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Complete List of Authors:	Peng, Anlin; Wuhan University Xiao, Jing; Wuhan No.3 Hospital Wang, Yuting; Puai Hospital, Huazhong University of Science and Technology Wang, Shi; Hubei University of Science and Technology Ding, Hong; Wuhan University, Department of pharmacy

Development of a novel and simple method for clinical therapeutic drug monitoring of aminophylline in human based on MWNTs-SiO_2/Au composites modified screen-printed electrode

Anlin Peng[1, 3], Yuting Wang[4], Jing Xiao[3], Shi Wang[2]**, Hong Ding[1]*

1 Department of Pharmacy, Wuhan University, Wuhan 430072, China
2 School of Pharmacy, Hubei University of Science and Technology, Xianning 437100，China
3 Department of Pharmacy Wuhan No.3 Hospital, Wuhan 430072, China
4 Department of Cardiology, Heart Center at Puai Hospital, Puai Hospital, Huazhong University of Science and Technology, Wuhan 430030, China

Abstract

The aminophylline is the drug that needs therapeutic drug monitoring in clinical. A novel disposable screen-printed electrode (SPE) modified with MWNTs-SiO2/Au composites for determination of aminophylline concentration in the blood samples was developed. The electrochemical performance of aminophylline at the MWNTs-SiO_2/Au SPE was explored by cyclic voltammetry (CV) and square wave voltammetry (SWV). The optimum experimental conditions were obtained and the currents of aminophylline presented a good linear property with the increasing of the concentrations under optimized conditions. The linear range of aminophylline was 5 μM to 200 μM with the detection limit 0.5 μM (S/N=3). The results of clinical blood samples were showed with good sensitivity and selectivity by the prepared sensor. Moreover, the drug concentration-time curve of aminophylline of clinical patient was attained at different time points such as 0.5h, 1h, and 2h by the proposed method. The results strongly suggested that the established sensor could be used for the practical monitoring in clinical individualized treatment.

Keywords: clinical therapeutic drug monitoring; aminophylline; disposable screen-printed electrode; clinical patient

1. Introduction

Theophylline and aminophylline are bronchodilator drugs and aminophylline is a modified form of theophylline, which contains the double salt of theophylline and ethylenediamine. Due to its better bioavailability, aminophylline is often applied to treat respiratory diseases including bronchial asthma, panting bronchitis, cardiogenic asthma.[1-4] Based on the reports,[1,2] the aminiphylline is the prodrug of theophylline and would release the theophylline quickly in the buffer solution (pH 7.4) and blood samples. So, the real working section of treatment is actually theophylline. While, due to the poor solubility in water, the ethylenediamine is used to increase the solubility of

* Corresponding authors: Prof. Hong Ding E-mail address: 912692441qq.com
Dr. Shi Wang E-mail address: wangshi413@gmail.com.

theophylline. Thus, the patients are often given the aminophylline treatment instead of theophylline. According to the literatures,[5,6] the therapeutic dose of the theophylline in blood serum is 5-20 μg/mL, but when the dose is more than 20 μg/mL, it could occasionally produce serous toxicity, including nausea, emesis, central nervous excitation et al..[7] Furthermore, the clinical cases show the patient would even die when the concentration of theophylline is 40 μg/mL or higher. In order to avoid this situation, clinical medication guideline of China states that it's required to monitor the clinical blood aminophylline concentration of patients.

Different analytical methods have been described for determination of aminophylline or theophylline. In general, they are high performance liquid chromatography,[8] spectroscopy,[9] chemiluminescence,[10] LC/MS[11] and electrochemical detection. [12-14] However, not all of these methods are suitable for the rapid detection of clinical blood aminophylline concentration. For example, HPLC or LC/MS method needs complicated pretreatment and time-consuming, which is not appropriate for the clinical rapid determination. In addition, spectroscopy and chemiluminescence methods cannot be used for clinical actual monitoring due to the low sensitivity and instability. However, electrochemical method presents the unique merits including high sensitivity and specificity, no complex pretreatment for analyte and fast detection.

In recent years, some researches have focused on the disposable screen-printed electrode (SPE) for drug determination. [14-17] Due to various advantages including low cost, small size, fast response, portability and disposability, the disposable SPE attracts more interest in electrochemistry[14-16] and is developed rapidly in different fields such as the monitor of glucose in blood and heavy metal in water.[17-19] Consequently, in our previous work, the disposable SPE is explored for the drug determination[20,21]. The result indicates that the disposable SPE could achieve therapeutic drug monitoring by electrochemical methods. Based on reported articles, [6,7, 12, 13], aminophylline or theophylline could be detected by electrochemical method. Therefore, we supposed that the SPE could detect the blood aminophylline concentration for clinical therapeutic drug monitoring.

In this work, a new sensor based on SPE is fabricated for analysis of aminophylline-containing blood samples. A goal is to achieve rapid detection for clinical samples without complicated pretreatment. And to our knowledge, there is no report concerning the fast determination of aminophylline in the clinical biological samples by using modified SPE, especially the clinical blood samples.

A lot of researches have exhibited that the multi-walled carbon nanotubes (MWNTs) could act as catalytic and enhance the sensitivity of detection due to the unique geometrical and chemical properties.[22-26] Furthermore, the previous studies reported that the MWNTs modified glassy-carbon electrode has shown superior performance for determination of theophylline or aminophylline with high sensitivity and specificity.[6,12,13] Nowadays, the nanoparticle composites have become the focus of sensor development because of their synergistic effect, such as the silicon dioxide (SiO_2) nanoparticles and colloidal nanoscale gold (nano-Au). SiO_2 nanoparticles, a new inorganic material, exhibit good ability of the immobilization to form films,

tunable porosity and the synergistic effect with other nanometer materials.[27,28] Many researches have used SiO_2 nanoparticles to develop electrochemical sensor.[29-31] Hence, it offers the theory basis for our experimental study. Nano-Au has been extensively applied to develop different sensors because of their unique structure and fascinating properties, including high effective surface area, excellent adsorption ability, good biocompatibility, and conductivity.[32,33] The literature reported,[34,35] AuNPs film could be confined on the SiO_2 nanoparticles by thermal treatments or carboxylation during acidic conditions for detection. While, the aminophylline sensor based on MWNTs, SiO_2 nanoparticles or/and nano-Au has not been reported. Therefore, this kind of attempt is carried out in present study.

In our work, a novel disposable SPE based on MWNTs-SiO_2/Au was described to detect aminophylline in clinical blood samples. The MWNTs-SiO_2/Au film combined the advantages of MWNTs, SiO_2 and nano-Au. MWNTs and nano-Au provided high sensitivity and selectivity, and SiO_2 made the composites film more firm because of the good mechanical and filming. This proposed sensor could offer a new and simple way for clinical therapeutic drug monitoring of aminophylline with few minutes. In addition, the modified SPE laid the foundation for the low cost, portable and miniaturized test tool, just like Glucose Test Strips.

2. Material and methods

2.1. Reagents and apparatus

Aminophylline was purchased from Sigma. The acidulated MWNTs（purity > 90%）was obtained from Chengdu Organic Chemicals Co., Lit, Chinese Academy Sciences (Sichuan, China). SiO_2 (15~20 nm, 99.9 %) was purchased from Aladdin Chemistry Co., Ltd (Shanghai). $HAuCl_4$ was obtained from Sigma. The stock solutions of aminophylline (1 mM) was prepared and stored at -20℃ prior to use. More diluted working solutions used in further studies were prepared daily by diluting different amounts of stock solutions with Tris-HCl buffer solution (0.05 M, pH 7.5). All chemicals and solvents used were of analytical grade.

As the Fig.1 showed, carbon working electrode, carbon auxiliary electrode and Ag/AgCl reference electrode were integrated on the strip like the Glucose Test Strips. The screen-printed electrode (SPE) is produced using polymeric commercial inks: ED427 (silver ink, Acheson, American), ED 423 SS (carbon ink, Acheson, American), CNC-01 (silver/silver chloride ink, CamNano, China), JUJO IN-15M (insulator ink, JUJO, Japan). This kind of SPE replaced the traditional three electrode system. The EC 570 electrochemical workstation (Gaoss Union Technology, Wuhan, China) was employed to record the voltammograms by SPE. Field Emission Scanning Electron Microscope (FE-SEM) instrument (Quanta 200, FEI Coropration, Holland) and UV-Vis spectroscopic experiment were performed for the characterization of modified SPE.

2.2 Synthesis of nanoscale gold

Based on the literature,[32,33] the colloidal nanoscale gold (nano-Au) was synthesized according to standard method with slight modification. Briefly, 100 mL of

0.01% $HAuCl_4$ solution was stirred in a three round-bottom flask in oil bath (at 100 ℃). After that, 2.0 ml sodium citrate (1%, wt %) was immediately added into the solution, and constantly heated and stirred for 10 min. The color of solution gradually turned to brownish red and then the nano-Au was obtained. The UV-Vis spectroscopic experiment was further carried out.

2.3 Preparation of MWNTs-SiO_2/Au SPE

The bare screen-printed electrode was coated with MWNTs-SiO_2/Au as follows. The 2 mg acidulated MWNTs and 2 mg silicon dioxide nanoparticles were mixed in 1 mL aqueous solution with ultrasonication for 2 h. Next, the 1.5 μL of MWNTs-SiO_2 was dropped on the carbon working electrodes, air-dried for 30 min at room temperature. After that, the MWNTs-SiO2/Au SPE was prepared by coating the working electrode surface with 1.5 μL colloidal nanoscale gold. The flow-process diagram of modification was shown in Fig.1. For comparison, MWNTs SPE and MWNTs-SiO_2 SPE were prepared and applied to the further investigation.

2.4 Electrochemical procedure

The appropriate amounts of aminophylline were added to the sample solutions containing 0.05 mol L^{-1} Tris-HCl (pH 7.5) buffer solution. The cyclic voltammetric curves (CV) and square wave voltammogram (SWV) were respectively recorded over the potential range from 0 V to 1.2 V and 0.1 to 1.2 V or 1.3Vversus the Ag/AgCl reference electrode. The accumulation step was carried out under open-circuit for 4 mins. All electrochemical experiments were performed at room temperature. The CV relevant parameters were as follows: initial potential = 0.0 V, end potential = 1.2V, sample interval = 1 mV, scan rate = 100 mV. And the SWV relevant parameters were set as: initial potential = 0.1 V, end potential = 1.2V or 1.3V, step width =5 mV, amplitude =25 mV, frequency =15 Hz.

2.5 Analysis of real sample

The clinical patient blood samples were acquired from the clinical volunteers in Wuhan NO.3 Hospital. According to the literature,[36,37] clinical blood samples were collected from the asthma patient volunteers after signing informed consent. The male volunteer was 82 kg and then administered slow intravenous drip at the dose of 0.25 g aminophylline. Considering patient's will and right, the blank blood sample was taken as 0 h measuring point before intravenous drip administration, and then the aminophylline-containing blood samples were obtained at 0.5, 1, 2 and 4 h after administration. Next, the samples were used for experiments without any pretreatment and diluted for the electrochemical assays. Each sample was diluted from 1.0 mL to 2.0 mL in electrolytic cell with Tris-HCl buffer solution (pH 7.5) for direct determination by square-wave voltammetry (SWV).

All experiments were performed in compliance with the relevant laws and institutional guidelines. Wuhan University and Wuhan NO.3 Hospital institutional committees have approved the experiments. Besides, informed consent was obtained for any experimentation with human subjects. The protocol number is Wuhan No.3

Hospital Ethics Committee 2014-063.

