of 5 and 300 U L^{-1} alpha fetoprotein were prepared by dissolving the content of the one vial alpha fetoprotein of each one in 1.0 mL of H_2O. Human serum samples were obtained from the New Al-Kasr-EL-Aini teaching Hospital Cairo University and Ain Shams Specialized Hospital, Ain shams University, Cairo, Egypt in accordance with WHO (World Health Organization) approved the protocol for human specimen collection and for the use of this material and related clinical information for research purposes. [All patients are consented and approved the using of their clinical samples in the research work].

A stock solution (1.0×10^{-2} mol L^{-1}) of the prepared optical sensor was prepared by dissolving the required weight of the binuclear Pt(abi)(bpy) in DMSO. The working standard solution (5.0×10^{-4} mol L^{-1}) of the optical sensor was prepared by appropriate dilution of the stock with deionized water. Stock solution (300 U L^{-1}) of alpha fetoprotein was prepared in distilled water. More diluted solutions (5–250 U L^{-1}) of Alpha Fetoprotein were prepared by diluting the stock solution with distilled water. The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ = 380/610 nm. Stock and working solutions were stored at 0 – 4 °C when not in use.

2.3. General procedures

2.3.1. Preparation of nano optical sensor Pt(abi)(bpy) complex doped in the sol-gel

Nano sensors prepared previously [35–37] by sol-gel method suffered from some disadvantages in which the cracking was taken place for the product and the casted optical sensor which is converted into small species. Some modification was introduced to the method in which the sol after reflux was transferred into a deep freezer at zero temperature to expel the trapped gases and to decrease the cracking process of the optical sensor. Even though, the cracking still takes place. To overcome this problem; the preparative method was modified by adding a plasticizer (DEDMS) at room temperature. The method of preparation in details: 10 mL of binuclear Pt(abi)(bpy) (5.0×10^{-4} $mol^{-1}L$) in ethanol. Part of this solution (8 mL) was mixed with TEOS (4 mL), DEDMOS (4 mL) and deionized water (2 mL). Glass vials (diameter 24 mm, height 48 mm) were filled with 9 mL of this solution and were covered with Parafilm. After 2 days, three small holes were pierced in the Parafilm. After 6 days, thin film was prepared from the partly hydrolyzed and condensed solution by spin-coating on small quartz slide (substrate) (width 8.5 mm; height 25 mm) to fit in a cuvette for the measurement of the luminescence intensity. The substrate was cleaned first by putting it in distilled water with a surfactant. In the next step, it cleaned ultrasonically for 30 min in distilled water with surfactant and then ultrasonically for 10 min in acetone, and finally it boiled for 10 min in 2-propanol. Before spin-coating, the substrate was rinsed with 2-propanol and spun dry. Then, the partially hydrolyzed and condensed sol-gel solution was dropped on the substrate with a syringe through a 2 cm filter and spin-coated at 2000 rpm for 30 s, see TEM image [38–49] [Fig. 1 a and b].

2.3.2. Recommended procedure

An appropriate amount (150 μL) of various standard concentrations of the alpha fetoprotein in distilled water was mixed with the optical sensor [binuclear Pt(abi)(bpy)] complex doped in sol-gel matrix in the cell. The luminescence spectra were then recorded at the excitation wavelength. The optical sensor was rinsed with distilled water after each measurement and the calibration plot was constructed by plotting the luminescence intensity at λ_{em} = 610 nm on the y axis against the reciprocal of alpha fetoprotein concentration on the x axis and all data were compared by data obtained by standard method (Supplementary data).

2.3.3. Standard method

AFP in serum was determined using an electrochemiluminescence kit (Roche Company, Switzerland) on an Elecsys 2010 electrochemiluminescence instrument (Roche Company).

2.4. Analytical application

The optical sensor was immediately mixed with an accurate volume (50 μL) of plasma / serum samples of HCC patients (20 persons), cirrhosis patient (20 persons), virus c patients (20 persons), virus B patient (20 persons) and healthy control (10 persons) and pH was adjusted at 5 by using the phosphate buffer [50]. The volume was completed to 3.0 mL with distilled water then the optical sensor [binuclear Pt(abi)(bpy)] complex doped in sol-gel matrix was immersed in each solution in the measuring cell and the emission intensity at 610 nm was measured against the reagent blank. The main characteristics of the patients (male/female) and control groups are given in Supplementary data. The sensitivity and the specificity of the AFP biomarker were determined by: