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# Application of new aptasensor modified with nanocomposite for selective estradiol valerate determination in pharmaceutical and real biological samples

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1           **Application of new aptasensor modified with**  
2           **nanocomposite for selective estradiol valerate**  
3           **determination in pharmaceutical and real biological**  
4           **samples**

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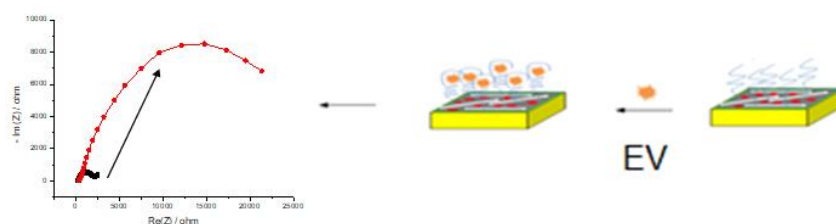
9  
10  
11           **Abstract**

12           In this study we developed a highly sensitive aptasensor based on polyaniline  
13           electro-polymerization, new nanomembrane composite, and aptamer (PANI-  
14           MWCNT@Chs-APT) for the electrochemical detection of estradiol valerate  
15           in real samples. The high sensitivity and selectivity of the aptasensor were  
16           demonstrated by evaluating its response to some potential interference  
17           having structural similarities. The selectivity of this aptasensor showed high  
18           selectivity for estradiol valerate among other interfering hormones.  
19           Electrochemical impedance spectroscopy combined with the standard added  
20           method used to characterize the modified gold electrodes as well as for the  
21           detection of estradiol valerate both in pharmaceutical and in women's blood.  
22           It showed that the response of the aptasensor rose with increasing estradiol  
23           valerate concentration, which highlighted the good sensitivity of the

1 aptasensor to estradiol valerate with a very low limit of detection of 0.56 fM.  
2 This was very promising for rapid analysis of estradiol valerate detection in  
3 pharmaceutical and biological samples.

4

5 *Graphical abstract*



6

7

8 **Keywords** Biosensor • Aptamer • Carbon Nanotubes • Chitosan • Blood  
9 samples

10 \_\_\_\_\_

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19

## 1 **Introduction**

2 Oestradiol (E2) is a major female sex hormone. According to Ettawfik  
3 Medical Analysis Laboratory, normal levels of E2 for menstruating women  
4 range from 18 to 147 pg/cm<sup>3</sup>. For postmenopausal, E2 levels is lower than  
5 10 pg/cm<sup>3</sup>. Lower levels of E2 in the body may suggest late puberty,  
6 hypopituitarism, hypogonadism, Turner syndrome, and menopause.  
7 Whereas E2 levels that are higher than normal may suggest tumors in the  
8 ovaries, early puberty, and scarring of the liver. Estradiol valerate (EV, Fig.  
9 1) is essentially the ester of E2 characterized by the presence of a substitution  
10 radical at carbon 17 such as the radical. Following absorption in the body,  
11 the ester was cleaved, and the natural estrogen (E2) resulted [1]. EV is very  
12 significant in clinical medicine and could be used to treat menopause  
13 syndrome and prostate cancer. This hormone can be utilized together with a  
14 progestogen for the inhibition of ovulation [2]. As a result, many clinical  
15 studies have reported the use of different detection techniques such as HPLC  
16 [3, 4] and LC-MS/MS [5], CG/MS [6, 7] for the quantification of EV in  
17 diverse samples. Thus, given the high cost, time requirements, and expensive  
18 instruments. The development of rapid, simple, selective, less expensive,  
19 sensitive, and specific analytical methods for the detection of EV is of great  
20 importance.

1           Several biosensors have been developed for electrochemical detection  
2 of natural E2 [8-12] and, to our knowledge; few research articles have been  
3 published for synthetic E2 detection [13-15]. Mofidi et al. [13] have  
4 developed an electrochemical sensor for EV in blood samples; a carbon paste  
5 electrode was modified with terbium carbonate nanoparticles for EV  
6 determination. The method showed a limit detection of  $0.1 \text{ ng/cm}^3$  with a  
7 linear range of  $13 \times 10^2 - 10 \times 10^3 \text{ ng/cm}^3$ . In this study, EV was extracted from  
8 the blood sample with a recovery percentage of 67%. Various  
9 electrochemical methods are based on aptamer-based biosensors for the  
10 detection of E2 in real samples [16-20].

11           Recently, many aptasensor based on nanomaterial electrochemical  
12 electrode-modifying has received a huge amount of attention due to their  
13 inherent advantages such as high specific surface area and excellent  
14 electrical performance of nanomaterials such as simplicity, high sensitivity,  
15 low cost, and high stability [21-24]. Hlavata et al. [25] have developed an  
16 electrochemical biosensor based on nanomaterials, by combining the  
17 benefits of chitosan and carboxylate single-walled carbon nanotubes for  
18 evaluation of damage to DNA induced by UV-C radiation. They were  
19 studied the role of these nanomaterials (with and without carbon nanotube)  
20 for the performance of biosensors and they deduced that the utilization of  
21 carbon nanotubes increasing the electro-conducting properties of the

1 biosensor. In another report, Nameghi et al. [26] have proposed a simple  
2 electrochemical aptasensor for E2 in a blood sample. In this sensor platform,  
3 a split DNA aptamer was applied as recognizing agents with E2. The  
4 aptasensor was indicated a wide linear range with detection limits of 0.7 pM  
5 and 0.5 pM for E2 in milk samples and tap water, respectively.

6 In this study, we developed a highly sensitive aptasensor based on  
7 PANI-MWCNT@Chs-APT composite for the electrochemical detection of  
8 estradiol valerate (EV) in real samples. The elaboration of the aptasensor  
9 involves a three-step modification process. First, we carried out the  
10 electrochemical polymerization of aniline (polyaniline (PANI)) on the gold  
11 electrode (AuE). Secondly, the MWCNT@Chs was prepared and dropped  
12 to the surface of electropolymerized gold to form  
13 MWCNT@Chs/PANI/AuE. This new membrane has been developed by  
14 Ying Ou et al.; the MWCNTs were functionalized by the polyelectrolyte  
15 Chs, via a noncovalent surface deposition method [27]. Finally, the free  
16 carboxyl groups on the MWCNT@Chs/PANI/AuE surface were conjugated  
17 with the  $\text{NH}_2$ -Aptamer to afford an aptasensor  
18 APT/MWCNT@Chs/PANI/AuE interface. Electrochemical impedance  
19 spectroscopy (EIS) was used for gold electrodes (AuE) characterization and  
20 it combined with a standard added method to evaluate the performance of

1 aptasensor developed. This method has seen a huge boost in popularity in  
2 recent times due to its extraordinary sensitivity.

3

4 < Figure 1 >

5

## 6 **Results and Discussion**

### 7 **Characterization of the aptasensor surface**

8 EIS could be the effective method to study the interface characteristics of  
9 modified electrodes. Figure 2 showed the Nyquist plots comparison of  
10 different modified electrodes of AuE, AuE/PANI, AuE/PANI/  
11 MWCNTs@Chs, and AuE/PANI/ MWCNTs@Chs/APT. The EIS of the  
12 bare AuE displays quite small semicircle diameters, implying a low  
13 resistance charge transfer ( $R_{CT}$ ) to the redox probe in  $[\text{Fe}(\text{CN})_6]^{3-}/$   
14  $[\text{Fe}(\text{CN})_6]^{4-}$  electrolyte solution. While the value of  $R_{CT}$  increases after the  
15 PANI was modified on the AuE electrode, this might be ascribed to the less-  
16 conductive polymers. However, the  $R_{CT}$  of PANI/MWNTs@Chs was  
17 decreased gradually because PANI could promote electron transfer. After  
18 aptamer immobilized on AuE/PANI/MWNTs@Chs, the  $R_{CT}$  rose  
19 significantly because the formed aptamer layer prevented the electron  
20 transfer. EIS data are commonly analyzed by fitting a Randles equivalent  
21 circuit model (Fig. 2 inset). This circuit was adapted by replacing the ideal

1 capacitor with a constant phase element (CPE). The presence of the diffusion  
2 limitations at low frequencies must also be presented in the circuit and was  
3 represented by a Warburg element ( $Z_w$ ). The obtained semi-circle indicates  
4 that multiple processes with different time constants occurred. The high-  
5 frequency values resulted from processes in the electrolyte or electrode bulk  
6 (conductivity), whereas the low-frequency values resulted from processes at  
7 the electrode-electrolyte interface (double layer, charge transfer) or the  
8 electrode surface (mass transport, adsorption, electrochemical reactions)  
9 [28].

10

11

&lt; Figure 2 &gt;

12

### 13 **Optimization of aptamer concentration**

14 The optimization of aptamer concentration is an essential step that affects  
15 the performance of the developed aptasensor. Therefore, three different  
16 concentrations of aptamer (0.40, 1.21, and 2.02 mM) were tested within the  
17 range of  $4 \times 10^{-16}$  to  $4 \times 10^{-10}$  mg/cm<sup>3</sup> of EV using the EIS device. Figure 3  
18 shows the variation of  $R_{ct}^f$  as function of the EV concentrations for the  
19 aptamer concentrations. The calibration curves (Fig. 3) show a comparison  
20 between the relative slopes of three aptasensors; as it can be observed, when



1 using the aptamer concentration (2.02 mM) the aptasensor presents a good  
2 linear response towards the different [EV], with high sensitivity.

3

4

< Figure 3 >

5

### 6 **Detection of EV in pharmaceutical samples**

7 To evaluate the effectiveness of aptasensor developed  
8 (APT/MWCNT@Chs/PANI/AuE) to quantify EV in pharmaceutical  
9 samples. Two pills of (progenovat<sup>®</sup>2mg) were dissolved in 25 cm<sup>3</sup> of PBS  
10 (5 mM pH 7.4) and sonicated for 10 min. Various EV concentrations in the  
11 range of 4×10<sup>-16</sup> to 4×10<sup>-9</sup> mg/cm<sup>3</sup> were prepared using PBS. EIS  
12 measurements were carried out to detect EV in these solutions at a frequency  
13 ranging from 100 kHz to 100 mHz. The aptasensor was incubated with  
14 different concentrations of EV for 30 min at room temperature, and then the  
15 response of the aptasensor was determined (Fig. 4(A)).

16 The normalized data was presented as the change to film resistance  
17 ( $R_{ct}^f$ ) as a function of EV concentrations is plotted in Fig. 4(B). The  $R_{ct}^f$  was  
18 calculated according to the equation:

$$19 \quad R_{ct}^f = \frac{R_{ct}^C - R_{ct}^{C=0}}{R_{ct}^{C=0}} \quad (1)$$

1 where  $R_{ct}^C$  and  $R_{ct}^{C=0}$  are the values of the charge transfer resistance before  
2 and after the incubation in EV concentrations.

3 Figure 4(A) showed that the Nyquist plot of the interface aptasensor at  
4 different concentrations of EV. The  $R_{CT}$  values were found to rise (from 12  
5 to 51.5 k $\Omega$ ) with increasing concentration of EV from  $4 \times 10^{-16}$  to  $4 \times 10^{-9}$   
6 mg/cm<sup>3</sup>. This was due to the increased complexation by adsorption of EV  
7 onto the aptasensor interface; it was directly related to the good sensitivity  
8 of the modified electrode to EV.

9 It could be seen in the calibration plot (Fig. 4(B)), a linear dependence  
10 between the  $R_{ct}^f$  values and the logarithmic values of EV concentration. The  
11 calibration curve equation was obtained by Origin 9.1 version via plotting  
12 and analyzing:

$$13 \quad R_{ct}^f / \Omega = 0.46 \text{ Log } ([EV] / \text{mg cm}^{-3}) + 7.41 \quad (2)$$

14 The aptasensor had a linear response in the range of EV concentrations  
15 between  $4 \times 10^{-16}$  to  $4 \times 10^{-9}$  mg/cm<sup>3</sup> with a good correlation coefficient of  
16 about 0.996 and a high sensitivity of 0.46  $\Omega$  per EV concentration decade.  
17 While the limit of detection was calculated to be 0.56 fM from the calibration  
18 curve by three times the standard deviation of the average value of the blank  
19 without adding the EV standard solution (n=5), divided by the sensitivity.  
20 This LOD was lower than those reported in the references [23, 31, 32, 34]  
21 and it was in good agreement with those attained by other sensors [29, 30,

1 36] (Table 1). This lower limit detection was attributed by Ke et al. [30];  
2 after estradiol reacts with aptamer, the interfacial electron transfer resistance  
3 was enlarged on the electrode surface thus the reason for the rise of  
4 impedance. These results indicated that the prepared aptasensor could be  
5 safely used for the determination of [EV] in the pharmaceutical samples.

6 In this work, the role of carbon nanotube for aptamer immobilization  
7 was investigated. The aptamer was directly fixed on the PANI layer, without  
8 nanotubes (Fig. 4(B)). These results showed that the performance of  
9 APT/PANI/AuE aptasensor could be significantly degraded with the absence  
10 of MWCNTS.

11

12

< Figure 4 >

13

#### 14 **Study of interferences**

15 The developed aptasensor was evaluated for the selectivity studies by  
16 comparing it with some interference having structural similarities with EV  
17 such as DCI and P4, all at various concentrations ranging from  $4 \times 10^{-16}$  to  
18  $4 \times 10^{-9}$  mg/cm<sup>3</sup>. Using 5 mM PBS electrolyte at pH 7.4. The specificity of  
19 the APT/MWCNT@Chs/PANI/AuE aptasensor for different interfering  
20 elements is shown in Fig. 5. From these results, we could deduce that this  
21 aptasensor had a high selectivity towards EV and E2, with a much lower

1 response to all other hormones tested. This indicates that we can use this  
2 aptasensor to detect the synthetic estradiol in real samples.

3

4

< Figure 5 >

5

### 6 **Detection of EV in real biological samples**

7 The developed aptasensor was used to estimate EV concentrations in the  
8 blood sample by the standard addition method combined with EIS. A blood  
9 sample (on the third day of menstruation) was centrifuged for 10 min. 1 cm<sup>3</sup>  
10 of serum obtained was diluted in 24 cm<sup>3</sup> of PBS pH 7.4. Different  
11 concentrations of EV were prepared in PBS from 4×10<sup>-16</sup> to 4×10<sup>-10</sup> mg/cm<sup>3</sup>.  
12 1 cm<sup>3</sup> of each EV concentration was added to each of the seven volumetric  
13 flasks of 2 cm<sup>3</sup>. To obtain the final volume of 2 cm<sup>3</sup>, 1 cm<sup>3</sup> of the serum  
14 sample was added to each of the seven volumetric flasks. The Nyquist plot  
15 of the platform aptasensor at different concentrations of EV in serum  
16 samples was shown in Fig. 6(A). It could be seen from the plot that the  
17 diameter of the curve increased with the growing concentration of EV,  
18 indicating a decrease in the conductance of the samples. The values of  $R_{CT}$   
19 rose with the increasing concentration of EV, which was responsible for the  
20 decrease in the double layer capacitance appearing at the electrode-  
21 electrolyte interface. The calibration curve equation was obtained:

1 
$$R_{ct}^f/\Omega = 6.473 \text{ Log } ([\text{EV}] / \text{mg cm}^{-3}) + 79.21 \quad (3)$$

2 The concentration of EV in the unknown serum sample obtained by the  
3 standard addition method was 43.15 pg/cm<sup>3</sup>.

4 The concentration of this plasma sample was also tested using the  
5 immuno-electrochimiluminescence "ECLIA" (Cobas, Roche). The result  
6 obtained was 41.25 pg/cm<sup>3</sup>. Both results were nearly the same with a  
7 recovery = 95 %. However, this result was in line with the estradiol levels  
8 determined in the blood samples of healthy women during menstruation  
9 range from (18 to 147 pg/cm<sup>3</sup>). These results indicated that the developed  
10 aptasensor could be applied in real pharmaceutical applications.

11

12 < Figure 6 >

13 < Table 1 >

14

## 15 **Conclusion**

16 In this work, a new sensitive and selective aptasensor was developed and  
17 successfully applied to determine EV in pharmaceutical and real female  
18 blood samples. We showed that the APT/MWCNT@Chs/PANI/AuE  
19 aptasensor exhibited excellent performances with high sensitivity and  
20 selectivity. A good linear relationship was also obtained between the  $R_{CT}$   
21 values and the logarithmic values of EV concentration with a lower LOD of

1 about 0.56 fM both in pharmaceutical and women's blood. Therefore, this  
2 work could provide a potential tool for the rapid detection of EV in real  
3 samples.

4

## 5 **Experimental**

6 (Progynova®) was employed from Delpharm Lille S.A.S (France).  
7 Progesterone (P4) was obtained from Laboratoires BESINS International  
8 (France). Methylprednisolone (DCI) was produced from Valdepharm  
9 (France). 17 $\beta$ -Estradiol (E2), multi-walled carbon nanotube (MWNTs),  
10 chitosan (Chs, 75% deacetylated), glutaraldehyde (GA) (grade II, 25%  
11 aqueous solution), potassium ferricyanide (K<sub>3</sub>[Fe(CN)<sub>6</sub>]), potassium  
12 ferrocyanide (K<sub>4</sub>[Fe(CN)<sub>6</sub>]), ammonia, phosphate buffer solution (pH 7.4),  
13 nitric acid, hydrogen peroxide solution, acetone, sulfuric acid, hydrogen  
14 peroxide, and ethanol were obtained from Sigma-Aldrich (France). Aniline  
15 and acetic acid were obtained from VWR International S.A.S (France). *N*-  
16 Hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N'*-  
17 ethylcarbodiimide (EDC) were received from Shanghai Yuanye  
18 BioTechnology Co., Ltd. (China). Gold substrates were provided by the  
19 French RENATECH network (LAAS, CNRS Toulouse). The single-strand  
20 DNA Aptanti-oestradiol was chosen according to the prior reported  
21 literature [37]; then, was obtained from Eurogentec, the sequence of the 76-

1 mersized amino-modified aptamer as follows: (5'-amino-modified): 5'-  
2 GCT-TCC-AGC-TTA-TTG-AAT-TAC-ACG-CAG-AGG-GTA-GCG-  
3 GCT-CTG-CGC-ATT-CAA-TTG-CTG-CGC-GCT-GAA-GCG-CGG-  
4 AAGC-3'). After Jenison et al and Kato et al.; the dissociation constants for  
5 (10ssDNA) aptamers were in the range of (0.1-3  $\mu\text{M}$ ) [38, 39]. The stock  
6 (EV, P4, and DCI) samples ( $4\pm 0.04 \text{ mg/cm}^3$ ) were obtained by dissolving in  
7 PBS buffer (5 mM, pH 7.4) and stored at 4 °C for experiments.

8 All the electrochemical measurements were carried out in a Faraday  
9 box at room temperature using an SP-300 Potentiostat/Galvanostat/FRA  
10 (BioLogic Science Instruments SAS, France) controlled by EC-Lab software  
11 with a three-electrode cell system, which consists of a gold electrode (AuE:  
12  $0.78 \text{ mm}^2$ ), a platinum wire (Pt), and an electrode calomel (saturated KCl)  
13 used as the working, counter, and reference electrodes, respectively.

14 Cyclic voltammetry (CV) was used for electrochemical  
15 polymerization of aniline on the surface of gold electrodes within potential  
16 scan limits of -0.2 to 1.4 V at a potential scan rate of  $100 \text{ mV s}^{-1}$ .

17 The electrochemical impedance spectroscopy (EIS) measurements  
18 combined with the standard added method were used to evaluate the  
19 performance of the aptasensor developed. Furthermore, the EIS was  
20 analyzed over a frequency range from 100 mHz to 100 kHz at a potential of  
21 128 mV and an amplitude of 10 mV. The obtained data EIS were fitted by

1 Randles equivalent circuit model. The validation of this model is obtained  
2 by comparing the experimental data to the theoretical EIS model using EC-  
3 Lab software with the Randomize + Simplex method. This device was then  
4 used to detect EV, both in pharmaceutical and blood women, these samples  
5 were diluted in PBS at pH 7.4 within the range  $4.0 \times 10^{-16}$  to  $4.0 \times 10^{-9}$  mg/cm<sup>3</sup>.  
6 The impedance response was recorded by successive incubations for 30 min  
7 of interface aptasensor in real samples containing different concentrations of  
8 EV.

9

## 10 **Elaboration of the aptasensor (APT/MWCNTs@Chs/PANI/AuE)**

### 11 *Electropolymerization of aniline on gold electrode*

12 Before modification, the gold electrodes were cleaned with acetone and  
13 sonicated in ultrapure water. Then, dipped into a piranha solution (3:1  
14 mixture of concentrated H<sub>2</sub>SO<sub>4</sub> with H<sub>2</sub>O<sub>2</sub>) for 4 min and washed with  
15 ethanol and ultrapure water. Next, the electrodes were electrochemically  
16 cleaned in 0.5 M H<sub>2</sub>SO<sub>4</sub> in the potential scanning between -0.5 V and 1.5 V  
17 for 5 min, followed by washing them with ultrapure water and drying under  
18 nitrogen. After that, the electrochemical polymerization of AuE electrode  
19 had been performed in 0.5 M sulfuric acid solution containing 0.1 M of  
20 aniline by potential cycling within the potential scan limits of -0.2 to 1.4 V



1 for 30 cycles at a potential scan rate of  $100 \text{ mV s}^{-1}$  (Fig. 7). This technology  
2 of functionalization was recently detailed in various papers [40-43].

3

4

< Figure 7 >

5

### 6 *Preparation of nanomembrane (Chs@ MWCNTs)*

7 First, the pristine MWCNTs (200 mg) were oxidized by  $80 \text{ cm}^3$  of a  
8 concentrated  $\text{H}_2\text{SO}_4$  (98 %) and  $\text{HNO}_3$  (65 %) solution mixed at a volume  
9 ratio of 3:1 under ultrasonic oscillation at  $70 \text{ }^\circ\text{C}$  for 3 h to obtain  
10 carboxylated HOOC-MWCNTs [44]. The resulting HOOC-MWCNTs were  
11 rinsed several times with double distilled water until the pH reached 7.0 and  
12 then dried in a vacuum freeze dryer. This process was for the open of the  
13 ends of long tubes and the introduction of carboxyl groups to the surface  
14 [45]. Secondly, the Chs@MWCNTs were prepared by the process of Ou et  
15 al. (2018) using a noncovalent surface-deposition and cross-linking method.  
16 Briefly, 100 mg of MWCNTs was dispersed in a Chs solution (0.1 g Chs in  
17  $100 \text{ cm}^3$  1 vol. % acetic acid solution). Diluted ammonia solution was added  
18 dropwise to the dispersion to adjust  $\text{pH} = 9$ . Subsequently, the obtained  
19 suspension was heated to  $60 \text{ }^\circ\text{C}$  and 0.02 g of GA was added to cross-link  
20 the surface deposited Chs for 1 h. Finally, the Chs@MWCNTs products  
21 were collected by centrifugation and then washed with dilute acetic acid to

1 remove the adsorbed and uncross-linked Chs [27]. Then, 5 mm<sup>3</sup> of  
2 Chs@MWCNTs dispersion was dropped by drop added to the surface of the  
3 electropolymerized gold electrode and dried at room temperature for 5 h.

4

#### 5 *Immobilization of aptamer*

6 Before the aptamer immobilization, NHS/EDC technique was used to  
7 activate the –COOH groups of the aptamer. Briefly, 50 mm<sup>3</sup> of aptamer (4.04  
8 mM) were mixed with 50 mm<sup>3</sup> of the PBS solution containing (0.1 M  
9 NHS/0.4 M EDC). 6 mm<sup>3</sup> of the activated aptamer was then dropped on the  
10 MWCNT@Chs/PANI/AuE electrode and maintained for 24 h at room  
11 temperature for immobilization. Afterward, the remained active carboxylic  
12 acid groups were deactivated by incubating them in the ethanolamine  
13 solution (1% in PBS) at room temperature for 20 min. This stage is very  
14 important to prevent nonspecific bonding at the detection step [46]. The  
15 whole construction procedure of the modified aptasensor was displayed in  
16 Fig. 8 and the resulting electrode is called APT/MWCNT@Chs/PANI/AuE  
17 electrode.

18

19

< Figure 8 >

20

1 EIS was used for modified electrodes (bare AuE, PANI/AuE,  
2 MWCNTs@Chs/PANI/AuE and APT/MWCNT@Chs/PANI/AuE)  
3 characterization using 5 mM of  $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$  (Fig. 2).  
4

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- 9

1 *Figure Captions*

2 **Fig. 1** Chemical structure of oestradiol (E2), estradiol valerate (EV),  
3 methylprednisolone (DCI), and progesterone (P4)

4

5 **Fig. 2** Nyquist plots of 5 mM  $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$  obtained at (a) bare  
6 AuE, (b) PANI/AuE, (c) MWCNTs@Chs/PANI/AuE, (d)  
7 APT/MWCNTs@Chs/PANI/AuE modified electrodes. The frequency range  
8 from 100 mHz to 100 kHz. The inset shows the equivalent circuit used to  
9 model impedance data

10

11 **Fig. 3** Optimization of EV-aptamer concentration; ( $\blacktriangle$ ) 0.40 mM, ( $\bullet$ ) 1.21  
12 mM, and ( $\blacksquare$ ) 2.02mM of aptamer

13

14 **Fig. 4** (A) Nyquist plots of APT/MWCNT@Chs/PANI/AuE aptasensor at  
15 various concentrations of EV in 5 mM PBS (pH 7.4). (B) Calibration curve  
16 of APT/MWCNT@Chs/PANI/AuE and APT/PANI/AuE aptasensors at  
17 various concentrations of EV in 5mM PBS (pH 7.4) (n=3)

18

19 **Fig. 5** Specificity of the aptasensor for the detection of (a) EV by comparing  
20 it with (b) E2, (c) DCI, and (d) P4. All at various concentrations range from  
21  $4 \times 10^{-16}$  to  $4 \times 10^{-9}$  mg/cm<sup>3</sup> in 5 mM PBS at pH 7.4 (n=3)



1

2 **Fig. 6** (A) Nyquist impedance plots ( $Z_r$  vs  $Z_i$ ) obtained from the standard  
3 addition method performed on a serum blood sample at 5 mM of PBS pH  
4 7.4 solution. (B) Calibration curve used to determine the concentration of  
5 EV in the unknown Serum sample by the standard addition method ( $n=3$ ),  
6  $R^2 = 0.996$

7

8 **Fig. 7** Cyclic voltammograms of electrochemical polymerization of aniline  
9 on the gold electrode from the aqueous solution of 0.5 M of sulfuric acid  
10 containing 0.1 M of aniline, as obtained at a potential scan rate of  $100 \text{ mV s}^{-1}$   
11 within potential scan limits of -0.2 to 1.4 V

12

13 **Fig. 8** Construction of the aptasensor APT/MWCNTs@Chs/PANI/AuE  
14 interface

15

16

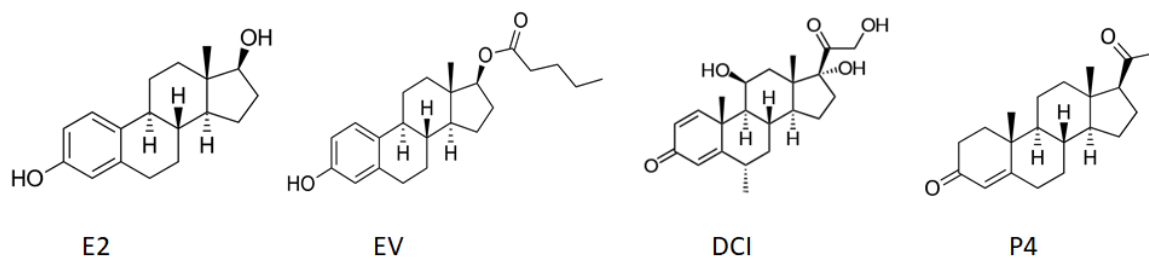
1 **Table 1** Performance comparison of APT/MWCNT@Chs/PANI/AuE for  
 2 the detection of estradiol with other sensors

3

Estradiol–sensor interfaces	Method	Linearity range /M	LOD /M	Ref.
6-Mercapto-1-hexanol, graphene, DNase I enzyme	DVP	$0.07 \times 10^{-12}$ - $10 \times 10^{-12}$	$50 \times 10^{-15}$	[16]
(CuO/CPE detector)	SWV	$60.0 \times 10^{-9}$ - $800.0 \times 10^{-9}$	$21.0 \times 10^{-9}$	[19]
GOx/APT/AuNPs/CuS/GCE	DVP	$5 \times 10^{-13}$ - $5 \times 10^{-9}$	$6 \times 10^{-14}$	[23]
APT–ERGO/GCE	SWV	$1.0 \times 10^{-15}$ - $0.23 \times 10^{-9}$	$0.5 \times 10^{-15}$	[29]
Hierachical dendritic gold	EIS	$10.01 \times 10^{-15}$ - $1.0 \times 10^{-9}$	$5.0 \times 10^{-15}$	[30]
(pNIPAm)	Sps	$3.2 \times 10^{-12}$ - $711.1 \times 10^{-12}$	$3.2 \times 10^{-12}$	[31]
GN	Fluorescence emission spectra	$0 - 73.42 \times 10^{-9}$	$2.75 \times 10^{-15}$	[32]
11-MUA / E2-BSA conjugate on gold sensor chip vs. Ab-E2	SPR	$0.036 \times 10^{-12}$ - $3.6 \times 10^{-12}$	$3.67 \times 10^{-12}$	[33]
Ru@ethyl-SiO <sub>2</sub>	ECL	$0.037 \times 10^{-12}$ - $367.12 \times 10^{-12}$	$1.8 \times 10^{-14}$	[34]
DNA-functionalized Au@Ag NPs	SERS	$1.0 \times 10^{-13}$ - $1.0 \times 10^{-9}$	$2.75 \times 10^{-15}$	[35]
Anti-E2/ ITO/ErG/AuNP	CV	$1 \times 10^{-3}$ – $0.1 \times 10^{-12}$	$0.1 \times 10^{-15}$	[36]
APT/MWCNT@Chs/PANI	EIS	$1.12 \times 10^{-15}$ - $1.12 \times 10^{-9}$	$0.56 \times 10^{-15}$	This work

- 4 APT–ERGO/GCE: electrochemically reduced graphene oxide  
 5 DPV: differential pulse voltammetry  
 6 CuO/CPE : copper(II) oxide-modified carbon paste electrode  
 7 GN: graphite nanoparticle  
 8 SPR: surface plasmon resonance  
 9 pNIPAm: poly(*N*-isopropylacrylamide) microgel-based etalons  
 10 SERS: surface-enhanced raman spectroscopy  
 11 Ru@ethyl-SiO<sub>2</sub>: Ru(bpy)<sub>3</sub><sup>2+</sup> Doped vinyl-SiO<sub>2</sub> nanoparticles (Ru@ethyl-SiO<sub>2</sub>) and E2 imprinted  
 12 polypyrrole (MIP)  
 13 ECL: electrochemiluminescence  
 14 GOx/APT/AuNPs/CuS: glucose oxidase / aptamer / gold nanoparticles / copper sulfide  
 15 nanosheets  
 16 Au@Ag CS NPs: gold-silver core-shell nanoparticles  
 17 Anti-E2/ ITO/ErG/AuNP: anti estradiol antibody/indium tin oxide/graphene/gold nanoparticle.  
 18

