بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

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تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

MONA MAGHRABY

New Nano optical sensors for the assessment of some biomarkers of cancer diseases in human body

Thesis

By

Ahmed Hossuany Hefny Mohammed

M.Sc. analytical chemistry

2017

In Partial Fulfillment of the Requirements for

The Degree of Ph. of Science

(Chemistry)

Submited

To

Chemistry Department

Faculty of Science

Ain Shams University

(2020)

New Nano optical sensors for the assessment of some biomarkers of cancer diseases in human body

To

Chemistry Department Faculty of Sciences - Ain Shams University

For The Degree of Doctor of Philosophy in Science

(Chemistry)

By

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(M.Sc.,Analytical Chemistry 2017)

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APPROVAL SHEET FOR SUBMISSION

Title of Ph. Sc. Thesis

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ACKNOWLEDGMENT

First and last thanks to Allah who give me the power to go forward in a way illuminated with his merciful guidance.

Words are not enough to describe my deep thanks to ***Prof.Dr. Mohamed Said Attia,*** *Professor of Analytical chemistry, Faculty of Science, Ain Shams University, for suggesting the point of the research and for managing of this work, his guidance and supervision in the course of the work, and for his stimulating criticisms and help in the preparation of the thesis. Who taught me how canI be a student seeks to research and knowledge, was supportive and did not spare something.*

I would like to express my deep thanks and gratitude to ***Prof.Dr. Ahmed Osman Youssef*** *Professor of Analytical Chemistry, Faculty of Science, Ain Shams University for his valuable help and support throughout the course of this work.*

I would like to express my thanks to ***Prof .Dr. Ragaa El-Sheikh Shohaib*** *Professor of Analytical Chemistry,Faculty of Science, Zagazige University, for giving me the chance to be one of her students and for her generous advices,valuable discussions*

I would like to express my thanks to ***Prof. Dr. Maged Abd El-Tawab Ahmed El-Kemary*** *Prof. of Photo and Nanochemistry Faculty of Science Kafrelsheikh University and Head of Kafrelsheikh University , for giving me the chance to be one of his students and for his generous advices,valuable discussions*

Also, I would like to thanks to ***Dr. Mona Naser Mohammed Abo omar*** *for his valuable help and support throughout this work..*

I would like to express my deepest gratitude and sincere thanks to the staff doctor in the New Al-Kasr-EL-Aini Teaching Hospital Cairo University and Ain Shams Specialized Hospital, Ain shams University, Cairo, Egypt which they give me Human samples which using in this research ***(Ahmed Hossuany Hefny Mohammed)***

Published

Paper

Highly selective optical sensor Eu $(TTA)_3$ phen embedded in poly methylmethacrylate for assessment of total prostate specific antigen tumor marker in males serum suffering prostate diseases

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Abstract

A low-cost, simple, and highly selective method was used for the assessment of total prostate specific antigen (tPSA) in the serum of prostate cancer patients. This method is based on quenching the intensity of luminescence displayed by the optical sensor Eu $(TTA)_3$ phen/poly methylmethacrylate (PMMA) thin membrane or film upon adding different concentrations of tPSA. The luminescent optical sensor was synthesized and characterized through absorption, emission, scanning electron microscopy (SEM) and X-ray diffraction (XRD), and is tailored to present red luminescence at 614 nm upon excitation at 395 nm in water. The fabricated sensor fluorescence intensity is quenched in presence of tPSA in aqueous media. The fluorescence resonance energy transfer (FRET) is the main mechanism by which the sensor perform. The sensor was successfully utilized to estimate tPSA in the serum of patients suffering prostate cancer in time and cost effective way. The statistical results of the method were satisfactory with 0.0469 ng mL^{-1} as a detection limit and 0.99 as a correlation coefficient.

Keywords: Optical Sensor; Eu $(TTA)_3$ **;** Prostate Cancer; Prostate-Specific Antigen; Quenching; Luminescence.

