Determination of Clobetasol Propionate by Multi-walled Carbon Nanotube-modified Carbon Paste Electrode using Cyclic Voltammetry

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We studied the electrochemical behaviour of Clobetasol Propionate (CP) on a multi-walled carbon nanotube-modified carbon paste electrode (MWCNTs-CPE) and developed a corresponding determination method. Sample solutions were scanned using cyclic voltammetry (CV) with a homemade MWCNTs-CPE as a working electrode in KH_2PO_4-$Na_2B_4O_7$ buffer solution (pH=4.90), and the peak potential and peak current of the cyclic voltammogram were recorded. CP showed a clear oxidation peak near 1.16 V, and the CP concentration exhibited an excellent linear relationship with peak current in the range of 1.0×10^{-4} to 1.0×10^{-6} mol/L, with a correlation coefficient of 0.999 and a detection limit of 3.45×10^{-8} mol/L. The content of CP in a facial mask was determined by this method, the recovery rate was between 98.0% and 106.2% in the presence of interfering substances, and the relative standard deviation (RSD) ranges between 2.0% and 4.1% (n=5). The MWCNTs-CPE showed a good electrochemical response to CP and possessed high sensitivity. This method can be applied to the determination of CP in cosmetics and pharmaceuticals.

1. INTRODUCTION

Clobetasol Propionate (CP), a white crystal or crystalline powder, is a synthetic high-efficacy topical glucocorticoid drug with strong anti-inflammatory, anti-itch, and capillary constriction properties [1,2]. The anti-inflammatory effect of CP is approximately 112.5, 2.3, and 18.7 times that of hydrocortisone, betamethasone sodium phosphate, and fluocinolone, respectively. In addition, CP can inhibit cell mitosis and effectively penetrate into the stratum corneum of skin and is a type of drug with numerous clinical applications, but this compound is a prohibited ingredient in cosmetic products [3-5].

At present, the main methods for detecting CP include thin-layer chromatography (TLC) [6,7], spectrophotometry [6,8], high-performance liquid chromatography (HPLC) [9-11], and HPLC-mass (HPLC-MS) [12,13]. However, the TLC and spectrophotometry methods have a low accuracy, the HPLC and HPLC-MS methods require sophisticated sample pretreatment, and the LC-MS instruments and measurement process are costly. In addition, these methods are not suitable for rapid on-site detection. In comparison, electrochemical methods are simple, fast, and accurate and have unique advantages over the abovementioned methods. To date, there have been only a few studies on the measurement of glucocorticoids by electrochemical methods. For example, Oliveira used cyclic voltammetry (CV) and square wave voltammetry to determine the content of dexamethasone in injections and eye drops, showing a recovery rate of 94.14 to 112.41% and a detection limit of 2.54×10^{-9} mol/L [14]; Smajdor used CV in combination with a mercury membrane electrode to determine the content of hydrocortisone in pharmaceuticals, demonstrating a recovery rate of 90 to 107% and a detection limit of 4.8×10^{-9} mol/L [15]. The low detection limits of these methods were attributed to the use of mercury-containing electrodes. Mercury is volatile, highly toxic to humans and, therefore, not recommended for use in general laboratories.

In this study, a simple and safe method for measuring CP by CV with a multi-walled carbon nanotube-modified carbon paste electrode (MWCNTs-CPE) [16,17] was established for the first time. The method was applied to the measurement of CP content in facial mask samples, and satisfactory results were obtained. The sensitivity of measurements can be improved with different modifiers or modification methods. Therefore, this method can be used for determination of trace amounts of CP in cosmetics and pharmaceuticals.

2. EXPERIMENTAL

2.1 Raw materials, reagents, and instruments

An Ingsens-1030 electrochemical workstation manufactured by Guangzhou Ingsens Sensor Technology Co., Ltd. was used. The homemade carbon paste electrode (CPE, Φ3 mm) or MWCNTs-CPE were used as the working electrode, and a Ag-AgCl (in saturated KCl) electrode and platinum electrode were purchased from Shanghai Chenhua Instrument Co., Ltd. and used as the reference electrode and the counter electrode, respectively. The KQ118 ultrasonic cleaner was manufactured by Jiangsu Kunshan Ultrasonic Instrument Co., Ltd. The pHS-25 pH meter was manufactured by Shanghai Zhiguang Instrument & Meter Co., Ltd.

