Int. J. Electrochem. Sci., 17 **(2022)** Article Number: 220564, doi: 10.20964/2022.05.58

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Sensor for Detection of Mifepristone and Cinnamic acid for Treatment of Uterine Fibroids using Immobilized Double-Stranded DNA on the AuNPs modified glassy carbon electrode

Wei Song

College of Life and Health, Nanjing Polytechnic Institute, Nanjing, 210048, China
E-mail: weisong@njpi.edu.cn

Received: 3 February 2022 / *Accepted:* 18 March 2022 / *Published:* 5 April 2022

This research was done to create immobilized dsDNA on Au/GCE for an electrochemical sensor of mifepristone and cinnamic acid (a component of guizhi fuling) as uterine fibroids medications. Deposition of Au nanoparticles (Au NPs) on GCE and immobilization of dsDNA on Au/GCE were accomplished using the electrodeposition approach. SEM and XRD structural investigations of electrodeposited Au NPs revealed that they were electrodeposited in a spherical shape with a fcc crystal structure. The CV and DPV electrochemical measurements indicated that Au NPs grew successfully on the GCE surface and that dsDNA was immobilized on Au/GCE in a stable manner. Further electrochemical investigations using DPV and ampermetery revealed that the simultaneous detection of mifepristone and cinnamic acid on dsDNA on Au/GCE was stable, selective, and sensitive. The linear range, detection limit, and sensitivity for determining mifepristone were 7.5 ng/ml and 0.40031 μA/mg ml^{-1} and 0-120 mg/ml, respectively, and for determining cinnamic acid, the linear range, detection limit, and sensitivity were 2.1 ng/ml and 1.42117 μA/mg ml^{-1} and 0-41.25 mg/ml, respectively. The practical capability and accuracy of dsDNA/Au/GCE were used for amperomtric determination of mifepristone and cinnamic acid in Mifeprex and Gui Zhi Fu Ling tablets, which indicated a high level of precision for practical ability in medicine sample analysis utilizing dsDNA/Au/GCE.

Keywords: Electrochemical sensor; Uterine fibroids; Mifepristone; Guizhi Fuling; Cinnamic acid; Au nanoparticles; dsDNA; Amperomtry

1. INTRODUCTION

Uterine fibroids, also known as leiomyomas, are uterine fibroids that are made up of connective tissue and muscle from the uterus's wall. Fibroids can grow as a cluster or as a single nodule [1, 2].

These tumors can form on the inside of the uterus's wall, inside of the major cavity, and even on the outside [3, 4]. The size, number, and location of fibroids within and around the uterus can all be different [5, 6]. They can be linked to a slender stem in some situations, giving them a mushroom-like look. Women with uterine fibroids that are rapidly growing or fibroids that are growing throughout menopause should be checked right away [7-9].

Most fibroids don't create any problems and don't require medical attention beyond frequent monitoring by your doctor [10-12]. Small fibroids are the most common type. Larger fibroids can cause bleeding between periods, chronic vaginal discharge, low back discomfort, painful bleeding during menstruation, constipation, infertility, and frequent urination, among other symptoms [13-15]. As a result, it is critical to detect uterine fibroids and identify medications for the treatment of larger fibroids [16]. Lupron, tranexamic acid, ulipristal acetate, and mifepristone are the most commonly used drugs to treat uterine fibroids [17-19].

Recent clinical studies have shown that 3 months of mifepristone (11ß-[p-(Dimethylamino) phenyl]-17ß-hydroxy-17ß-(1-propynyl) estra-4,9-dien-3-one) treatment can significantly reduce the mass of uterine fibroids, resulting in complete amenorrhea and a reduction in clinical symptoms. Clinical studies in recent years have shown that 3 months of mifepristone (11ß-[p-(Dimethylamino) phenyl]-17ß-hydroxy-17ß-(1-propynyl) estra-4,9-dien-3-one) treatment can significantly reduce the mass of uterine fibroids to achieve complete amenorrhea and reduce clinical symptoms. Moreover, Guizhi Fuling, as a Chinese herbal medicine, is widely used for uterine fibroids in China [20, 21]. This Chinese herbal formula is composed of Cinnamomum cassia, Poria, Paeonia suffruticosa Andrews, Paeonia lactiflora Pall and Persicae Semen [22, 23]. The major compound of Cinnamomum cassia is cinnamic acid (C-H-CH=CHCOOH) which is a well-known antioxidant and is supposed to have several health benefits due to its strong free radical scavenging properties [24-26]. Cinnamic acid has antimicrobial activity [27, 28]. However, it may be harmful if inhaled and cause respiratory tract irritation or be harmful if swallowed [29]. Thus, the determination of the cinnamic acid concentration in herbal medicine is necessary.

Combined mifepristone and Guizhi Fuling Formula may be an effective approach for reducing fibroid volume and uterine size when compared to mifepristone alone [20, 30]. Moreover, the dosage and timing are the important issues for effective treatment [31, 32]. Accordingly, many studies have been conducted to determine the content of the chemical drugs and essential elements in herbal medicines using polarography [33], UV-Visible spectrophotometry [34], high-performance liquid chromatography [35], mass spectrometry [36], X-ray fluorescence spectrometry [37], atomic spectrometry [38], chemometry [39], and electrochemical methods [40-42]. Among them, electrochemical methods are rapid, simple and low-cost analyses and capability the modification of the electrode surface using composites and nanomaterial make then attractive sensing techniques because of their improved sensitivity and selectivity. Therefore, this study was conducted to synthesize immobilized dsDNA on Au/GCE for an electrochemical sensor of mifepristone and cinnamic acid as medicines for the treatment of uterine fibroids.

2. EXPERIMENTAL

2.1 Modified electrode preparation

Before the electrodeposition process, the GCE surface was polished using alumina slurry (99.99%, 1.0 and 0.3 μm, Sigma-Aldrich) on a polishing cloth for 20 minutes. Then, the electrode was ultrasonically washed in a mixture of ethanol (99%, Guangxi Kunya Biotechnology Co., Ltd., China) and deionized water in an equal volume ratio for 15 minutes, and dried under nitrogen flow. Electrodeposition was performed using a potentiostat (Autolab PGSTAT 302N, Metrohm, Autolab B.V., Utrecht, The Netherlands) using the cyclic voltammetry (CV) technique at a potential range from −1.4 to 1.4 V at scan rate of 10 mV/s for 10 cycles in electrochemical cell contained the equal volume ratio of 3M $HAuCl_4 \cdot 3H_2O$ (≥99.9%, Sigma-Aldrich) and 0.1M KCl (≥99%, Sigma-Aldrich) as electrodeposition electrolyte [43]. The GCE, Ag/AgCl (3M KCl) and Pt wire were used as working, reference and counter electrodes, respectively. After that, the Au/GCE was immersed for 35 minutes in the 0.1M native calf thymus double stranded DNA (dsDNA, Sigma-Aldrich) solution, which was prepared by dissolving the dsDNA in 0.1M phosphate buffer solution (PBS) pH 7. In order to immobilize of dsDNA, the immersion of Au/GCE was continued at a potential of 1.5 V for 15 minutes.

2.2. Real samples preparation

The Mifeprex tablets were purchased from a local community pharmacy; each tablet contained 200 mg of mifepristone. For preparation of the real sample solution with a concentration of 10 mg/ml mifepristone, 10 tablets (2 g mifepristone) were powdered and were dissolved in 200 ml of 0.1 M PBS pH 7. Then, the amperometric measurements were conducted at a potential of 0.42 V under successive addition of mifepristone (99%, Shanghai Hualian Pharmaceutical Co. Ltd., China) to determine the initial mifepristone content in a prepared real sample of Mifeprex tablets. Gui Zhi Fu Ling tablets (Shop Guang Ci Tang® Chinese medicine products) also were prepared, and 20 tablets were ultrasonically dissolved in 20 ml of 0.1 M PBS pH 7. The mixture was filtered and centrifuged at 1000rpm for 5 minutes. Next, the attained supernatant was filtered and used as a real sample for the determination of Guizhi Fuling in Gui Zhi Fu Ling tablets using amperometric measurement at a potential of 0.34 V in 0.1 M PBS pH 7 under successive addition of cinnamic acid (≥99%, Sigma-Aldrich). The standard addition method was used to study accuracy through the relative standard deviation (RSD) and recovery values.

2.3 Analyses

CV, DPV and amperometry measurements were conducted on the Autolab with potentiostat in 0.1M PBS pH 7 containing 50 mM $Co(bpy)_3^{3+}$ that the 0.1 M PBS pH 7 was prepared of Na_2HPO_4 (≥99.0%, Merck, Germany) and NaH_2PO_4 (99%, Merck, Germany), and 50 mM $Co(bpy)_3^{3+}$ was prepared from mixture of Tris(2,2'-bipyridyl)-Cobalt(III) perchlorate $[Co(bpy)_3(ClO_4)_3]$ (Sigma-Aldrich) in ligand 2,2'-bipyridyl (≥99%, Sigma-Aldrich) in methanol (99.8%, Merck, Germany) in equal volume ratio [44]. Furthermore, the enzyme-linked immunosorbent assay kit (ELISA, Air Plants

Bio., Tokyo, Japan) was used for analyses of mifepristone in human blood serum. Scanning electron microscopy (SEM, JSM-6700F, Japan) and X-ray diffraction (XRD; Bruker D8 Advance, Billerica, MA, USA) with CuK radiation (λ= 1.5418 Å) were used to analyze the structural properties of the Au nanostructured electrode.

3. RESULTS AND DISCUSSION

Figure 1a shows the SEM image of Au NPs on the GCE surface. As observed, the Au NPs were electrodeposited in fairly uniform coverage and spherical-shape with an average size of 50nm. Figure 1b shows the XRD pattern of powder of the electrodeposited Au NPs. As seen, there are diffraction peaks at 38.88°, 44.61°, 64.85° and 78.02° which are assigned to the (111), (200), (220) and (311) planes, respectively (JCPDS card No. 04-0784). These are reflections of the face-centered cubic (fcc) structure of the Au NPs.

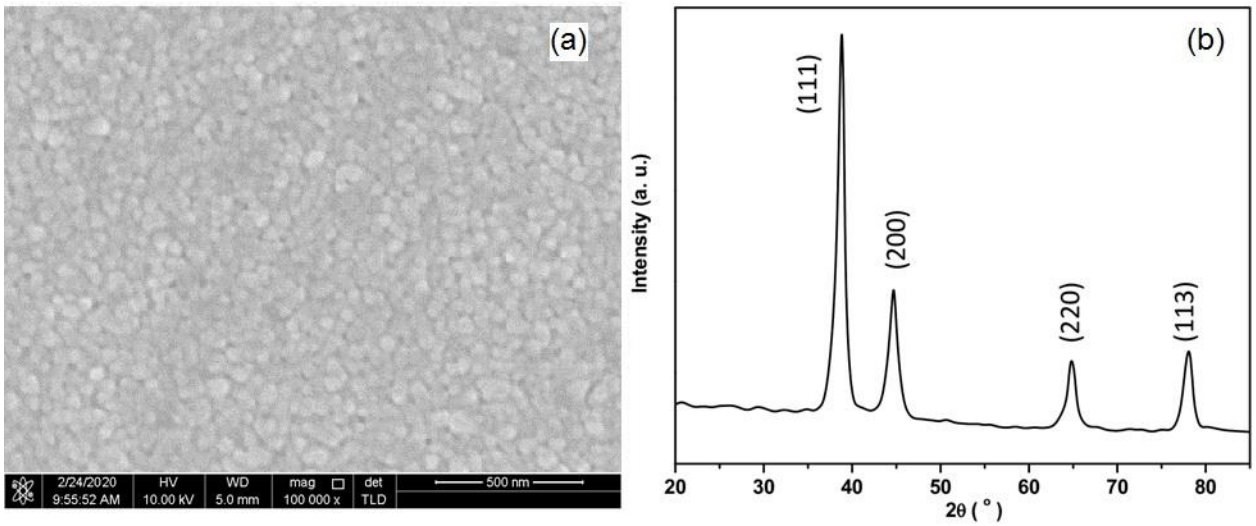

Figure 1. (a) SEM image of Au NPs on GCE and (b) XRD pattern of powder of electrodeposited Au NPs.

Figure 2 presents the obtained CV curves during the electrodeposition of Au NPs on the GCE surface at a potential range from −1.4 to 1.4 V at a scan rate of 10 mV/s for 10 cycles in 3M $HAuCl_4 \bullet 3H_2O$ containing 0.1M KCl. As seen from the first recorded CV, there is a cathodic peak at potential of +0.33 V which attributed the reduction of Au^{3+} into atomic Au^0 and the nucleation of Au NPs on the surface of GCE [45, 46]. It is observed from the second CV curve that the reduction peak is shifted to positive potential value (+0.28V), corresponding to the growth of Au NPs. In the reverse scan, there are a strong anodic peak at the potential of + 0.93 V that it is associated with the oxidation of Au NPs. The recorded continuous CV shows that the anodic and cathodic peak currents increase with increasing the number of cycles during the electrodeposition process, indicating the successful growth of Au NPs on the GCE surface [47, 48].

The CV experiments of GCE, Au/GCE, and dsDNA/Au/GCE were carried out at a potential range of 0.2 to 0.4 V at a scan rate of 50 mV/s in 0.1M PBS pH 7, containing 50 mM $Co(bpy)_3^{3+}$ as a probe to recognize surface-bonded DNA [49, 50].