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1. Introduction

The PSA protease is manufactured by the prostatic gland cells wether normal or malignant. Its function is digesting the gel formed in seminal fluid after ejaculation (Schroder et al., 2014). In case of prostate cancer, men exhibit elevated levels of total PSA and lower levels of the free form (fPSA). The fPSA/tPSA ratio can have a contribution in deciding if the elevation in the level of PSA is caused by prostate malignancy (Partin et al., 1996). The PSA test is sensitive to prostate cancer but is not specific where false positive results may occur in other diseases as prostate benign hyperplasia, prostatitis, prostate intraepithelial neoplasia, acute urinary retention, and renal failure (Nadler et al., 1995). Owing to the suboptimal performance of tPSA test, its significance as a sole test for diagnosis of prostate cancer is not recommended as it may direct the suspected patient to administer drugs that may affect the quality of life or lead him to perform unnecessary invasive biopsies (Tkac et al., 2019). The role of tPSA test could be used as a stand-alone test to detect possible recurrence of prostate cancer, and monitor disease progression following treatment, irrespective of the treatment modality. Furthermore, tPSA can detect early-stage of prostate cancer that would be missed by digital rectal examination (Tkac et al., 2019; Van Der Kwast et al., 2003). The tPSA normal level lies below 4.0 ng mL^{-1} (Tkac et al., 2019).

Recently, different approaches were tackled to overcome the over diagnosis of tPSA as independent test including biomarker panels such as Prostate Health Index and/ or a combined platform of biomarkers and some clinical manifestations and variables as the 4Kscore also known as 4-kallikrein and Stockholm 3 test (Ferro et al., 2020; Jin et al., 2020). These approaches utilized the tPSA levels as an important parameter. Thus the development of novel analytical methods and fabrication of cheap but yet sensitive sensors for the accurate estimation of tPSA is always in demand.

Several procedures have been described for the determination of tPSA in serum samples, such as electrochemical immunosensor (Ge et al., 2013), immunoassay (Huhtinen et al., 2004), immuno-chromatography (Yuhi et al., 2006), enhanced Raman scattering (Chen et al., 2012), surface plasmon resonance, integrated microfluidic systems (Grubisha et al., 2003), digital rectal examination, and fluorescence microscopy (Kerman et al., 2007). However, these methods have definite disadvantages where the interactions between antigen and antibody are accompanied with high constants of affinity, leading to single-use systems. Although an immunosensor is the most specific and highly sensitive method used in the laboratory (Panini et al., 2008), it is a time-consuming and more expensive technique. Many recently developed methods depend on nanoscale biosensors for cancer detection at its earliest stages (Attia et al., 2019). In the present work, the optical sensor Eu $(TTA)_3$ phen

embedded in polymethylmethacrylate (PMMA) matrix is used for sensitive determination of tPSA as a prostate cancer marker in human serum. We determined tPSA concentration in blood serum by fluorescence quenching of this optical sensor. This is a relatively simple and inexpensive technique providing a quick reproducible analysis and is relatively free from interference with coexisting substances.

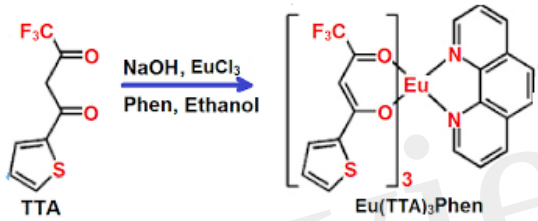

Fig.1. Structure of Eu(TTA)$_3$phen.

2. Experimental

2. 1. Apparatus

A spectrofluorophotometer [Shimadzu RF5301PC] was used for scanning all luminescence spectra within range of (200–800 nm). A spectrophotometer [Shimadzu UV 2450] was used for scanning the absorption spectra in 1.0 cm matched silica cells within the range of (200–800 nm). Morphology investigation was executed using SEM [Sirion, FEI] supported by an EDX detector [S-3400 N II, Japan]. Phases and crystallinity characterization of the Eu(TTA)$_3$phen nanostructure was accomplished via X-ray diffractometer specified by Cu-kα radiation of λ =1.5412 Å, 30 mA and 40 KV [Shimadzu 6000, Japan] the 2θ ranged between 10°- 80° with a scanning rate of 2°/min at room temperature.

2.2. Sample collection and delivery

The samples of patients were supplied in agreement with WHO approved protocol for the collection of human specimens and their use in the field of research by two reputable hospitals in Egypt, Ain Shams Specialized Hospital and the New Al-Kasr-EL-Aini Teaching Hospital. The approval and consent forms of the patients to use their own samples were fulfilled before starting the experiments. The experimental clinical samples included human serum samples of different patients with normal and abnormal PSA concentrations to diagnose prostate carcinoma.