3. Result and discussion

3.1 Characterization of modified screen-printed electrode (SPE)

The surface morphology of screen-printed electrode was evaluated by scanning electron microscopy (SEM). The SEM images of MWNTs SPE (A), MWNTs-SiO_2 SPE (B) and MWNTs-SiO_2/Au (C) were shown in Fig.2. The multi-walled carbon nanotubes ranged from 150 to 200 nm in diameter (Fig.2A). From the Fig.2B, it could be observed that the nanostructured SiO_2 was polymerized to the carboxylated MWNTs. Due to the size effect, the surface area obviously increased. The synthesized colloidal nanoscale gold was developed to immobilize the composite film on the working electrode in the Fig.2.C. The colloidal nanoscale gold not only displayed excellent electro catalytic competence, but also produced good film-forming property. In addition, the Fig.2.D indicated the UV-vis absorption spectra of synthesized nano-Au. The colloidal nanoscale gold solution appeared an absorption peak at 530 nm due to the characteristic absorption peak of AuNPs. The diameter is about 50 nm relevantly. The result was consistent with the literatures.[38.39]

3.2 Electrochemical activity of aminophylline at MWNTs-SiO_2/Au SPE

First, the electrochemical characterization of the analyte at screen-printed electrode (SPE) was performed by means of cyclic voltammetry. As shown in Fig.3.A, the bare SPE exhibited a small background current (curve b) in the blank Tris-HCl buffer. While, one irreversible oxidation peak of 100 μM aminophylline (curve a) appeared at the bare SPE. This result told that the SPE could be applied for determinatio n of aminophylline. Next, the CV curves of 100 μM aminophylline at the different modified SPE were showed as Fig.3.A. Compared with the CV curve a, it could be obtained that the peak currents of aminophylline enhanced relatively at the modified SPE (curves c, d, e). But the oxidation peak obtained by CV method was not outstanding and the sensitivity was not high. So we choose the SWV method due to the higher sensitivity.

Then, the detailed electrochemical behavior of aminophylline (20 μM) at MWNTs-SiO_2/Au SPE was characterized with SWV in 0.05 M Tris-HCl buffer (pH 7.5). While, the oxidation peak (curve d) at about 0.92V was obtained at MWNTs-SiO_2/Au. As shown in the Fig.3.B, at bare SPE, a wide anodic peak (curve a) at about 0.98V was observed. Whereas, the MWNTs SPE (curve b) well improved the oxidation peak at about 0.96 V of 20 μM aminophylline. The increase could be ascribed to the high surface area and excellent electro conductivity of MWNTs. Besides, the peak currents enhanced further at MWNTs-SiO_2 SPE due to the nano-size effect of SiO_2 nanoparticles (curve c). But the enhancement in current response was not huge. In our previous experiment, the MWNTs film and the MWNTs-SiO_2 were possibly rinsed off by the electrochemical detection samples. So, as the fixed film and catalytic material, the colloidal nanoscale gold acted as an important role. The MWNTs-SiO_2/Au SPE was more stable and sensitive to detect the aminophylline samples. Curve d in Fig.3.B strongly suggested that MWNTs-SiO_2/Au could effectively catalyze the electrooxidation of aminophylline and greatly improve

the peak shape. The large increase and the small shift in oxidation potential could be attributed to the large surface area, more stable subtle electronic properties of MWNTs and the ion-exchange characters of SiO_2 nanoparticles. Meanwhile, the colloidal nanoscale gold could combine the advantages of all of them and accelerate electron transfer significantly. Hence, the MWNTs-SiO_2/Au SPE could be used to determinate the real samples.

3.3 Influence of pH

The influence of pH values of supporting electrolyte on the behavior of aminophylline at MWNTs-SiO_2/Au SPE was investigated by SWV (Fig. 4). It was observed that the oxidation peak current of 50 μM aminophylline increased to the maximum at pH 7.0-7.5. However, when the pH further increased, the oxidation peak current decreased. This indicated the pH 7.0-7.5 was favorable to the electrochemical reaction. While, the pH of 7.5 was closer to the pH of blood samples. Therefore, the pH 7.5 was chosen as the optimum pH value for the following sample determination.

Furthermore, the anodic peak potential of aminophylline was found to shift to negative values along with the increasing pH. The plot of peak potential versus pH was obtained with a linear regression equation Ep (V) = 1.22849-0.04051pH (R=0.9951) in the range of pH 6.5-9.0. The value of slope revealed that the number of electron transfer was equal with that of hydrogen ions taking part in the electrode reaction.[6]

3.4 Influence of the scan frequency

The effect of different scan frequencies was studied for determination of 50 μM aminophylline. The constant scan increment and the pulse amplitude were 6 mV and 25 mV, respectively. From Fig.5, it can be seen that the peak current increased first and then decreased with increasing frequency in the range of 10–40 Hz. The peak current reached the maximum at 15Hz. Also, the peak potential E_p shifted to a little more positive values along with the increasing of frequency, the peak potential versus the scan frequency was found to be linear with following equations: Ep(V) = 0.8754+0.0017f (Hz). When the 15Hz was chosen for the determination, the peak potential was about 0.90V.

3.5 Influence of accumulation time

Accumulation time is usually an important factor to enhance the determining sensitivity. Hence, the influence of accumulation time on the peak current was also studied by SWV. As shown in Fig.6, without applying potential in the working electrode, the peak currents of 50 μM aminophylline increased with accumulation time up to 4 min, and then peak current increased much slightly as further increasing accumulation time owing to the saturation occurred in binding sites at MWNTs-SiO_2/Au SPE. Thus, duration of 4 min was selected as accumulation time for further real sample detection.

3.6 Calibration curve and real sample analysis

During the experiment, the disposable sensor was similar to the Glucose Test Strips, and each screen printed electrode (SPE) was discarded after a single use. SWV curves of aminophylline at MWNTs-SiO_2/Au SPE were attained after the optimized determination condition was established. Prior to the practical clinical blood assays, various concentrations of aminophylline in the 0.05 M Tris-HCl (pH 7.5) were examined with SWV. And the SWV relevant parameters were as follows: initial potential = 0.1 V, end potential = 1.2V, step width =5 mV, amplitude =25 mV, frequency =15 Hz. The MWNTs-SiO_2/Au SPE presented an oxidation wave at about 0.92 V. From the Fig.7, we could observe that the peak currents of the aminophylline were increased linearly with the increasing of the analytic concentration. The linear response for aminophylline was 5 μM to 200 μ M with a regression equation of Ip (μA) = 0.0368c (μM) +1.0535 (R=0.9968). Based on the signal-to-noise ratio of 3, the detection limit was found to be 0.5 μM.