1 *Figure 1*



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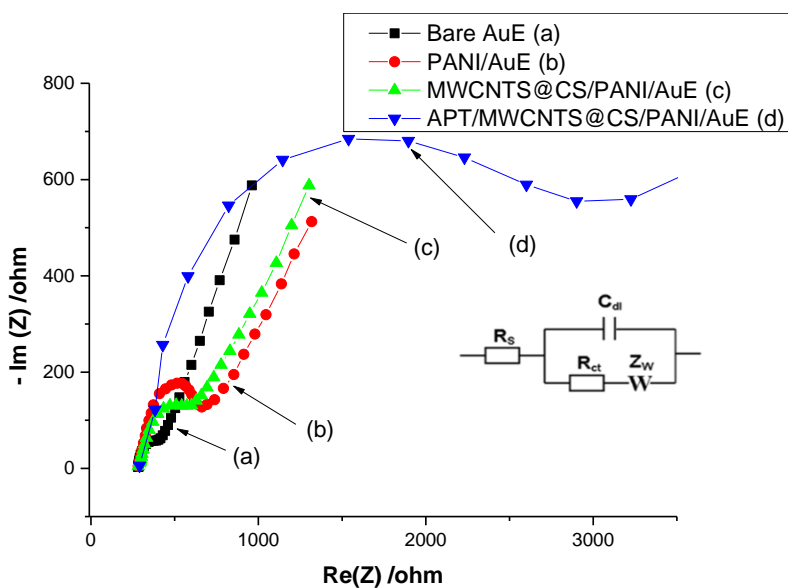
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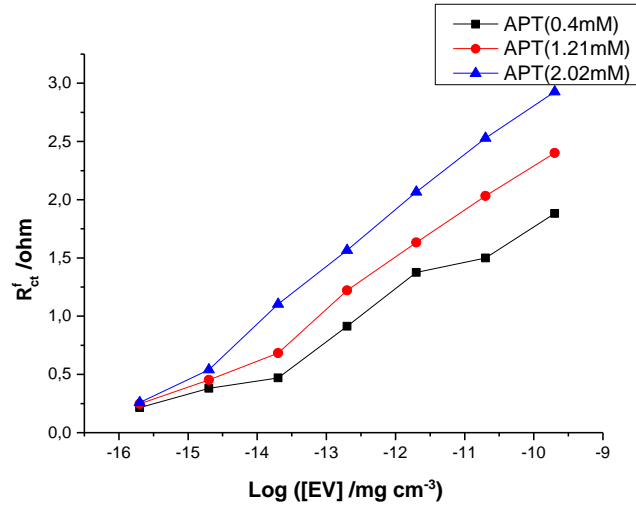
7 *Figure 2*



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1 *Figure 3*



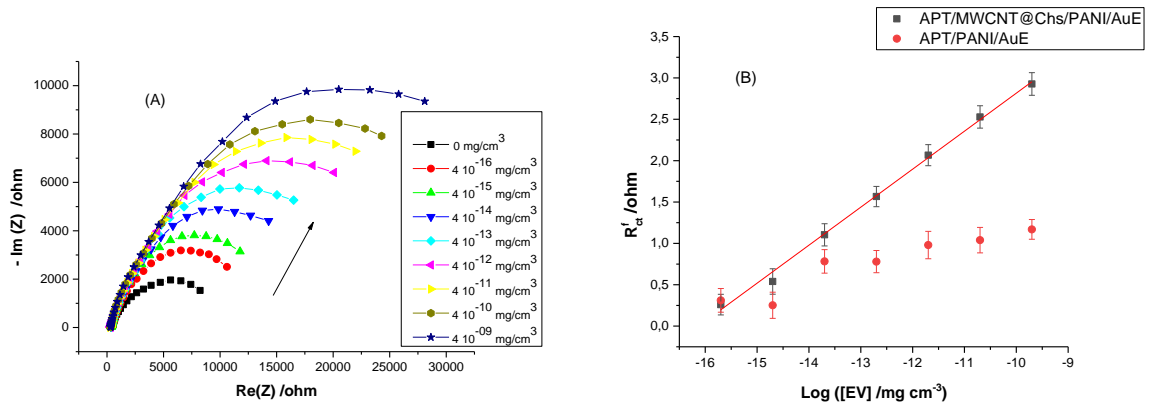
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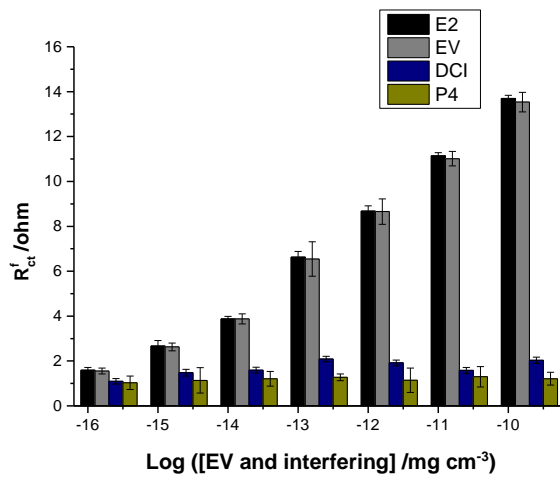
6 *Figure 4*