2.3. Materials and reagents

Uric acid, glucose, urea, albumin, KCl, and NaCl were purchased from Sigma-Aldrich. Poly methylmethacrylate (PMMA), polyethylene glycol (PEG), and tetramethoxysilane (TMOS) were purchased from Alfa-Aesar. Total prostate-specific antigen (tPSA) (1mg) was purchased from (Ortho-Clinical Diagnostics). A system of Milli Q- Plus was used for the production of pure distilled water [Millipore Corporation, USA]. Pure solvents of analytical grade were utilized throughout the whole work. [Aldrich, USA]

A quantity equivalent to 53.4 ng mL^{-1} of tPSA stock solution was obtained via dissolution of 1 mg of tPSA in 2 mL deionized water and stored at 4 °C. More diluted solutions (0.1–31.5 ng mL^{-1}) of tPSA were obtained through diluting the previously prepared stock solution using deionized water. The optimum temperature for saving the prepared solutions stable ranged between 0 - 4 °C.

For the fabrication of the luminescent sensor, a specific amount $Eu(TTA)_3$ was accuretly weighed and dissolved in DMSO to prepare a stock solution with a final concentration of (5×10^{-3} mol L^{-1}). The stock solution was further diluted by DMSO to obtain a working solution of concentration (1×10^{-4} mol L^{-1}).

2.4. General procedures

2.4.1. Preparation the optical sensor $Eu(TTA)_3$ phen complex embedded in PMMA matrix

Doping of the optical sensor ($Eu(TTA)_3$ phen) in PMMA was done via adding 1.50 g PMMA to 10 mL $CHCl_3$ with continuous stirring 30 minutes until complete dissolution at 60 °C. Then, 200 µl of $Eu(TTA)_3$ phen was added with continuous stirring for 30 minutes until a homogenous matrix was formed.

The solution was finally casted in a 60 mm x 15mm petri dish petri dish and kept at 25 °C till complete dryness. The thin film thickness was 0.1 mm, and its width and height were 8.5 and 25 mm, respectively.

2.4.2. Recommended procedure

Standard solutions of different concentrations tPSA were accuretly prepared in water. A constant definite sized $Eu(TTA)_3$ Phen/PMMA film was sunk in each solution sequentially in the spectrofluorometer cell carefully to avoid its fracture. The film should be rinsed with water after each measurement.

The luminescence intensity of the solutions was measured in a quartz cell of 1 cm thickness of the spectrofluorometer, at λ_{ex} = 395 nm, and the calibration graph was fitted via plotting the values of $(F_0/F - 1)$ at λ_{em} = 614 nm vs. tPSA concentration.

2.5. Determination of tPSA

2.5.1. Standard method for tPSA

Assay Principle

The principle procedure of the assay was reported previously, for which the PSA test is a two-site immunoenzymatic similar to a sandwich assay Kuriyama, (Kuriyama et al., 1980).

In the one reaction flask, a serum sample is added to two anti-PSA monoclonal, one conjugated with alkaline phosphate and the second one was used as a coat for paramagnetic nanoparticles.

A linkage between serum PSA and its anti monoclone fixed on the solid phase along together with other interactions between specific antigenic sites of PSA with the anti-PSA monoclone conjugate.

Magnetic separation of the solid phase from unbound materials occurred by washing. A chemiluminescent substrate, Lumi Phos – 530, was dripped in the same flask leading to generation of light that could be measured using a simple luminometer. The relationship between PSA concentrations in the sample and light generation was found to be proportional and a multi-point calibration curve was used for calculating the analyte concentrations.

Assay Protocol

All prepared reagents were mixed thoroughly without foaming before use. All measurements were performed in duplicate. Quantities of 25.0 µL of standards, samples, or controls were placed inside each well with 5 minutes incubation period in temperature range between 18 to 25 °C. Then, 100.0 µL PSA was placed in each well separately and merged by stirring the plates (10 sec), before incubation at room temperature for one hour (18–25 °C). A quantity of 100.0 µL of solution of TMB-substrate was added all the wells before incubation 20 minutes within 18–25 °C. A quantity of 100 µL/well stop solution was added (in the same order as for the substrate solution). Absorbance (OD) was obtained at 450 nm (at 630 nm for the blank).

2.6. Proposed method for tPSA

For measuring the tPSA concentrations in samples of serum, the film of the optical sensor is inserted in the quartz cell of the fluorimeter then a volume of 200 µL of each is added and diluted with 1.5 mL distilled water. The intensity of emission for the sensor was recorded at 614 nm before and after serum was added.