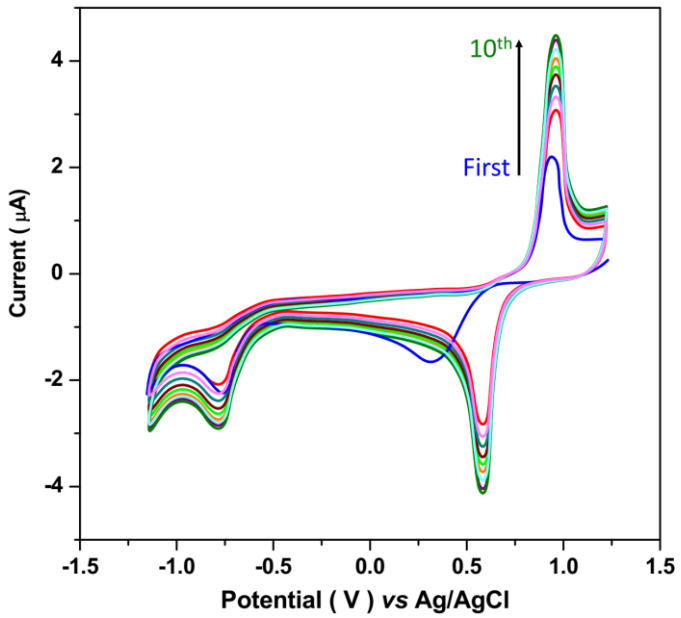

Figure 2. CV curves during the electrodeposition of Au NPs on GCE surface at potential range from −1.4 to 1.4 V at scan rate of 10 mV/s for 10 cycles in 3M $HAuCl_4 \cdot 3H_2O$ containing 0.1M KCl.

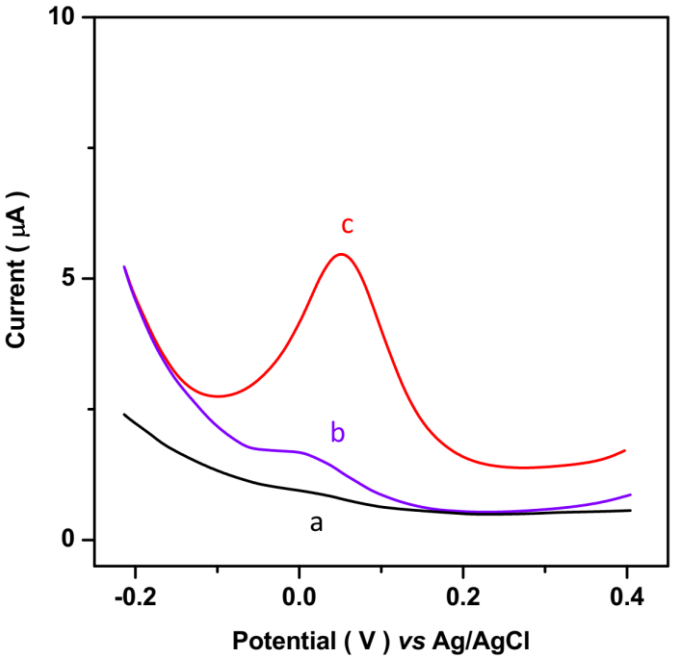

Figure 3. CV curves of (a) GCE, (b) Au/GCE and (c) dsDNA/Au/GCE at potential range from −0.2 to 0.4 V at scan rate of 50 mV/s in 0.1M PBS pH 7 containing 50 mM $Co(bpy)_3^{3+}$.

It is observed from Figure 4, the CV curve of bare GCE does not show any peak, and the CV curve of Au/GCE shows a negligible peak at 0.07 V. However, the CV curve of dsDNA /Au/GCE shows the well-defined anodic peak at 0.09 V, corresponding strong interact of $Co(bpy)_3^{3+}$ with immobilized DNA on Au/GCE and highly sensitive response of the dsDNA /Au/GCE to trace Co^{2+} ion [49, 51-53]. Therefore, it is evidence to the strong immobilization of DNA on Au/GCE.

Figure 4 depicts the DPV curves of GCE, Au/GCE and dsDNA/Au/GCE at 50mV/s scan rate in 0.1M PBS pH 7 which contains 5mg/ml mifepristone. As seen from Figure 4a and 4b, there is a very weak anodic peak at 0.44 V for Au/GCE, and no remarkable anodic peak is observed for bare GCE or the existence of mifepristone [54]. However, Figure 4c displays the significant anodic peaks at 0.42 V due to accumulation of mifepristone onto the surface via interaction with immobilized DNA on Au/GCE which increases the oxide peak [55-57]. The different values of the peak current and potential for Au/GCE and dsDNA/Au/GCE are attributed to the immobilization of the dsDNA on the electrode surface, which interacts with mifepristone and increases the signal of electrochemical reaction [58-60]. Additionally, the electrodeposited high porous layer of Au nanoparticles with high electron conductivity and large specific surface area provide biocompatible DNA immobilizing platform which acted as the electron transfer mediator and catalyze the oxidation reaction of mifepristone, and improve fast electron transfer [61-63].

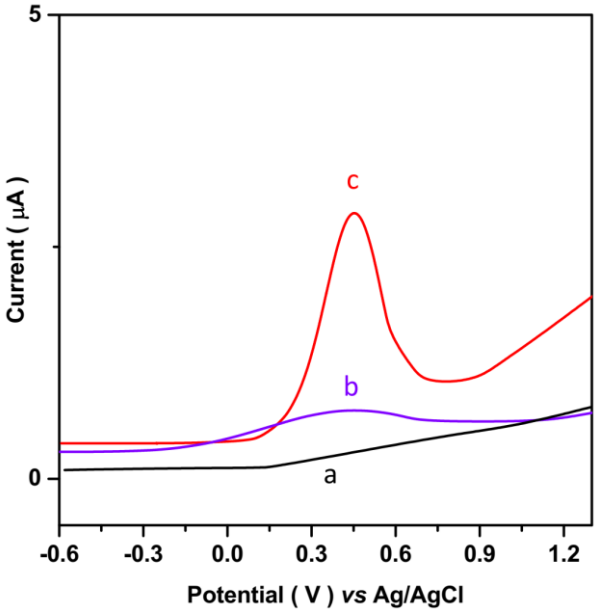

Figure 4. DPV curves of GCE, Au/GCE and dsDNA/Au/GCE at 50mV/s scan rate in 0.1M PBS pH 7 inclosing 5mg/ml mifepristone.

Figure 5 exhibits the electrochemical response of GCE, Au/GCE and dsDNA/Au/GCE at 50mV/s scan rate in 0.1M PBS pH 7 containing 5 mg/ml cinnamic acid. As observed from the DPV curves in Figures 5a and 5b, there is no peak for GCE, and Au/GCE shows a negligible peak at 0.35 V, and the obvious oxidation peak appears at 0.34 V for cinnamic acid on dsDNA/Au/GCE, indicting the

lesser potential and upper oxidation peak current than that on Au/GCE. Comparison between the DPV curves in Figures 4c and 5c reveals that there is a difference between the anodic peak potentials of mifepristone (0.42 V) and cinnamic acid (0.34 V) on dsDNA/Au/GCE, illustrating the ability of the electrochemical sensor to simultaneous determine mifepristone and cinnamic acid.