In order to investigate the practical application of the fabricated electrode, the calibration procedure of the human blood samples was established. The clinical patient blood sample was diluted 1:1 with the supporting electrolyte. For example, 1.0 mL blood sample was diluted to 2.0 mL with the supporting electrolyte containing different concentration aminophylline. Then, 0, 10, 20, 40, 60, 80, 100 and 200μM aminophylline-containing blood samples were attained. Based on the voltammetry procedure mentioned above, the calibration curves of blood aminophylline samples were obtained and are shown in Fig.8. Due to the biological fouling of blood sample, [40] the accumulation of aminophylline on the surface of modified SPE might be influenced in a certain degree. Relevantly, the detection range and limit in blood sample were also different. From Fig.8, after repeated five times, the SWV curves indicated that the peak current was linear with the aminophylline concentration in the range 10 μM to 200 μM , and the linear regression equation was expressed as Ip (μA) = 0.0270c (μM) +0.2597 (R=0.9954) with the detection limit of 5 μM. The curve a reminded that the oxidation peaks at about 0.2V and 0.7V appeared in the blank blood sample. According to the reports,[7] the obtained peak might be due to the oxidation of uric acid and xanthine. After the addition of different concentration aminophylline to the blank blood samples, the oxidation peak was gained at about 1.03 V. This result was similar to the previous study and the aminophylline detection was not disturbed.[6,7]

Moreover, as listed in the Table 1, the method developed in this study was compared with other methods in the literature.[6-13,35,41-44]To our knowledge, no paper had been reported about the SPE for determination of clinical blood samples. This was the first study for detection of clinical aminophylline using MWNTs-SiO_2/Au SPE. The experimental data indicated that MWNTs-SiO_2/Au SPE could be successfully applied to directly determinate clinical whole blood samples. Moreover, compared to serum samples, the whole blood sample detection in this work does not

need deproteinization, centrifugalization and quantification pretreatments. It not only simplifies the clinical aminophylline detection process, but also reflects more accurate aminophylline concentration in blood. This method could bring many advantages in instantaneous measurement and monitoring for doctors.

3.7 Interference, reproducibility and stability

To assess the selectivity of the MWNTs-SiO_2/Au SPE, the various interfering substances such as glucose, uric acid and so on in the biological samples were studied in the presence of 50 μM. The result indicated that 200 μM glucose, ascorbic acid, uric acid, 500 μM cations and anions had no interference to the aminophylline (the relative error of signal peak change below 5%). These findings proved that the proposed sensor had good selectivity for the determination of real clinical samples.

The repeatability of MWNTs-SiO_2/Au SPE was investigated for responding to 50 μM aminophylline. The relative standard deviation (RSD) of 6.84% on different electrodes was gotten for ten assays samples. In addition, the stability of MWNTs-SiO_2/Au SPE was evaluated by recording its response to 50μM aminophylline over 30 days. After stored at 4 ℃ refrigerator for 30 days, the current response of the sensor still remained up to 94.2% (n=5). The result demonstrated the reliability of the fabrication sensor and its excellent reproducibility and high stability.

3.8 Application for clinical therapeutic drug monitoring

In our work, the time course of aminophylline about the clinical patient blood samples was also carried out to verify the practicability of MWNTs-SiO2/Au SPE. As mentioned in the experiment section, the aminophylline concentrations of the clinical blood samples were monitored. Before intravenous drip administration 0 h, the aminophylline concentration is detected zero for eliminating previous aminophylline residual reference interference. After intravenous drip administration, blood samples were obtained at different time points of 0.5 h, 1 h, and 2 h. The Drug Concentration-Time Profile was shown in the Fig.9. The aminophylline declined in whole blood sample over time, which the result was in agreement with the reports.[33,34] Besides, the blood concentration of aminophylline was lower than the detection range after dosing aminophylline 4 hours. The experimental dates further indicated the proposed method could be applied for the clinical therapeutic drug monitoring.

4. Conclusion

In this work, a new and simple method was performed to detect the aminophylline concentration for clinical therapeutic drug monitoring by sensor modified with MWNTs-SiO_2/Au composites. The MWNTs-SiO_2/Au film exhibited well-defined redox response to aminophylline oxidation with excellent stability and efficient electrocatalytic activity. The electrochemical oxidation peak of aminophylline at modified sensor was attained at about 1.0 V by square wave voltammetry. The oxidation peak currents of aminophylline were linear with concentrations. The detection results of clinical samples indicated that the proposed sensor displayed

excellent characteristics, such as low production cost, easy realization of industrialization, high sensitivity, and rapid simple analysis procedures. Based on the above points, the established sensor provided possibility to produce the clinical medical diagnosis apparatus and instrument for determination of clinical aminophylline, just like Glucose Test Strips.

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Statement

All experiments were performed in compliance with the relevant laws and institutional guidelines. Wuhan University and Wuhan NO.3 Hospital institutional committees have approved the experiments. Besides, informed consent was obtained for any experimentation with human subjects. The protocol number is Wuhan No.3 Hospital Ethics Committee 2014-063.

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Figure captions

Figure 1 Preparation process of screen printed electrode (SPE) and the flow-process diagram of modification (a—PVC sheets; b—silver conducting paths; c—working electrode; d—auxiliary electrode; e—reference electrode; f—insulation layer)

Fig.2 FE-SEM images of MWNTs modified SPE (A), MWNTs-SiO_2 modified SPE (B), MWNTs- SiO_2/Au modified SPE (C) and UA-vis absorption spectra of Au (D)

Figure 3.A Cyclic voltammogram curves of blank sample (curve b), 100 μM aminophylline at bare SPE (curve a), MWNTs SPE (curve c), MWNTs-SiO_2 SPE (curve d) and MWNTs-SiO2/Au SPE (curve e) in 0.05 M Tri-HCl buffer (7.5), scan rate: 100mV/s.
Figure 3.B Square wave voltammograms of 50 μM aminophylline at bare SPE (curve a), MWNTs SPE (curve b), MWNTs-SiO_2 SPE (curve c) and MWNTs-SiO2/Au SPE (curve d) in 0.05 M Tri-HCl buffer (7.5), scan frequency:15 Hz.

Figure 4 Square wave voltammogram curves of 50 μM aminophylline at different pH: (a to f) 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 on the electrochemical response of 50 μM aminophylline in 0.05M Tri-HCl buffer at the MWNTs-SiO2/Au SPE; Insert: the currents of aminophylline response to pH, the relationship of the peak potential Ep of against pH.

Figure 5 Square wave voltammograms of 50 μM aminophylline at different frequency：(a-g) 10, 15, 20, 25, 30, 35 and 40 Hz; scan increment of 6mV and pulse amplitude of 25mV; the other experimental conditions are the same as those described above.