The CP standard was purchased from the China's National Institute for the Control of Pharmaceutical and Biological Products. Graphite powder, MWCNTs, liquid paraffin, KH_2PO_4, and $Na_2B_4O_7$ were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.; most of the facial mask samples were purchased from general supermarkets in Guangzhou, and the positive facial mask samples were provided by the Guangzhou Institute for Drug Control. The reagents used in the experiment were of analytically pure grade, and the water was deionised water.

2.2 Solution preparation

2.2.1 Preparation of buffer solution

KH_2PO_4 (6.800 g) was accurately weighed and dissolved in a small amount of deionised water, and then deionised water was used to dilute the solution to the mark in a 250 mL volumetric flask to obtain 0.2 mol/L KH_2PO_4 buffer solution, which was stored in a refrigerator (4°C) for future use. The pH was adjusted with $Na_2B_4O_7$ as needed.

2.2.2 Preparation of standard solution

The CP standard (0.4670 g) was accurately weighed and first dissolved with a small amount of absolute ethanol, and then a buffer solution was used to dilute the solution to the mark in a 50 mL brown volumetric flask to obtain a 1×10^{-7} mol/L CP standard solution, which was placed in a refrigerator (4°C) for future use. The solution was diluted with buffer solution to the required concentration.

2.3 Preparation of MWCNTs-CPE

After MWCNTs and graphite powder were uniformly mixed at a ratio of 1:7 (m:m), an appropriate amount of liquid paraffin was added, and the mixture was stirred and ground into a non-flowable paste, placed in a glass tube with a diameter of approximately 3 mm, and pressed tightly. A copper wire was inserted to establish an electrical contact and fixed with tape. The surface of the front of the electrode was smoothed and polished with a piece of smooth paper for subsequent use.

2.4 Experimental methods

A certain amount of test sample was accurately measured in a 25 mL beaker, followed by adding KH_2PO_4-$Na_2B_4O_7$ buffer solution to a total volume of 10mL. The three-electrode system was inserted, and CV measurement of the test sample solution was performed at a set scan rate within the potential range of 0.5 V to 1.4 V. The peak potential (E_p) and peak current (I_p) were recorded. At the end of the measurement, the electrode was rinsed with deionised water.

3. RESULTS AND DISCUSSION

3.1 Electrochemical behaviour of CP

CV measurements with the same 1×10^{-5} mol/L CP standard solution were performed using a bare CPE and MWCNTs-CPE, and the results are shown in Figure 1. The bare-CPE results show an indistinct oxidation peak at 1.16 V, indicating a low response of CP on the bare CPE. When the MWCNTs-CPE was used, the peak current significantly increased by 1-fold compared with that of the bare CPE, and the peak shape became more distinct, indicating that the modification can significantly improve the sensitivity of the electrode, At the same time, we also found that the anti-interference ability of

MWCNTs-CPE is stronger than that of bare CPE, the possible reason is that MWCNTs can improve the electron transfer rate and increase the surface area of the electrode, therefore, it is often used as electrode modification material.
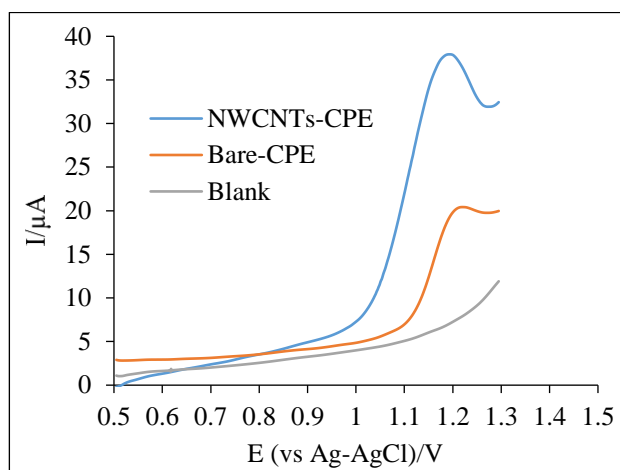