3. Discussion and Results

3.1. The absorption and emission spectra

The thin film of Eu $(TTA)_3$ phen /PMMA matrix in distilled water exhibits two absorption bands at 280 and 395 nm owing to π-π* transitions of the organic moieties; 1, 10-phenanthroline and 2-thenoyltrifluoroacetone (Rajamouli et al., 2017).

While upon excitation at 395 nm, the optical film exhibits six emission bands at 580, 590, 614, 650, 696, and 705 nm. These emission bands are due to excited Eu^{3+} and are characteristic to the transitions from $^5D_0$ to $^7F_0$, $^7F_1$, $^7F_2$, $^7F_3$, and $^7F_4$, respectively. The transfer of energy from the antenna to Eu^{3+} results in the red emission of Eu complex in which TTA acted as β-diketone ligand with a high absorption coefficient bound to the metal ion, while the phen ligand has an agonist shielding effect, minimizes the radiationless rate of decay and significantly enhances the complex luminescence intensity (Lunstroot et al,. 2010). The sensitivity of the optical sensor towards the tPSA depends on the ratio between the two emission bands at 590 nm (magnetic-dipole), where the $^5D_0 \rightarrow {}^7F_1$ transition is not affected by the coordinate environment, and 614 nm (electric dipole), in which the transition

$^5D_0 \rightarrow {}^7F_2$ is the most intense peak, suggesting that Eu (III) occupies an inversion centered site. Any variation in the surrounding chemical environment of the sensor affects the electric dipole band (Hamed et al., 2009) of the Eu (III).

The excitation of the Eu-complex embedded in PMMA was obtained via the population of the ligands to their singlet states followed by consequent decay through the intersystem crossing (ISC) to the triplet state. The triplet state ultimately decays via a Dexter-type transfer if an antenna is found in the bonding distance field to lanthanide ion within 10 Å or less (Heine et al., 2013). The overlap between energy levels of antenna triplet state and the Eu(III) resonance level is an efficient triplet >5000 cm^{-1} (Latva et al., 1997), while a most favorable difference in energy between antenna triplet state and Eu(III) resonance level promote the activation of ligand-to-metal ET route (Rajamouli et al., 2017).

In the light of the above illustration, it may be concluded that PMMA exhibits transparent, flexible, and excellent optical properties. Hence, it could be a good host to the Eu-complex to enhance luminescence intensity of the complex due to increasing disorder in the local environment by the surrounding polymer that decreases the symmetry environment around Eu ions. However, the binding of the Eu-complex to polymer via branched groups, such as C=O and C-O, also leads to enhanced fluorescence intensity of the Eu-complex. (Shuhui et al., 2006)

3.2. Surface characterization of Eu complex embedded in PMMA

The XRD pattern for Eu $(TTA)_3$ phen/PMMA film showed sharp diffraction peaks at 2Ɵ = 15°, 20.6°, 21.9°, 26.6°, and 30.6° show the formation of the crystalline structure of Eu$(TTA)_3$phen. The maximum sharp peak intensity at 45° associated with the polymer indicates that crystallinity has its maximum degree owing to high degree of atom ordering in the polymer blends. The results suggest the fixed crystal structure and chemical bonds were formed between the Eu-complex and the polymer matrix (Manjusha et al., 2018).

While the SEM image of the as-synthesized thin film assured that the microcrystals of Eu $(TTA)_3$ phen in the PMMA polymer matrix are homogenously mixed with a spherical shape, a more disordered environment, and less uniformity around Eu (III) ions of the surrounding polymer.

Demonstration of elemental analysis of Eu $(TTA)_3$ phen/PMMA thin film, which contains Eu, F, O and C elements, due to TTA and phen, confirmed that polymer blend and Eu- complex were synthesized successfully (Dandekar et al, 2015).

3. 3. Analytical parameters

The emission spectra of Eu $(TTA)_3$ phen/PMMA thin film in organic solvents of different polarity was examined. In aqueous solution, the sensor displays high red emission intensity despite the low concentration of complexes in the matrix. The enhanced emission of Eu ions is attributed to increasing the efficiency of the ions upon excitation due to increasing intensity of the ligand π-π* transitions, which leads to the photoluminescence of Eu resulting from radiative transition from $^5D_0$ and $^5D_1$ levels (Watson et al.,1975). An additional reason for increasing emission intensity in water is possibly the stabilization of electrons in the $^5D_0$