Figure 6 Relationship between accumulation time and peak currents of 50 μM aminophylline in 0.05M pH 7.5Tris-HCl buffer (pH 7.5), scan frequency 15Hz.

Figure 7 Square wave voltammograms of different concentration aminophylline in 0.05 M Tris-HCl (pH 7.5): (curve a to h) 0, 5, 10, 20, 40, 80, 100, 200μM (scan frequency 15Hz).

Figure 8 Square wave voltammograms of different concentration aminophylline in clinical blood samples: (curve a to h) 0, 10, 20, 40, 60, 80, 100, 200μM (scan frequency 15Hz).

Figure 9 The clinical blood sample concentration-time course of aminophylline before and after administration of aminophylline (0.25g/82kg).

Table1. Comparison of the linear range and detection samples obtained at the MWNTs-SiO_2/Au SPE for determination of aminophylline with other methods.

Methods	Linear range (μM)	Detection samples	Reference
HPLC	10.0-400.0	Capsules	[8]
spectroscopy	60.0-230.0	Injection	[9]
chemiluminescence	0.05-5.0	Serum samples	[10]
LC/MS	0.28-28.00	Rabbit plasma	[11]
MIP/CNPs-SO_3H GCE	0.05-30.0	Drug sample	[6]
AT-AVNps GCE	20.0-240.0	Human blood serum	[7]
K_2PtCl_6 CPE	17.5-87.5	Cosmetic and tablet	[12]
Nafion/lead oxide pyrochlore GCE	20.0-100.0	Tea and tablet	[13]
GNP-CHIT-IL hybrid/r-GO GCE	0.05-2.0	Tea and tablet	[35]
MnOx/NH_2-IL/Chit GCE	1.0-120.0	Table sample	[41]
GNP/l-cys/Gr/Nafion GCE	0.04-60.0	Tea and tablet	[42]
1,4-BBFT/IL GPE	12.0-1200.0	Tea and blood serum	[43]
CdSe GCE	40.0-700	Food and tea drink	[44]
MWNTs-SiO_2/Au SPE	10.0 -200.0	Clinical whole blood	This work

HPLC: high performance liquid chromatography

LC/MS: liquid chromatography/mass

MIP/CNPs-SO_3H GCE: molecularly imprinted polymer/Carbon nanoparticles-SO_3H glassy carbon electrode

AT-AVNps GCE: aminotriazole grafted gold nanoparticles films on glassy carbon electrode

K_2PtCl_6 CPE: potassium hexachloroplatinate modified carbon paste electrode

Nafion/lead oxide pyrochlore GCE: Nafion®/lead – ruthenium oxide pyrochlore

chemically modified glassy carbon electrode

GNP-CHIT-IL hybrid/r-GO GCE: gold nanoparticle – chitosan –ionic liquid/graphene modi fied glassy carbon electrode

MnOx/NH2-IL/Chit GC: manganese oxide nanoparticles/ionic liquid/chitosan nanocomposite modified glassy carbon electrode

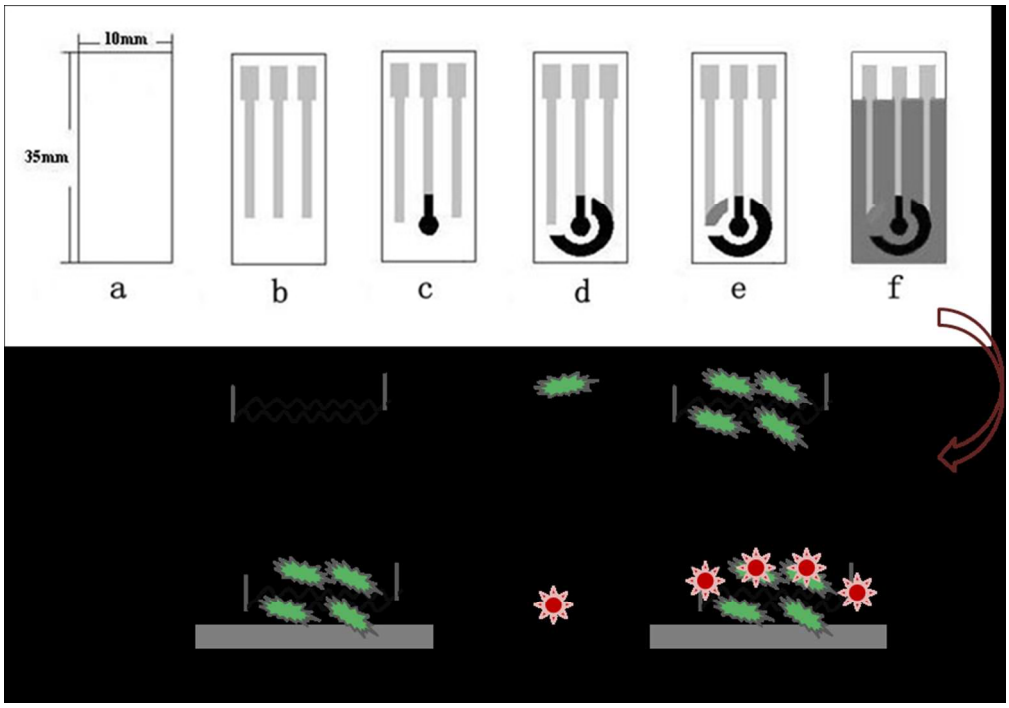
GNP/l-cys/Gr/Nafion GCE: gold nanoparticles/l-cysteine/Graphene/Nafion modified glassy carbon electrode

1,4-BBFT/IL GPE: 1-(4-bromobenzyl)-4-ferrocenyl-1H-[1,2,3]-triazole/ionic liquid modified graphene paste electrode

CdSe GCE: cdSe microparticles modified glassy carbon electro

LIVE SUBJECT STATEMENT:

The author should include in the Methods/Experimental section of the manuscript a statement that all experiments were performed in compliance with the relevant laws and institutional guidelines, and also state the institutional committee(s) that have approved the experiments. The author should also include a statement that informed consent was obtained for any experimentation with human subjects.