Figure 1. Cyclic voltammograms of 1.0×10^{-5} mol/L CP at MWCNTS-CPE and bare CPE in KH_2PO_4-$Na_2B_4O_7$ buffer solution (pH=4.90), and bare CPE in blank KH_2PO_4- $Na_2B_4O_7$ buffer solution at a scan rate of 200 mV/s.

3.2 Influence of buffer solution

Buffer solution plays a key role in the transfer of protons and electrons, Britton-Robinson (BR) solution, KH_2PO_4-$C_6H_8O_7$, NH_4Cl-NH_3, phosphate-buffered saline (PBS), H_3PO_4, Na_2HPO_4-$C_6H_8O_7$, $Na_2B_4O_7$-H_3BO_3-$NaCl$, H_3BO_3-KCl-Na_2CO_3, H_3BO_3-KCl, KH_2PO_4-$Na_2B_4O_7$, $C_8H_5KO_4$, and KH_2PO_4-$Na_2B_4O_7$ were used as base buffer solutions for the CV measurement of 1×10^{-5} mol/L CP standard solution. KH_2PO_4-$Na_2B_4O_7$ buffer produced an optimal peak shape, the largest peak current, and good stability. Therefore, KH_2PO_4-$Na_2B_4O_7$ buffer was selected as the base solution for measurements.

3.3 Influence of pH

With 0.20 mol/L KH_2PO_4-$Na_2B_4O_7$ buffer solution as the base solution, the pH was adjusted in the range of 4.50-6.32 with $Na_2B_4O_7$. The CV measurement of 7.5×10^{-6} mol/L CP standard solution was performed (Figure 2A), and the patterns of peak current and peak potential as a function of pH were recorded. Overall, the peak current gradually decreased with increasing pH (Figure 2B), When the pH was 4.90, the peak current was large, and the peak shape was good and stable. Therefore, the buffer pH used in subsequent experiments was set at 4.90.