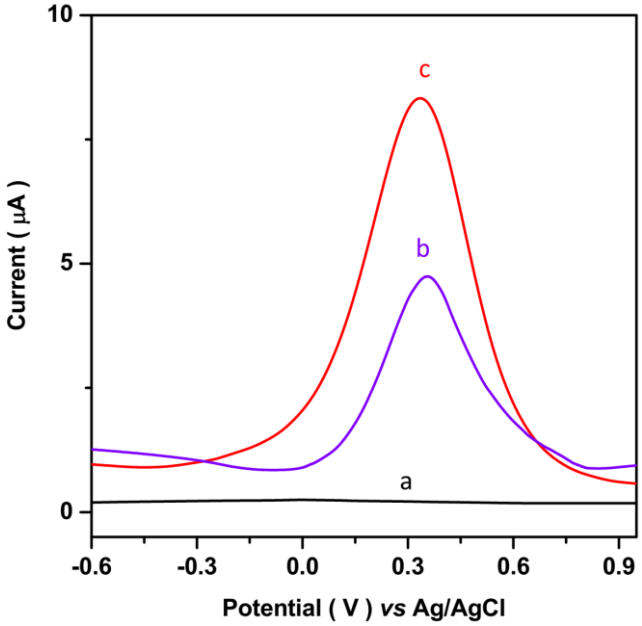

Figure 5. DPV curves of GCE, Au/GCE and dsDNA/Au/GCE at 50mV/s scan rate in 0.1M PBS pH 7 inclosing 5mg/ml cinnamic acid.

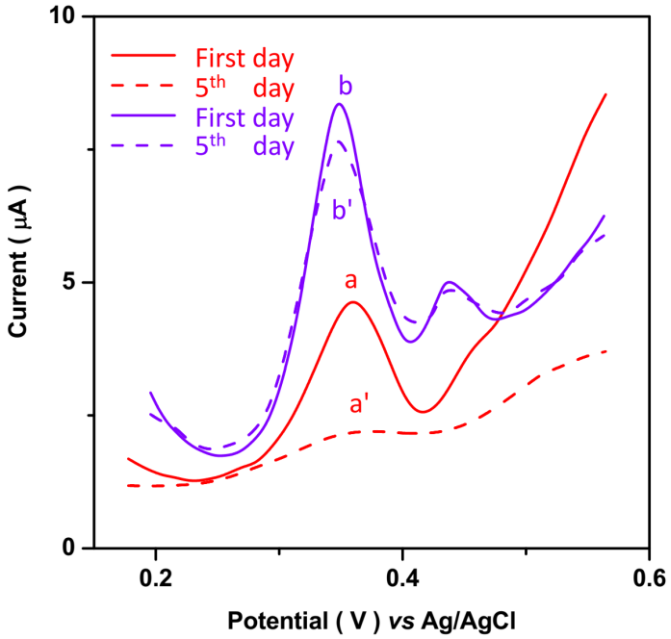

Figure 6. DPV response of the (a and a') Au/GCE and (b and b') dsDNA/Au/GCE at 50mV/s scan rate in 0.1M PBS pH 7 inclosing 5mg/ml cinnamic acid and 5mg/ml mifepristone during storage for five successive days (The first day (solid line) and 5th day (dash line)).

The stability of responses of Au/GCE and dsDNA/Au/GCE was investigated by recording DPV response of the electrodes at scan rate of 50mV/s in 0.1M PBS pH 7 containing 5mg/ml cinnamic acid and 5 mg/ml mifepristone during storage for five successive days. Figures 6a and 6a' depict that the weak responses of Au/GCE toward mifepristone and cinnamic acid are completely diminished after five days. Figures 6b and 6b' show the responses of dsDNA/Au/GCE toward both analytes are decreased ~ 8% after five days, indicating the great stability responses of dsDNA/Au/GCE due to synergetic effect of immobilized DNA on the Au nanoparticles [64-66]. These results demonstrate that dsDNA-modified electrodes could provide enhanced electrochemical responses to cinnamic acid and mifepristone. The Au nanostructured electrode also improves electric conductivity and has a large effective surface area and can influence redox reactions [62, 67, 68]. Therefore, the dsDNA/Au/GCE was used for the following electrochemical studies [69, 70].

Further studies were conducted on the amperometry technique to study the sensing properties of dsDNA/Au/GCE. Figures 7a and 7b show the amperometry measurements and obtained calibration plots of dsDNA/Au/GCE to successive additions of mifepristone and cinnamic acid in 0.1 M PBS pH 7.0 at potential of 0.42 V and 0.34 V, respectively. From Figure 7a, it can be seen that the sensor shows a fast response to the addition of mifepristone at potential of 0.42 V, and the electrocatalytic current is linearly increased with increasing mifepristone content in the electrochemical cell. The linear range, detection limit and sensitivity to determine mifepristone are evaluated at 7.5ng/ml and 0.40031 μA/mg ml^{-1} and 0-120 mg/ml, respectively. Figure 7b also shows the same observation for successive additions of cinnamic acid at potential of 0.34 V and its calibration plot indicated that the linear range, detection limit and sensitivity to determine cinnamic acid are 2.1 ng/ml and 1.42117 μA/mg ml^{-1} and 0-41.25 mg/ml, respectively.

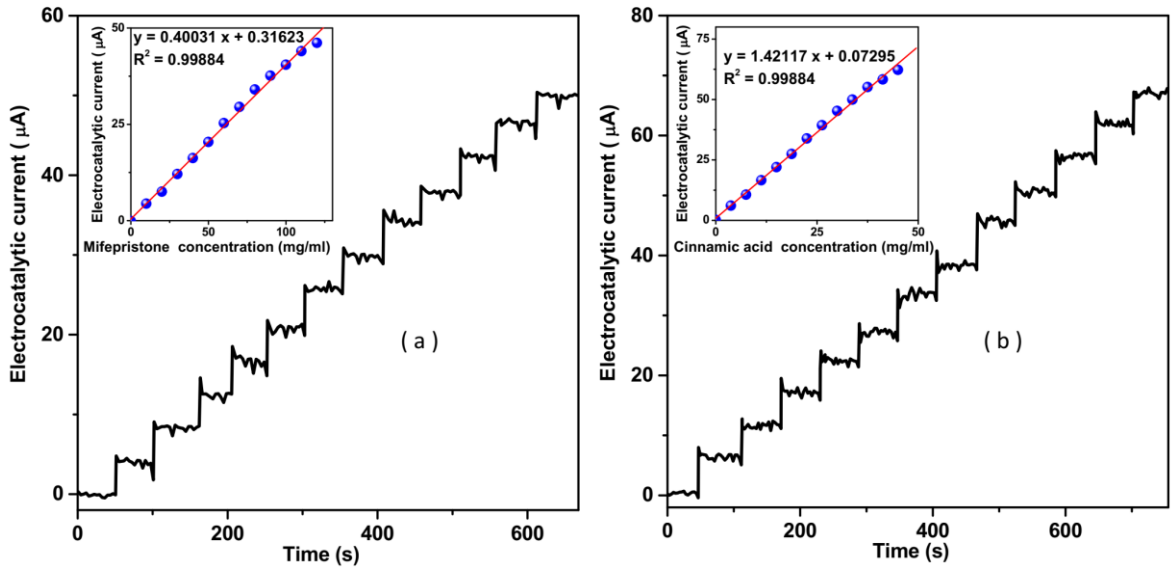

Figure 7. Amperometric responses and obtained calibration plots of dsDNA/Au/GCE in 0.1 M PBS pH 7.0 to successive additions of (a) mifepristone at potential 0.42 V and (b) cinnamic acid at potential of 0.34 V.