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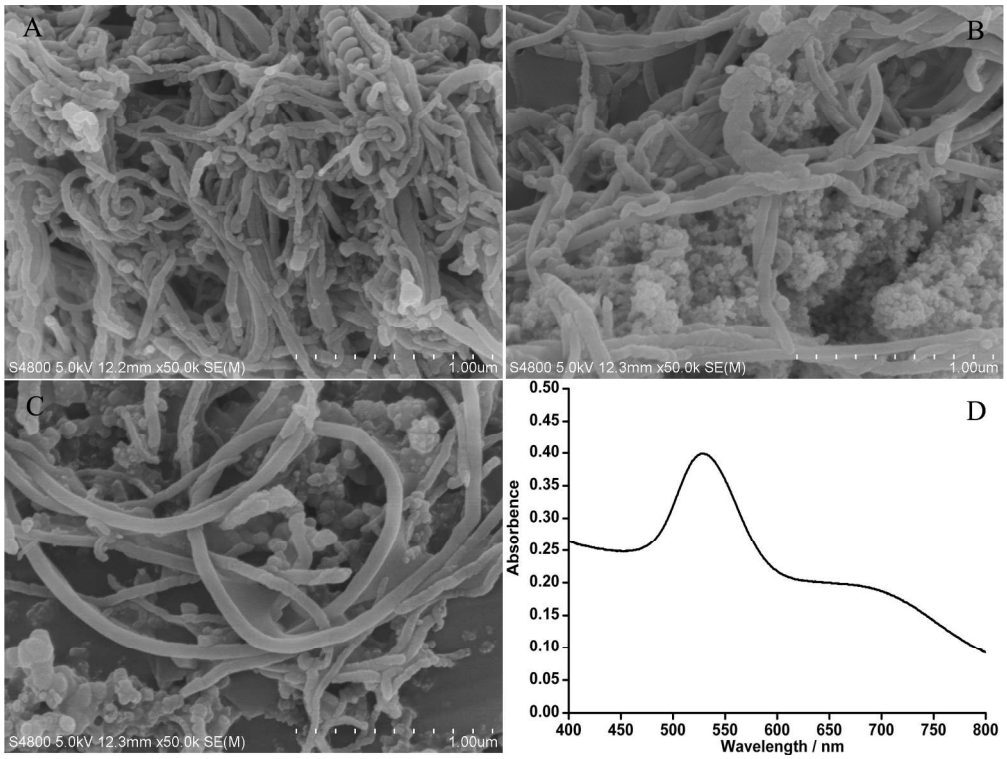

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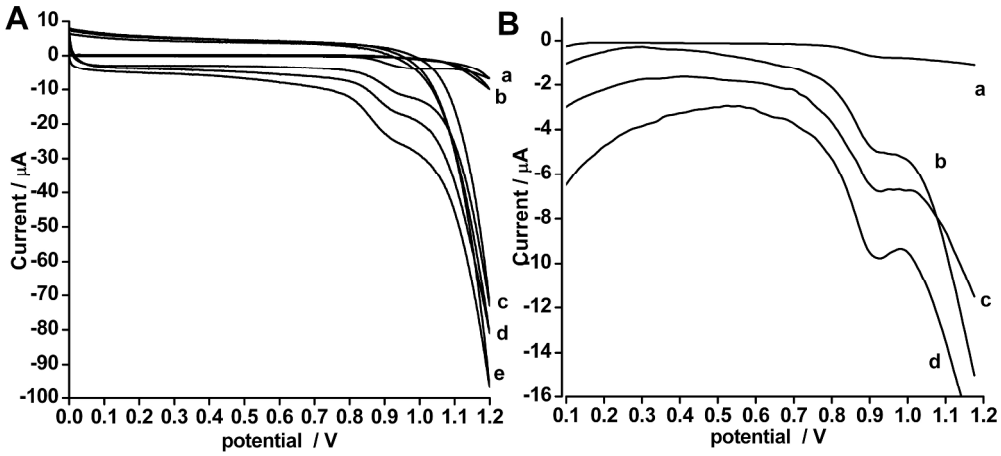

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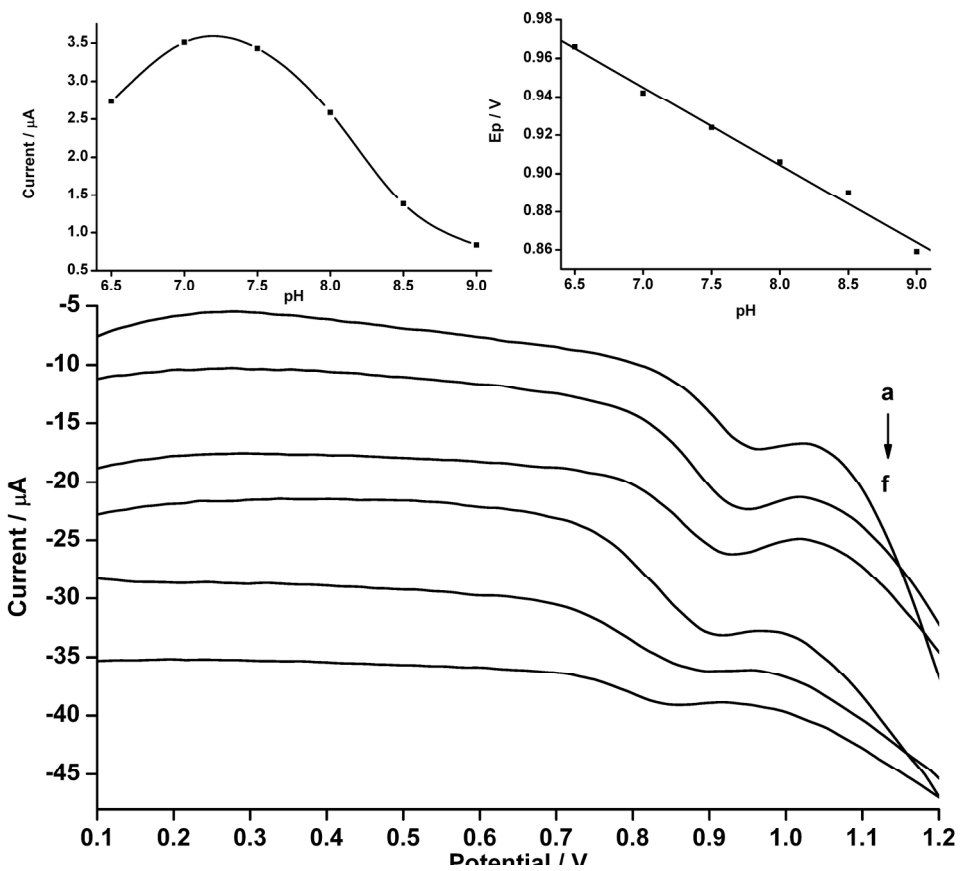