In addition, the peak potential Ep of CP gradually showed a negative shift with increasing pH, indicating that protons are involved in the electrode reaction process of CP. Ep and pH were strongly linearly related (Figure 2C) through the equation Ep=1.4268-0.0505 pH (r=0.9968). The slope 0.0505 is approximately equal to 0.059, indicating that the reaction of CP on the electrode is a redox reaction involving equal numbers of electrons and protons, this result agreed with the reported literatures [17,18].
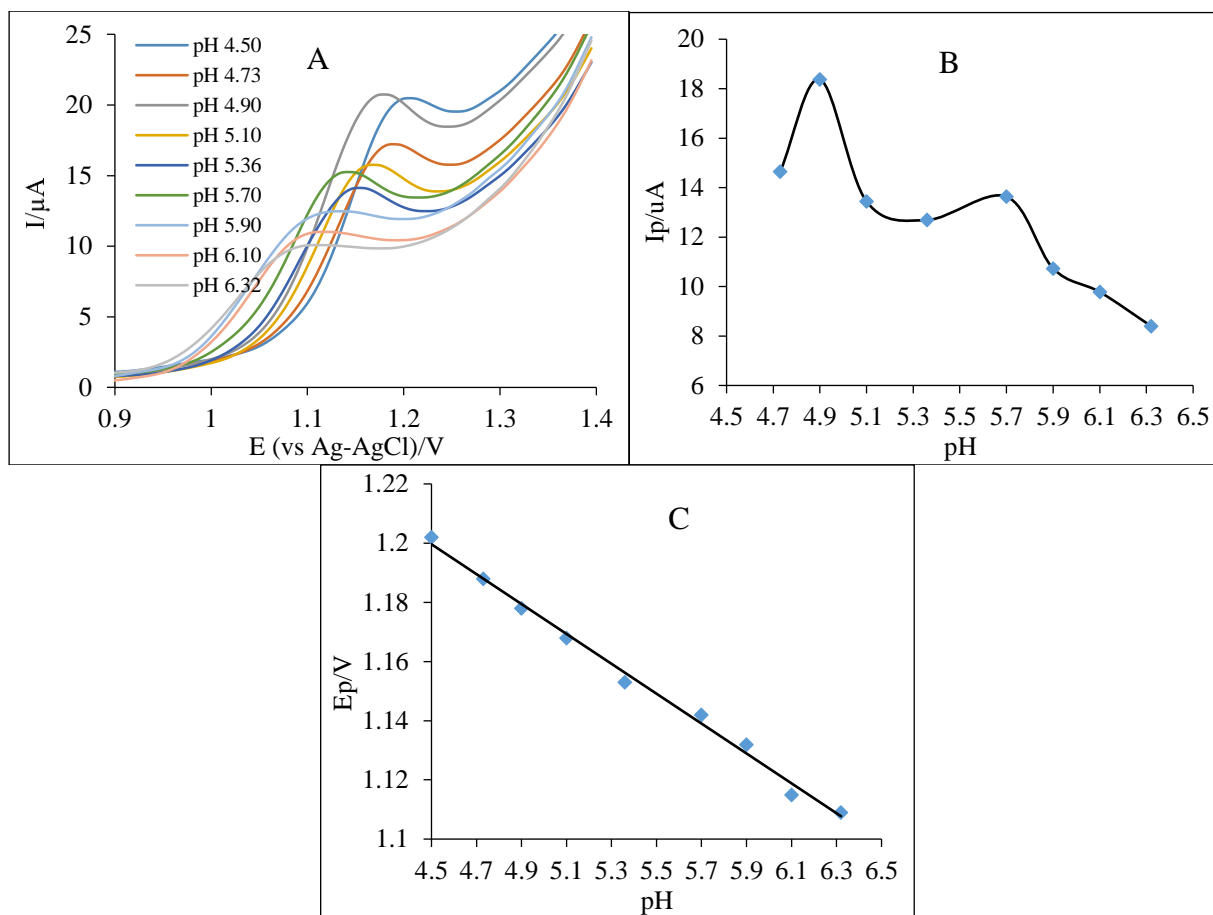
Figure 2. (A) Cyclicvoltammograms of 7.5×10^{-6} mol/L CP in KH_2PO_4-$Na_2B_4O_7$ buffer solution at pH 4.50-6.32. The effect of pH on the (B) I_p and (C) E_p.