The obtained sensing properties of the dsDNA/Au/GCE are compared with those of previously reported mifepristone and cinnamic acid sensors in Table 1. The comparison reveals that the resulted limit of detection of dsDNA/Au/GCE is comparable, and linear range is significantly higher than other method that it related to improvement of the electrochemical signal with Au NPs which provided a high superficial biocompatible plataform for DNA immobilization [71-73].

Table 1. Comparison of obtained sensing properties of dsDNA/Au/GCE with those of previously reported mifepristone and cinnamic acid sensors

Technique	**Analyte**	**Detection limit (ng/ml)**	**Linear range (mg/ml)**	**Ref.**
Amperometry using dsDNA/Au/GCE	Mifepristone	7.5	0-120	This work
Amperometry using dsDNA/Au/GCE	Cinnamic acid	2.1	0-41.25	This work
DPV using DNA/carbon paste electrode	Mifepristone	43	0.086– 0.860	[41]
SSOP using Hg electrode	Mifepristone	8.6	0.0172–0.430	[33]
Visible spectrophotometry using Chromium (VI) solution	Mifepristone	3011.4	0.01-0.1	[34]
HPLC	Mifepristone	-	0–0.001	[35]
HPLC-UV	Mifepristone	3	0–0.001	[74]
UALLME-DES	Cinnamic acid	0.48	4.8×10^{-7}–0.001	[75]
LC/MS/MS	Cinnamic acid	0.50	4×10^{-7}–4×10^{-4}	[36]
HPLC DAD	Cinnamic acid	5	2.35×10^{-5}– 1.107×10^{-4}	[76]
RP-HPLC	Cinnamic acid	15	0.0015–0.150	[77]
UHPLC-MS/MS	Cinnamic acid	0.1	5.8×10^{-6}-5.8×10^{-3}	[78]

SSOP: Single-sweep oscillator- polarography; HPLC: High-performance liquid chromatography; HPLC-UV: HPLC method with UV, UALLME-DES: Ultrasonic-assisted liquid–liquid microextraction method based on deep eutectic solvent; LC/MS/MS: Liquid chromatography/tandem mass spectrometry; HPLC DAD: High-performance liquid chromatography with diode-array detection; RP-HPLC: Reversed-phase high-performance liquid chromatography

Table 2 also shows the results of the study of the interference effect on the electrochemical responses of dsDNA/Au/GCE to determine the mifepristone and cinnamic acid [79]. As seen from Table 2, the amperometrtic responses of the electrode are remarkable in 0.1 M PBS pH 7.0 to addition the 10 mg/ml of mifepristone and cinnamic acid at potentials of 0.42 V and 0.34 V, respectively. Moreover, the response of sensor is very weak and negligible to additions the 50 mg/ml of Al^{3+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Ni^{2+}, Zn^{2+} , nitrite, dopamine, glucose, ascorbic acid, and some suggested uterine fibroids drugs such as lupron, ulipristal acetate, tranexamic acid, oriahnn and leuprolide [80-82]. Therefore, the results indicate that the presented substances in Table 2 didn't show any interference

effects on amperometric determination of mifepristone and cinnamic acid, and that the sensor exhibits the great selectivity.

Table 2. Amperometric response of dsDNA/Au/GCE in 0.1M PBS at 0.42V and 0.34V for successive adding of mifepristone, cinnamic acid and different substance

Electrode	**Added(mg/ml)**	**Amperometric current response(μA) at 0.42 V**	**RSD(%)**	**Amperometric current response(μA) at 0.34 V**	**RSD(%)**
mifepristone	10	4.0121	±0.0319	0.2491	±0.0109
cinnamic acid	10	0.0810	±0.0060	1.4210	±0.0860
Al^{3+}	50	0.0777	±0.0012	0.0198	±0.0012
Ca^{2+}	50	0.0189	±0.0022	0.0905	±0.0032
Cu^{2+}	50	0.0180	±0.0013	0.1001	±0.0083
Fe^{3+}	50	0.0294	±0.0032	0.1019	±0.0092
K^{+}	50	0.0077	±0.0010	0.0319	±0.0012
Mg^{2+}	50	0.0391	±0.0015	0.0420	±0.0017
Ni^{2+}	50	0.1010	±0.0092	0.0517	±0.0070
Zn^{2+}	50	0.0866	±0.0027	0.0518	±0.0013
Nitrite	50	0.1071	±0.0081	0.0124	±0.0011
Dopamine	50	0.0444	±0.0009	0.0647	±0.0010
Glucose	50	0.1131	±0.0097	0.0132	±0.0008
Ascorbic acid	50	0.0550	±0.0013	0.1051	±0.0093
Lupron	50	0.0077	±0.005	0.1024	±0.0075
Ulipristal acetate	50	0.0461	±0.0010	0.0132	±0.0011
Tranexamic acid	50	0.0365	±0.0008	0.0752	±0.0011
Oriahnn	50	0.0217	±0.0007	0.0451	±0.0009
Leuprolide	50	0.0287	±0.0011	0.0244	±0.0006

The results of the study on the practical capability and accuracy of dsDNA/Au/GCE for amperomtric determination of mifepristone and cinnamic acid in prepared samples of Mifeprex and Gui Zhi Fu Ling tablets are shown in Figures 8(a and a') and 8(b and b'). As observed, the initial mifepristone (Figures 8a and 8a'), and cinnamic acid (Figures 8b and 8b') concentrations in prepared samples are 9.975 and 0.972 mg/ml respectively. The obtained value for the prepared sample of Mifeprex is close to 1 mg/ml mifepristone content, and obtained value for the prepared sample of Gui Zhi Fu Ling tablets indicates that each Gui Zhi Fu Ling tablet contains ~1µg of cinnamic acid. Table 3 presents the results of analytical studies for the determination of recovery (≥97.47%) and (≤4.21%) values for both of pharmaceutical samples through the standard addition method which demonstrates the great precision of the proposed method to practical ability in the analysis of medicine samples using dsDNA/Au/GCE [83].