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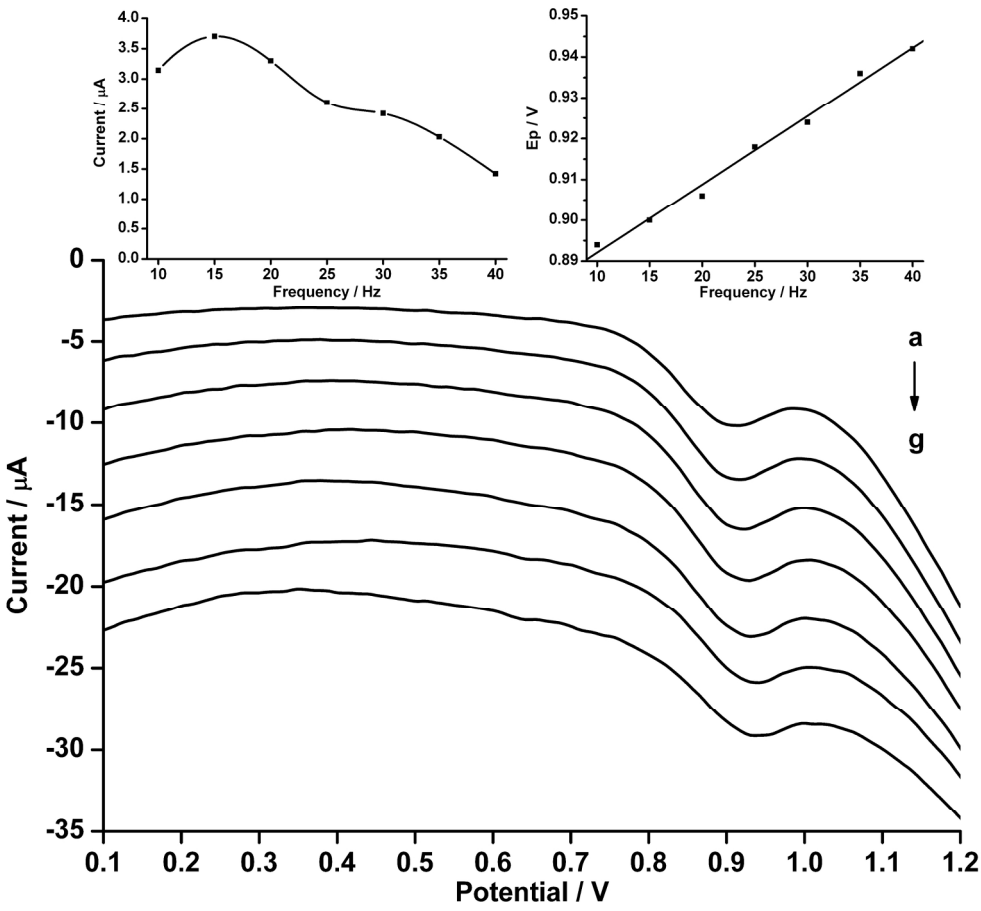

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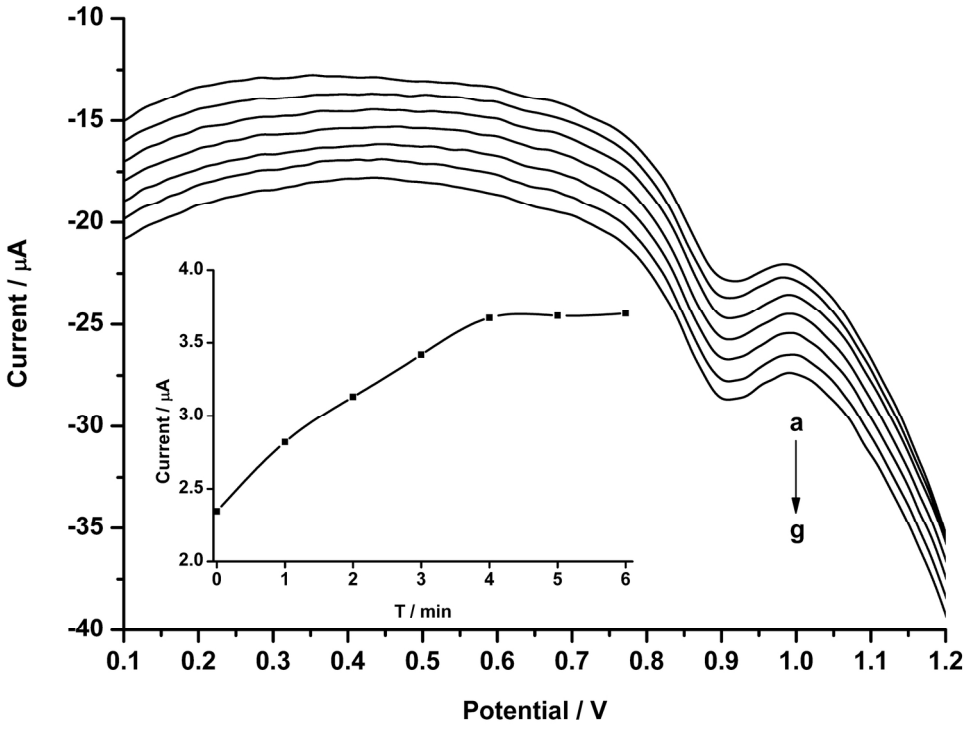