3.4 Effect of amount of MWCNTs

The MWCNTs and the graphite powder were mixed at ratios of 1:5, 1:6, 1:7, 1:8, and 1:9, and five working electrodes were prepared according to the method described in Subsection 1.3. The same 1×10^{-5} mol/L CP standard solution was scanned, and the results are shown in Figure 3. When the ratio of MWCNTs to graphite was 1:7, a good peak shape and large peak current were obtained, and the electrode surface was not easily deformed or peeled off, its mechanical stability is better, so it can be used and stored for a longer time. Therefore, the ratio of MWCNTs to graphite in this experiment was set to 1:7.
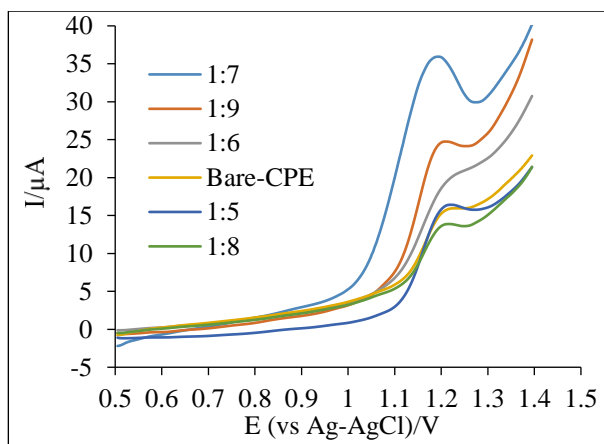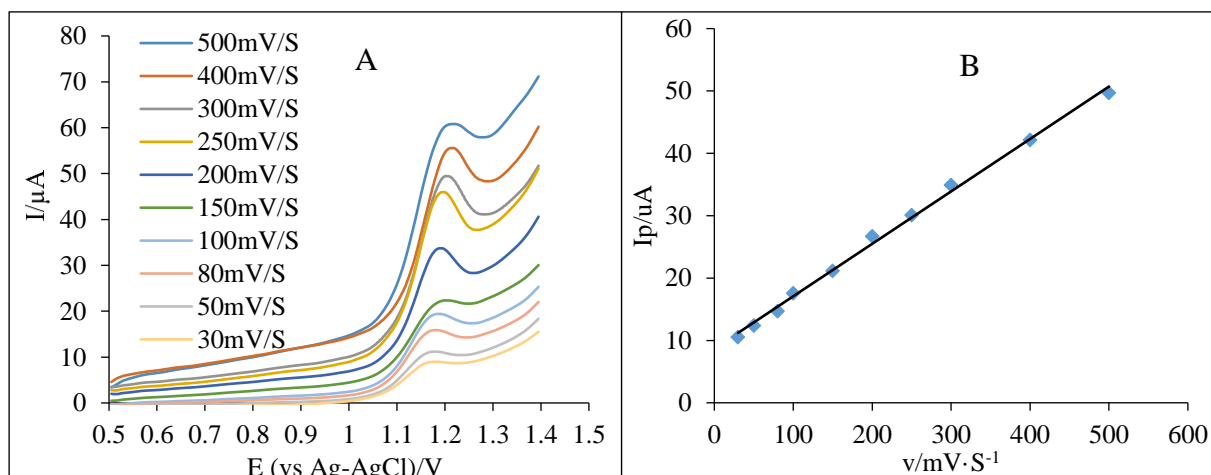
Figure 3. Effect of the MWCNTs amount on the Ip of 1.0×10^{-5} mol/L CP in KH_2PO_4-$Na_2B_4O_7$ buffer solution (pH=4.90).

3.5 Effect of scan rate

The same 1×10^{-5} mol/L CP standard solution was scanned while holding other experimental conditions constant and changing only the scan rate (v, 30 to 500 mV/s) (Figure 4A), and the impacts of varying scan rate on peak potential and peak current were observed. The peak current of CP increased with increasing scan rate. The I_P vs. v diagram (Figure 4B) shows a straight-line relationship, given by the equation I_P=8.676+0.084v (r=0.998), indicating that the oxidation process of CP on the CPE was primarily controlled by adsorption [19].