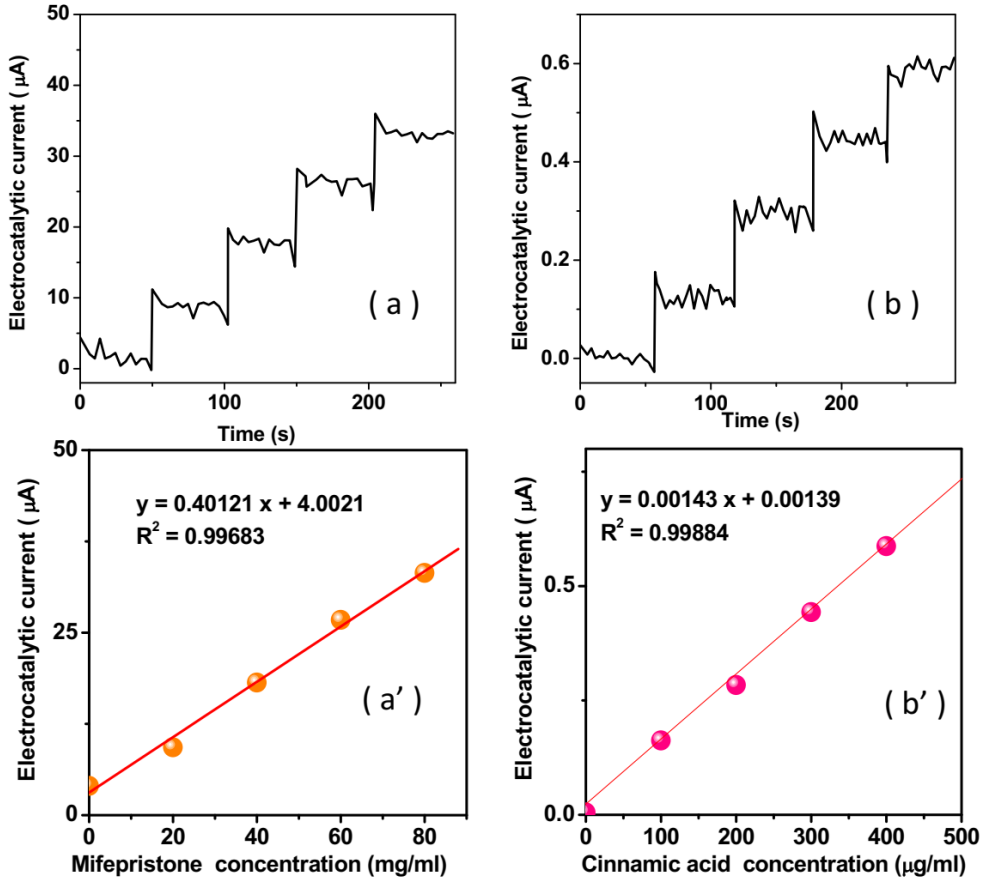

Figure 8. Results of amperometric studies using dsDNA/Au/GCE in prepared real samples of (a and a') Mifeprex successive additions of 20 mg/ml of mifepristone at potential 0.42 V and (b and b') Gui Zhi Fu Ling tablets successive additions of 100 µg/ml of cinnamic acid at potential of 0.34 V.

A second study was carried out to determine the presence of Guizhi Fuling in the blood serum of four patients aged 25 to 30 years with uterine fibroids who had mifepristone treatment at Capital Healthcare Aiyuhua Hospital for Women and Children (Beijing, China). The blood serum samples

were centrifuged at 1500 rpm for 15 minutes and the obtained supernatants were used to prepare 0.1 M PBS pH 7.0. Subsequently, the proposed sensor was used to determine the concentration of Guizhi Fuling in the prepared real samples using an amperometry technique. Table 4 shows the results of an average of five determinations of Guizhi Fuling for each sample through the dsDNA/Au/GCE and ELISA techniques, respectively. The comparison between the obtained results from the amperometric dsDNA/Au/GCE sensor (RSD = 4.71%) is very close to the results of the ELISA method, illustrating the good agreement and high accuracy between the two techniques.

Table 3. Result of analytical studies for determination of mifepristone and cinnamic acid in pharmaceutical samples

Sample	**Added (mg/ml)**	**Measured (mg/ml)**	**Recovery (%)**	**RSD (%)**
Mifeprex	20.0	19.7	98.50	4.08
	40.0	39.8	99.50	3.78
	60.0	58.8	98.00	4.03
	80.0	78.9	98.62	3.81
Sample	**Added (µg/ml)**	**Measured (µg/ml)**	**Recovery (%)**	**RSD (%)**
Gui Zhi Fu Ling	100.0	99.5	99.50	3.22
	200.0	195.7	97.85	3.94
	300.0	299.1	99.70	4.13
	400.0	389.9	97.47	4.21

Table 4. Results of determinations of Guizhi Fuling content in prepared real samples of blood serum from four patients aged 25 to 30 years with uterine fibroids who underwent mifepristone treatment with the amperometric dsDNA/Au/GCE sensor and ELISA techniques.

Sample	**Content of Guizhi Fuling in prepared real serum sample (µM)**			
	dsDNA/Au/GCE	**RSD (%)**	**ELISA**	**RSD (%)**
S1	1.021	±2.32	1.072	±3.72
S2	2.123	±2.77	2.257	±4.13
S3	1.588	±4.71	1.428	±3.15
S4	2.723	±3.24	2.879	±2.51

4. CONCOUSION

This study was conducted for the synthesis of immobilized dsDNA on Au/GCE for the electrochemical sensor of mifepristone and cinnamic acid. The electrodeposition method was used for the modification of the Au NPs on GCE and the immobilization of dsDNA on Au/GCE. Results of structural analyses of electrodeposited Au NPs showed that Au NPs were electrodeposited in spherical-shape and fcc crystal structure. Results of electrochemical analyses using CV, DPV and ampermetery showed a stable, selective and sensitive simultaneous determination of mifepristone and cinnamic acid on dsDNA on Au/GCE. Results indicated that the linear range, detection limit and the sensitivity to

determine mifepristone were evaluated 7.5 ng/ml and 0.40031 μA/mg ml^{-1} and 0-120 mg/ml, respectively, and the linear range, detection limit and the sensitivity to determine cinnamic acid were 2.1 ng/ml and 1.42117 μA/mg ml^{-1} and 0-41.25 mg/ml, respectively. The results of the study on the practical capability of dsDNA/Au/GCE to determination of mifepristone and cinnamic acid in prepared sample of Mifeprex and Gui Zhi Fu Ling tablets showed the acceptable value of recovery and values for both pharmaceutical samples which demonstrated the great precision of proposed method to practical ability in the analysis of medicine samples using dsDNA/Au/GCE.

ACKNOWLEDGEMENT
The research is supported by: The Scientific Research Project in 2018 of Nanjing Polytechnic Institute "Study of the determination of organic pollutants by electrochemical nucleic acid aptamer sensors" (NHKY-2018-13).