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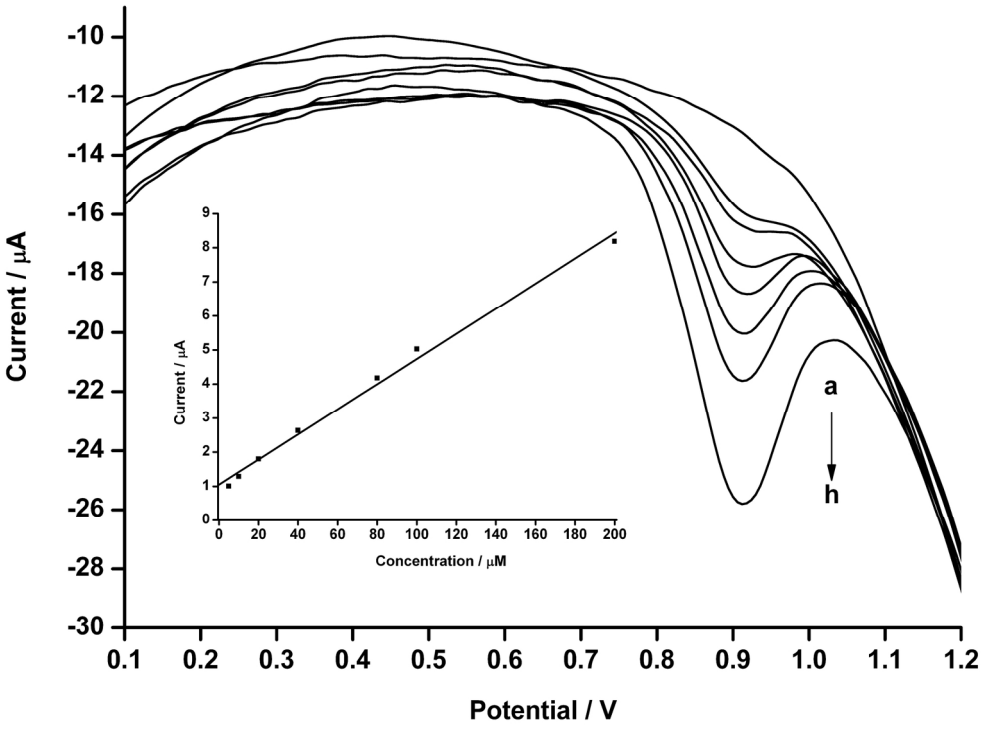

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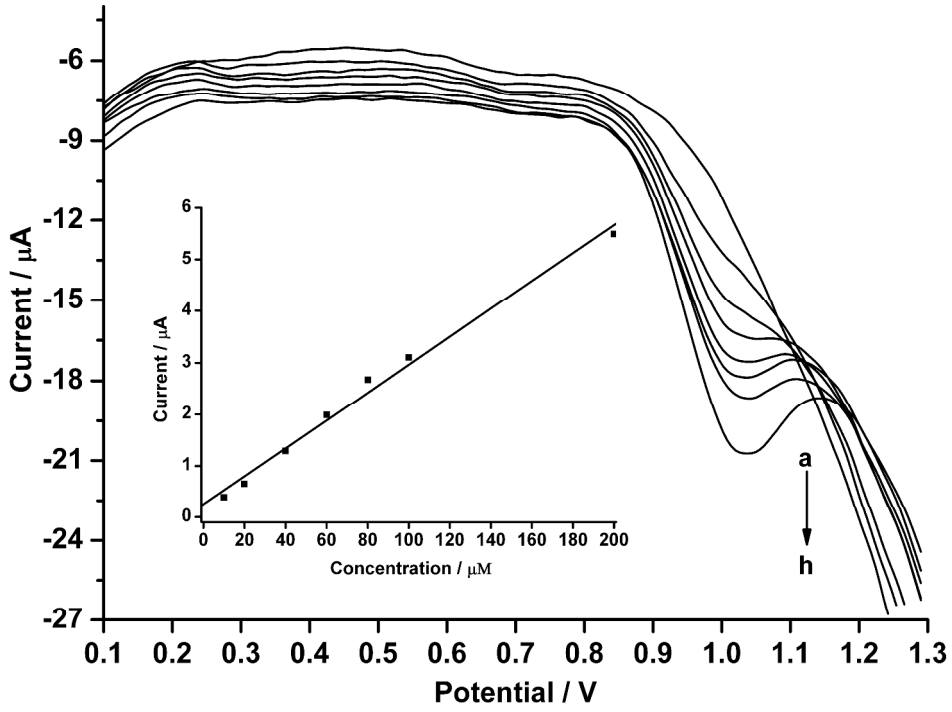

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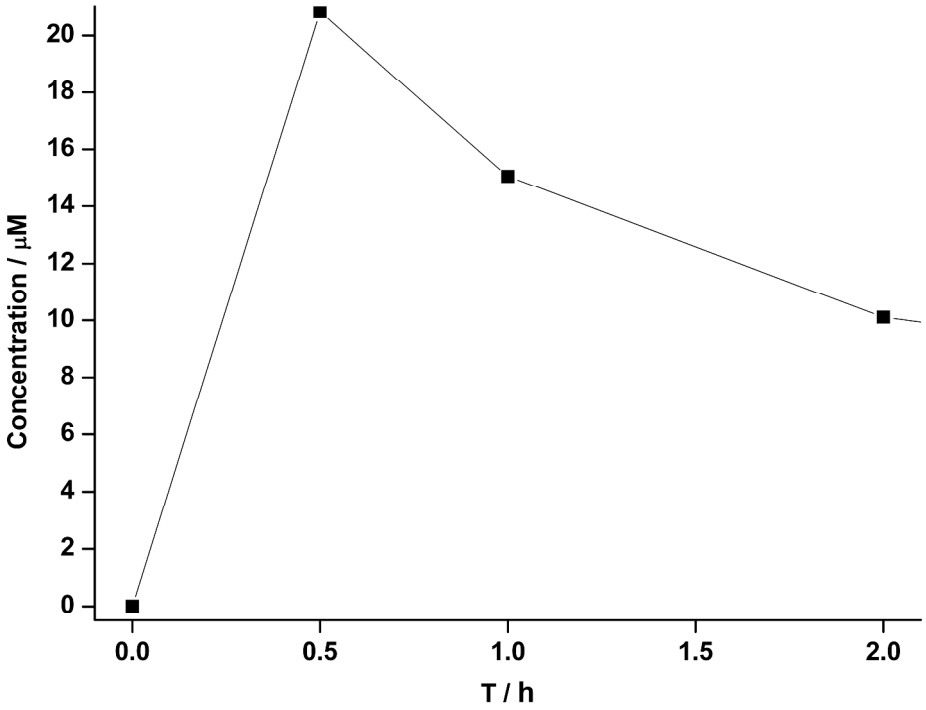

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