When the scan rate was less than 150 mV/s, the peak current was small, and the peak shape was poor. When the scan rate was greater than 250 mV/s, the baseline current was large. When the scan rate was greater than 250 mV/s, the peak shape gradually became poorer. Therefore, the scan rate selected in this experiment was 200 mV/s.
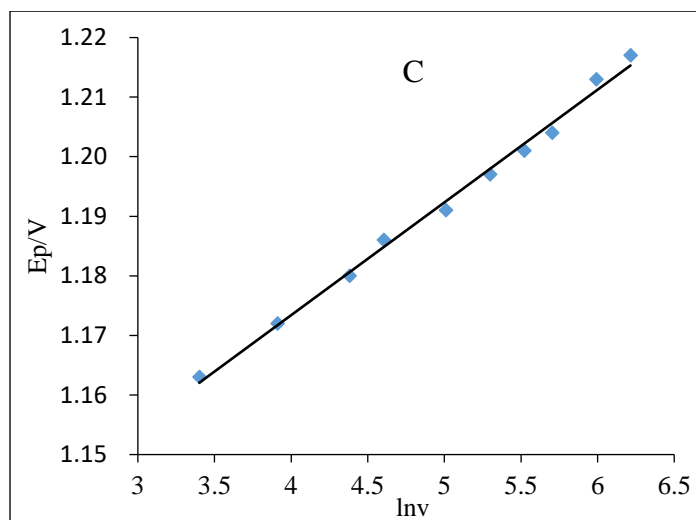
Figure 4. (A) Cyclic voltammograms of 1.0×10^{-5} mol/L CP in KH_2PO_4-$Na_2B_4O_7$ buffer solution (pH=4.90) at different scan rate of 30 to 500 mV/s. (B) Linear relationship of Ip versus scan rate. (C) Linear relationship of Ep versus lnv.
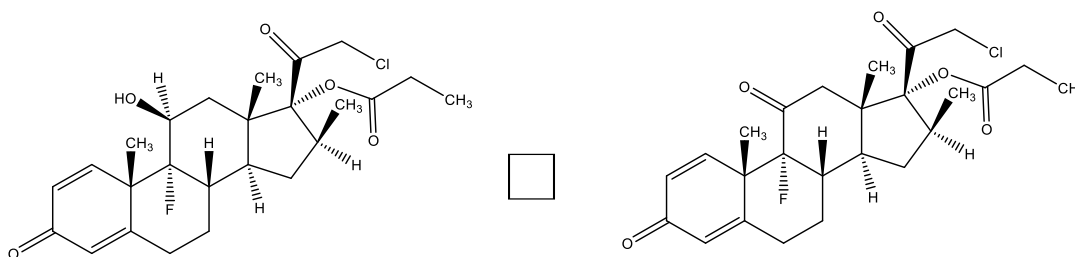
Besides, as the scan rate increased, the peak potential of CP also gradually showed a positive shift. The E_p vs. lnv diagram (Figure 4C) shows a straight-line relationship, given by the equation $E_P = 1.090 + 0.0205$lnv (r= 0.996).

According to the irreversible electrode reaction, the peak potential E_p is related to the scan rate v through the following equation [19]:

$$E_p = E^\theta - \frac{RT}{\alpha n F}\left[0.78 - \ln\frac{k^0}{D^{1/2}} + \ln(\frac{\alpha n F v}{RT})^{1/2}\right]$$

$\frac{1}{2} \times \frac{RT}{\alpha n F} = 0.0205$, R is the gas constant (8.314 J/K), T is the temperature in Kelvin (298), and F is the Faraday constant (96487 C/mol). The value of αn was calculated to be 0.63. Assuming $\alpha = 0.5$ [20], the number (n) of electrons involved in the reaction is $1.26 \approx 1$. Combining this result with the earlier conclusions, we speculate that the reaction mechanism of CP on MWCNTs-CPE is as follows (Scheme 1).

Scheme 1. Oxidation mechanism of CP at MWCNTs-CPE

In addition, combining the following equation and the linear relation between Ip and v (Fig.4B), the absorption capacity of CP on the MWCNTs-CPE surface was calculated as follows:

$$I_{pa} = \frac{n^2 F^2 \Gamma A v}{4RT}$$

Where Γ is the absorption capacity ($M \cdot cm^2$), A is the electrode surface area (cm^2), and n, F, v, R, and T have the same meanings as before. The absorption capacity of CP on the MWCNTs-CPE surface was calculated to be $1.26 \times 10^{-9} \cdot cm^2$, which was ten times that of the literature [21]. The results show that the sensitivity of the MWCNTs-CPE was high.

3.6 Interference test

For the interference test, 100-fold Mg^{2+}, Zn^{2+}, K^+, Na^+, SO_4^{2-}, Cl^- and CO_3^{2-} was added to a 1.0×10^{-5} mol/L CP standard solution and scanned using the bare CPE and MWCNTs-CPE, respectively. The results showed no interference in the detection of CP and that the MWCNTs-CPE exhibits higher anti-interference ability than the bare CPE.

3.7 Electrode precision and repeatability

The freshly prepared modified electrode was evaluated by continuously measuring the CP standard solution ten times. As the relative standard deviation (RSD) was 0.5%, the modified electrode was highly precise. Five electrodes were prepared with the same condition at the same time. RSD was 3.2% when measuring the same CP standard solution, which indicated good repeatability.