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1 *Figure 5*



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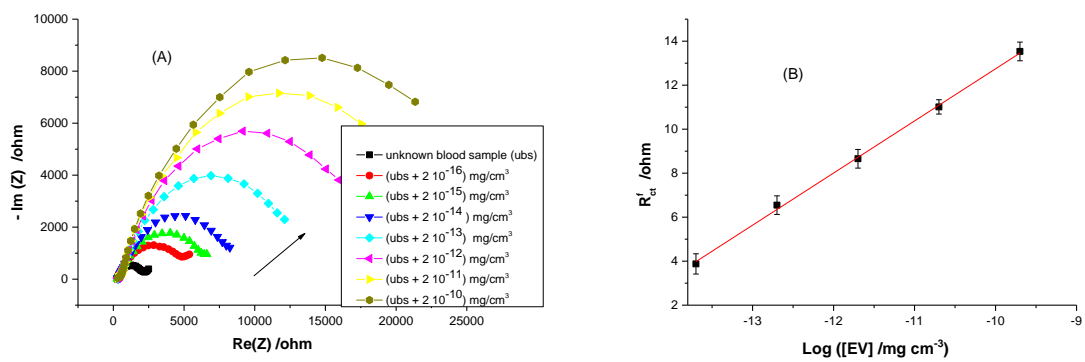
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7 *Figure 6*

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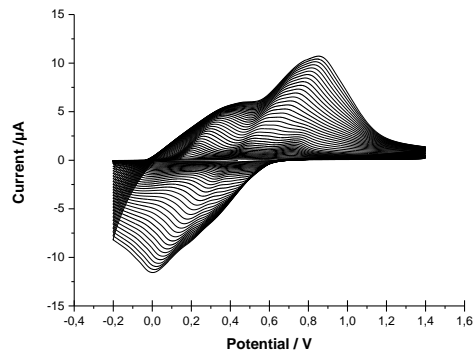


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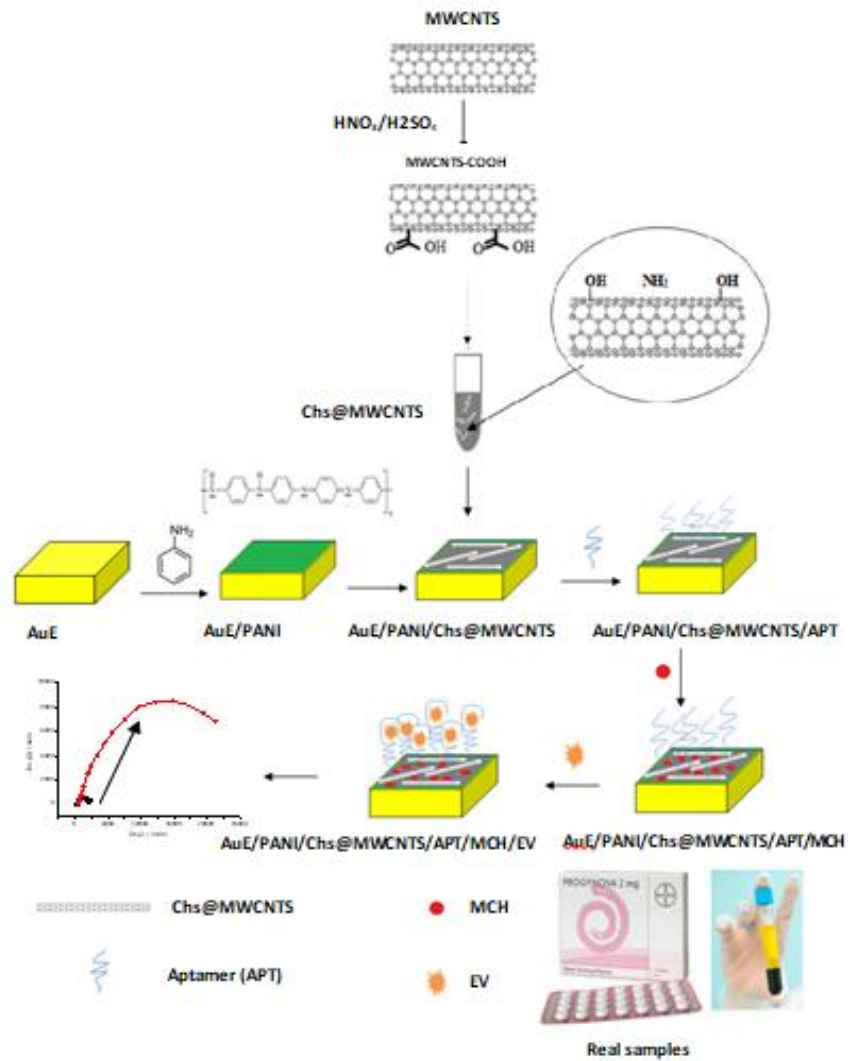
1 *Figure 7*



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4 *Figure 8*



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