References

1. S. Tamura, T. Hayashi, H. Tokunaga, N. Yaegashi, K. Abiko and I. Konishi, *Current Issues in Molecular Biology*, 43 (2021) 1188.
2. X. Zhang, L. Zhang, W. Zhang, S. Xue and Y. Tang, *Journal of Power Sources*, 518 (2022) 230741.
3. H. Karimi-Maleh, R. Darabi, M. Shabani-Nooshabadi, M. Baghayeri, F. Karimi, J. Rouhi, M. Alizadeh, O. Karaman, Y. Vasseghian and C. Karaman, *Food and Chemical Toxicology*, 162 (2022) 112907.
4. J. Rouhi, C.R. Ooi, S. Mahmud and M.R. Mahmood, *Materials Letters*, 147 (2015) 34.
5. Q. Shen, Y. Hua, W. Jiang, W. Zhang, M. Chen and X. Zhu, *Fertility and sterility*, 100 (2013) 1722.
6. W. Tai, L. He, X. Zhang, J. Pu, D. Voronin, S. Jiang, Y. Zhou and L. Du, *Cellular & molecular immunology*, 17 (2020) 613.
7. M. Engman, S. Granberg, A. Williams, C. Meng, P. Lalitkumar and K. Gemzell-Danielsson, *Human Reproduction*, 24 (2009) 1870.
8. X. Zhang, Y. Tang, F. Zhang and C.S. Lee, *Advanced energy materials*, 6 (2016) 1502588.
9. H. Qi, Z. Hu, Z. Yang, J. Zhang, J.J. Wu, C. Cheng, C. Wang and L. Zheng, *Analytical chemistry*, 94 (2022) 2812.
10. K. Annapuranam, *City, Culture and Society*, 22 (2020) 100338.
11. L. Jiang, Y. Wang, X. Wang, F. Ning, S. Wen, Y. Zhou, S. Chen, A. Betts, S. Jerrams and F.-L. Zhou, *Composites Part A: Applied Science and Manufacturing*, 147 (2021) 106461.
12. A. Bahrami, S. Jafarnejad, H. khoshnezhad Ebrahimi, S.M.M. Dezfouli and R. Pahlevani, *Systematic Reviews in Pharmacy*, 11 (2020) 905.
13. V. Tanos and K. Berry, *Best Practice & Research Clinical Obstetrics & Gynaecology*, 46 (2018) 12.
14. A.K. Franta, T.R. Lenters, D. Mounce, B. Neradilek and F.A. Matsen III, *Journal of Shoulder and Elbow Surgery*, 16 (2007) 555.
15. M. Khosravi, *Journal of Eating Disorders*, 8 (2020) 1.
16. M. Akbari and R. Elmi, *Case reports in medicine*, 2017 (2017) 1.
17. R. Moroni, C. Vieira, R. Ferriani, F. Candido‐dos‐Reis and L. Brito, *Annals of medical and health sciences research*, 4 (2014) 185.

18. X. Hu, P. Zhang, D. Wang, J. Jiang, X. Chen, Y. Liu, Z. Zhang, B.Z. Tang and P. Li, *Biosensors and Bioelectronics*, 182 (2021) 113188.
19. M. Nazeer, F. Hussain, M.I. Khan, E.R. El-Zahar, Y.-M. Chu and M. Malik, *Applied Mathematics and Computation*, 420 (2022) 126868.
20. N.-N. Chen, M. Han, H. Yang, G.-Y. Yang, Y.-Y. Wang, X.-K. Wu and J.-P. Liu, *BMC complementary and alternative medicine*, 14 (2014) 2.
21. W. Liu, Y. Zheng, Z. Wang, Z. Wang, J. Yang, M. Chen, M. Qi, S. Ur Rehman, P.P. Shum and L. Zhu, *Advanced Materials Interfaces*, 8 (2021) 2001978.
22. L. Sun, L. Liu, S. Zong, Z. Wang, J. Zhou, Z. Xu, G. Ding, W. Xiao and J. Kou, *Journal of Ethnopharmacology*, 191 (2016) 273.
23. P. Xu, J. Cao, C. Yin, L. Wang and L. Wu, *Supramolecular Chemistry*, 33 (2021) 63.
24. H.-G. Lee, Y. Jo, K. Ameer and J.-H. Kwon, *Food Science and Biotechnology*, 27 (2018) 1607.
25. H. Maleh, M. Alizadeh, F. Karimi, M. Baghayeri, L. Fu, J. Rouhi, C. Karaman, O. Karaman and R. Boukherroub, *Chemosphere*, (2021) 132928.
26. S. Haddadi, R. Shahrokhirad, M.M. Ansar, S. Marzban, M. Akbari and A. Parvizi, *Anesthesiology and pain medicine*, 8 (2018) e61041.
27. J.A. Hoskins, *Journal of Applied Toxicology*, 4 (1984) 283.
28. Z. Zhang, Y. Lou, C. Guo, Q. Jia, Y. Song, J.-Y. Tian, S. Zhang, M. Wang, L. He and M. Du, *Trends in Food Science & Technology*, 118 (2021) 569.
29. W. Chan and C. Lin, *Journal of chemical education*, 84 (2007) 1982.
30. M. Husairi, J. Rouhi, K. Alvin, Z. Atikah, M. Rusop and S. Abdullah, *Semiconductor Science and Technology*, 29 (2014) 075015.
31. S. Khosravi and S.M.M. Dezfouli, *Journal of Critical Reviews*, 7 (2020) 275.
32. M. Akbari, R. Moghadam, R. Elmi, A. Nosrati, E. Taghiabadi and N. Aghdami, *Journal of Ophthalmic and Vision Research*, 14 (2019) 400.
33. Z. Yonghuai and L. Haiyan, *Chinese Journal of Analytieal Chemistry*, 2 (1997) 1.
34. A. Giri, B. Saritha, V. Reddy and S. Reddy, *International Journal of Pharmacy and Therapeutics*, 5 (2014) 277.
35. C. Stith and M.D. Hussain, *Journal of Chromatography B*, 794 (2003) 9.
36. J. Zhang, M. Chen, W. Ju, S. Liu, M. Xu, J. Chu and T. Wu, *Journal of pharmaceutical and biomedical analysis*, 51 (2010) 685.
37. D. Desideri, M.A. Meli and C. Roselli, *Microchemical Journal*, 95 (2010) 174.
38. G. Gentscheva, T. Stafilov and E. Ivanova, *Eurasian Journal of Analytical Chemistry*, 5 (2010) 104.
39. H.A. Gad, S.H. El-Ahmady, M.I. Abou-Shoer and M.M. Al-Azizi, *Phytochemical Analysis*, 24 (2013) 1.
40. L. Fu, Y. Xu, J. Du, D. Cao and Q. Liu, *International Journal of Electrochemical Science*, 14 (2019) 4383.
41. K. Gu, J. Zhu, Y. Zhu, J. Xu and H.Y. Chen, *Fresenius' Journal of Analytical Chemistry*, 368 (2000) 832.
42. H.K. Ebrahimi, S. Sohrabi, S. Jafarnejad, S. Iranmanesh and S. Esmaeilian, *Systematic Reviews in Pharmacy*, 11 (2020) 899.
43. C. Jayakumar, C.M. Magdalane, K. Kaviyarasu, M.A. Kulandainathan, B. Jeyaraj and M. Maaza, *Journal of nanoscience and nanotechnology*, 18 (2018) 4544.
44. L. Dollimore and R. Gillard, *Journal of the Chemical Society, Dalton Transactions*, (1973) 933.
45. L.E. Betancourt, Á.M. Ortiz-Rodríguez, J. Corchado-García and C.R. Cabrera, *ACS Applied Energy Materials*, 2 (2018) 287.
46. M. Alimanesh, J. Rouhi and Z. Hassan, *Ceramics International*, 42 (2016) 5136.
47. T. Zhang, X. Zhu, D.-D. Ye, R. Chen, Y. Zhou and Q. Liao, *Nanoscale*, 12 (2020) 20270.