3.8 Analytical application

3.8.1 Standard curve and detection limit

CV measurements of different concentrations of CP standard solutions were performed under the optimal experimental conditions identified above (Figure 5A). The CP concentration showed a strong linear relationship with the peak current in the range of 1.0×10^{-4} to 1.0×10^{-6} mol/L (Figure 5B), and the regression equation was y=7.755x+11.25 (r=0.999, unit for y: μA, unit for x: $\times 10^{-5}$ mol/L). The CP concentration was fixed at 10^{-6} mol/L, and the corresponding peak current at this concentration was measured for 12 replicates. The relative standard deviation (RSD) of the peak current was 1.15%, and the detection limit (C_d) was calculated to be 3.45×10^{-8} mol/L.
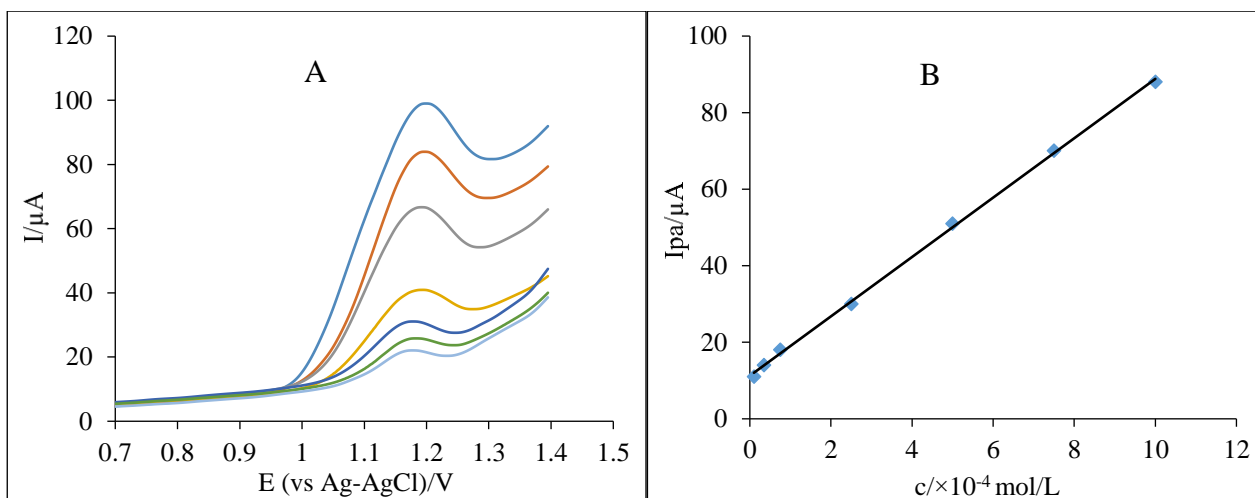
Figure 5. (A) Cyclic voltammograms of CP in KH_2PO_4-$Na_2B_4O_7$ buffer solution (pH=4.90) at different concentrations of 1.0×10^{-4} to 1.0×10^{-6} mol/L. (B) Linear relationship of Ip versus CP concentration.

3.8.2 Sample determination and recovery rate

The facial mask was squeezed out, and 1 mL of sample was transferred into a 25 ml beaker, followed by the addition of 9 mL of KH_2PO_4-$Na_2B_4O_7$ buffer solution (pH=4.90) to produce the test sample solution.