48. H. Ebrahimi, S. Jafarnejad, S. Sohrabi, A. Abbasi and S. Esmaeilian, *Journal of Critical Reviews*, 7 (2020) 685.
49. N. Zhu, H. Cai, P. He and Y. Fang, *Analytica chimica acta*, 481 (2003) 181.
50. A. Baradaran-Rafii, M. Akbari, E. Shirzadeh and M. Shams, *Journal of ophthalmic & vision research*, 10 (2015) 90.
51. A. Kirsch-De Mesmaeker, J.-P. Lecomte and J.M. Kelly, *Electron Transfer II*, (1996) 25.
52. S.M.M. Dezfouli and S. Khosravi, *Pakistan Journal of Medical & Health Sciences*, 14 (2020) 1236.
53. J. Rouhi, S. Mahmud, S.D. Hutagalung and N. Naderi, *Electronics letters*, 48 (2012) 712.
54. A. Medghalchi, M. Akbari, Y. Alizadeh and R.S. Moghadam, *Journal of current ophthalmology*, 30 (2018) 353.
55. M. Llaguno-Munive, M. Romero-Piña, J. Serrano-Bello, L.A. Medina, N. Uribe-Uribe, A.M. Salazar, M. Rodríguez-Dorantes and P. Garcia-Lopez, *Cancers*, 11 (2019) 16.
56. L.-N. Song, M. Coghlan and E.P. Gelmann, *Molecular endocrinology*, 18 (2004) 70.
57. Y.-M. Chu, U. Nazir, M. Sohail, M.M. Selim and J.-R. Lee, *Fractal and Fractional*, 5 (2021) 119.
58. K. Gu, J. Zhu, Y. Zhu, J. Xu and H.-Y. Chen, *Fresenius' journal of analytical chemistry*, 368 (2000) 832.
59. R. Mosaddegh, S. Nabi, S. Daei, F. Mohammadi, G. Masoumi, S. Vaziri and M. Rezai, *Open access emergency medicine: OAEM*, 11 (2019) 205.
60. J. Rouhi, S. Mahmud, S.D. Hutagalung, N. Naderi, S. Kakooei and M.J. Abdullah, *Semiconductor Science and Technology*, 27 (2012) 065001.
61. H. Li, S. Pan, P. Xia, Y. Chang, C. Fu, W. Kong, Z. Yu, K. Wang, X. Yang and Z. Qi, *Journal of biological engineering*, 14 (2020) 1.
62. H. Malekzad, P.S. Zangabad, H. Mirshekari, M. Karimi and M.R. Hamblin, *Nanotechnology Reviews*, 6 (2017) 301.
63. Y.-M. Chu, B. Shankaralingappa, B. Gireesha, F. Alzahrani, M.I. Khan and S.U. Khan, *Applied Mathematics and Computation*, 419 (2022) 126883.
64. G. Ghotra, B.K. Nguyen and J.I. Chen, *ACS Applied Nano Materials*, 3 (2020) 10123.
65. L. Mereuta, A. Asandei, I.S. Dragomir, I.C. Bucataru, J. Park, C.H. Seo, Y. Park and T. Luchian, *Scientific reports*, 10 (2020) 1.
66. A. Roghani, *AIMS Public Health*, 8 (2021) 655.
67. M.M. Collinson, *International Scholarly Research Notices*, 2013 (2013) 1.
68. S. Khosravi, S.M.H. Sadati, V.A.S. Sherafat and Z. Bazargani, *Pakistan Journal of Medical & Health Sciences*, 14 (2020) 1753.
69. J. Rouhi, S. Mahmud, S.D. Hutagalung and S. Kakooei, *Journal of Micro/Nanolithography, MEMS, and MOEMS*, 10 (2011) 043002.
70. R.S. Moghadam, M. Akbari, Y. Alizadeh, A. Medghalchi and R. Dalvandi, *Middle East African Journal of Ophthalmology*, 26 (2019) 11.
71. M. Vidotti, R.F. Carvalhal, R.K. Mendes, D. Ferreira and L.T. Kubota, *Journal of the Brazilian Chemical Society*, 22 (2011) 3.
72. L. Zhang, C. Ye, G. Zhou, W. Chen, X. Xu and Z. Li, *International Journal of Electrochemical Science*, 12 (2017) 6763.
73. N. Pantelic, D. Stanković, B. Zmejkovski, G.N. Kaluđerović and T. Sabo, *International Journal of Electrochemical Science*, 11 (2016) 1162.
74. Z. Guo, D. Wei, G. Yin, S. Wang, S. Zhao, Y. Chu and J. Zhai, *Journal of Chromatography B*, 856 (2007) 312.
75. T. Khezeli, A. Daneshfar and R. Sahraei, *Talanta*, 150 (2016) 577.
76. G.C. Moreira and F. de Souza Dias, *Microchemical Journal*, 141 (2018) 247.

77. R. Sladkovský, P. Solich and L.r. Opletal, *Journal of Pharmaceutical and Biomedical Analysis*, 24 (2001) 1049.
78. B. Ji, Y. Zhao, Q. Zhang, P. Wang, J. Guan, R. Rong and Z. Yu, *Journal of Chromatography B*, 1001 (2015) 107.
79. F. Husairi, J. Rouhi, K. Eswar, C.R. Ooi, M. Rusop and S. Abdullah, *Sensors and Actuators A: Physical*, 236 (2015) 11.
80. T.D. Lewis, M. Malik, J. Britten, A.M. San Pablo and W.H. Catherino, *BioMed research international*, 2018 (2018) 1.
81. T.H. Zhao, M.I. Khan and Y.M. Chu, *Mathematical Methods in the Applied Sciences*, (2021) 1-19.
82. O.H. Eskandanı, *Turkish Journal of Computer and Mathematics Education (TURCOMAT)*, 12 (2021) 4808.
83. K. Eswar, J. Rouhi, H. Husairi, M. Rusop and S. Abdullah, *Advances in Materials Science and Engineering*, 2014 (2014) 1.