Table 1. Application of MWCNTs-CPE for the detection of CP in facial mask samples

Sample	Original (mol/L)	Added (mol/L)	Found (mol/L)	Recovery (%)	RSD (%)
1-1	0	4.00×10^{-6}	4.12×10^{-6}	103.0	2.1
1-2	0	5.00×10^{-6}	5.31×10^{-6}	106.2	3.0
1-3	0	6.00×10^{-6}	5.88×10^{-6}	98.00	4.1
2-1	2.23×10^{-6}	1.80×10^{-6}	3.93×10^{-6}	95.52	2.2
2-2	2.23×10^{-6}	2.20×10^{-6}	4.38×10^{-6}	97.76	3.7
2-3	2.23×10^{-6}	2.60×10^{-6}	4.72×10^{-6}	95.07	2.1

The three-electrode system was inserted, scanning was carried out under the optimal experimental conditions described above, and the content was calculated according to the standard curve obtained in Subsection 3.1. In addition, a standard spike recovery experiment was carried out, and the CP standard solution and the sample solution were uniformly mixed with ratios of 80%, 100%, and 120%. Measurements were performed under the same experimental conditions, and the standard spike recovery rate was measured (Table 1).

As seen from Table 1, the overall recovery rate ranged between 95.07% and 106.2%, and the RSD ranged between 2.1 and 4.1. The recovery rate and RSD showed large fluctuations, mainly because the reproducibility of the modified electrode was slightly poor. In subsequent studies, the experimental conditions should be strictly controlled to improve the reproducibility of the electrode.

3.8.3 The proposed method compared with reported in literature

The determination method of CP is mainly as high-performance liquid chromatography. Compare with references, the proposed method (#) is not good enough, the reason is that glucocorticoids are difficult to detect by electrochemical methods, In addition, we use electrode materials without mercury, it has lower sensitivity but higher safety, at the same time, it can also meet the needs of real sample detection.

Table 2. Comparison of *the proposed method* with references for CP and other glucocorticoids detection

ref.	object	method	linear range	recovery /%	LOD	RSD /%
6	CP	TLC	0.10-0.50 µg/band	99.60±1.09	0.01 µg/band	1.09
		spectrophotometry	2.50-25.00 µg/mL	99.44±1.60	0.58 µg/mL	1.61
7	CP	TLC	---	98.7-101.0	0.01 µg/spot	---
8	CP	spectrophotometry HPLC	20-150 µg/mL	---	---	---
10	CP	HPLC	0.5-15.0 µg/mL	---	0.02 µg/mL	---
11	CP	HPLC	---	78-91	0. 4-1.6 µg/L	5.9-8.0
12	CP	HPLC-MS	0.04-10 ng/mL	102-109	---	---
13	CP	HPLC-MS	10-1000 ng/mL	---	0.75-3 ng/mL	---
14	dexamethasone	voltammetry	49.8-610 nmol/L	---	2.54 nmol/L	---
15	hydrocortisone	voltammetry	0.02-1.2 µmol/L	---	4.8 nmol/L	4.6
#	CP	voltammetry	10^{-4}-10^{-6} mol/L	95.07-106.2	3.45×10^{-8} mol/L	2.1-4.1

4. CONCLUSION

In this study, the voltammetric behaviour of CP on MWCNTs-CPE was studied, and an electrochemical analysis method for the direct determination of CP using this electrode was established. The experiments showed that the MWCNTs could promote the transfer of electrons, improve the sensitivity of the CPE, and enhance the peak current. The oxidation of CP on MWCNTs-CPE was an irreversible adsorption process. The number of transferred electrons was 1, and one proton participated in the reaction. The optimal conditions for the measurement were as follows: ratio of MWCNTs in the modified electrode to graphite powder was 1:7 (m:m), buffer solution was KH_2PO_4-$Na_2B_4O_7$ (pH=4.90), scan rate at 200 mV/s, scanning range from 0.50 to 1.40 V, and oxidation peak potential at near 1.16 V. The CP concentration showed a strong linear relationship with the peak current in the range of 1.0×10^{-4} to 1.0×10^{-6} mol/L, and the detection limit was 3.45×10^{-8} mol/L. The content of CP in a facial mask was measured by this method, and the results were satisfactory.

The working electrode used in the method was homemade, simple, and cheap, the surface of the electrode was easily renewed, and the sensitivity was high. Since the volume of the electrode and the

space occupied by the surface were small, only a small amount of sample was needed. In addition, the sample requirements in this method were low, and sample pretreatment was not needed. Therefore, this method has good application prospects.

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