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Determination of codeine in urine and drug formulations using a clay-modified screen-printed carbon electrode

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Abstract

Both flow-injection analysis and square-wave stripping voltammetry were evaluated for the determination of codeine in pharmaceutical formulations using a nontronite clay-modified screen-printed carbon electrode. Compared with a bare screen-printed carbon electrode, the nontronite clay-modified screen-printed carbon electrode exhibited a marked enhancement of the current response of codeine. A linear calibration plot was obtained over the 2.5–45 μM range (correlation coefficient = 0.999) in pH 6.0 phosphate buffer solution with a detection limit of 20 nM ($S/N = 3$) by square-wave voltammetry (SWV). While, in flow-injection analysis, the linearity was over 5–120 ng range with a detection limit of 1 ng in 20 μl loop. The nontronite clay-modified screen-printed carbon electrode can be either disposable or reused since the renewal gave a good reproducible surface. Quantitative analysis was performed by the standard addition method for codeine content in both urine and commercially available drugs.

Keywords: Codeine; Clay; Screen-printed carbon electrode; Drug; Urine

1. Introduction

Codeine has long been used as an effective analgesic and antitussive agent in pharmaceutical preparations [1–3]. Previous approaches, including gas chromatography, chemiluminescent, and high performance liquid chromatography, were reported for codeine determination [4–13]. However, these methods are time consuming or solvent-usage intensive. New pharmaceutical preparations appearing require fast, sensitive, simple, inexpensive, and specific method for the quantitative determination of codeine from oral formulations. Upto now, more sensitive and selective methods for the determination of codeine are still needed to be developed.

We report here the determination of codeine using a nontronite clay-modified screen-printed electrode (CMSPE) by both square-wave voltammetry (SWV) and flow injection analysis (FIA). Due to the special constraints and surface chemi-

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cal effects induced by clay lead to new patterns of reactivity and selectivity, the utilization of the material is promising. Indeed, clay modified electrode (CME) can also be useful in analytical detection. For example, montmorillonite CME were used to the detection of inorganic cations such as Fe^{3+} and $Ru(NH_3)_6^{3+}$ [14,15]. It was also observed that nontronite CME incorporated with methyl viologen exhibited excellent catalytic activity for the electroreduction of hydrogen peroxide where methyl viologen behaved as an electron transfer mediator [16]. Infact, due to colloidal clays' appreciable surface area, intercalation properties, low cost and high stability, their uses in analytical purposes certainly deserve an extensive study.

The purpose of the present work is to construct a sensitive and selective analytical method to determine codeine in a simple, fast, and inexpensive way. The working electrodes used were SPEs. SPE technology offers the advantages of inexpensive, simple, and rapid and it allows mass production of reproducible electrodes. Thus, fouling, one of the drawbacks of electrode-based reactions can be avoided by using disposable SPEs. Due to the reactivity and selectivity of the clay and the advantages of SPE, it is possible to carry the routine pharmaceutical and biomedical measurement of codeine by CMSPE. In the present study, the CMSPE is applied to the determination of codeine in urine and pharmaceutical formulations by both SWV and FIA. FIA was chosen due to the advantages of high speed and simple technology. The optimal experimental conditions such as pre-concentration time, pre-concentration potential, clay composition, pH, and SW parameters were thoroughly investigated. Practical analytical utility was illustrated by selective measurements of codeine in urine and commercially available drugs.

2. Experimental

2.1. Chemical and reagents

Standard clay mineral, nontronite (SWa-1, ferruginous smectite), was purchased from the Source Clay Minerals Repository (University of Missouri, MO). Codeine phosphate, $C_{18}H_{24}NO_7P$, and all the other compounds used in this work were prepared from ACS-certified reagent grade chemicals without further purification in doubly distilled deionized water. A stock solution was prepared by dissolving 97 mg of codeine phosphate (weighted accurately) in 50 ml of water. An aliquot was diluted to the appropriate concentrations with pH 6 phosphate buffer (0.05 M) before actual analysis. Codeine can be extracted from poppies of the genus *Papaveraceae somniferum* by a proprietary process involving a series of acidic, alkaline and solvent extractions [12]. Thus, codeine is stable in solution.

2.2. Apparatus

Electrochemistry was performed on a CHI-620 electrochemical analyzer (CHI, Austin, TX, USA). A BAS VC-2 electrochemical cell was employed in these experiments. The three-electrode system consisted of either a CMSPE or a bare SPE working electrode, an Ag/AgCl reference electrode (Model RE-5, BAS), and a platinum wire auxiliary electrode. Since dissolved oxygen did not interfere with the anodic voltammetry, no deaeration was performed.

The flow injection system consisted of a carrier reservoir, a Cole-Parmer masterflex microprocessor pump drive, a Rheodyne model 7125 sample injection valve (20 μl loop). A CHI model CH-660 electrochemical workstation was connected in the FIA experiments.

The SPEs were fabricated using carbon inks (Acheson, Japan). The electrodes were printed in a group of 16 (with a 1 mm gap between each) onto a polypropylene (PP) base. Each electrode consisted of an 8×2.5 mm working area with a 17×1.5 mm connecting strip. The SPEs were equilibrated in the test solution containing codeine before measurement. SW voltammograms were obtained by scanning the potential from $+0.6$ to $+1.3$ V at a SW frequency and amplitude of 15 Hz and 45 mV, respectively. At a step height of 4 mV, the effective scan rate is 60 mV/s. The codeine quantitation was achieved by measuring the peak current of the oxidation peak

after background subtraction. After the detection, the electrode was cycled from +0.6 to +1.3 V in the supporting electrolyte solution, until the analyte peak became nil to renew the electrode surface. Normally it took three to five cycles.

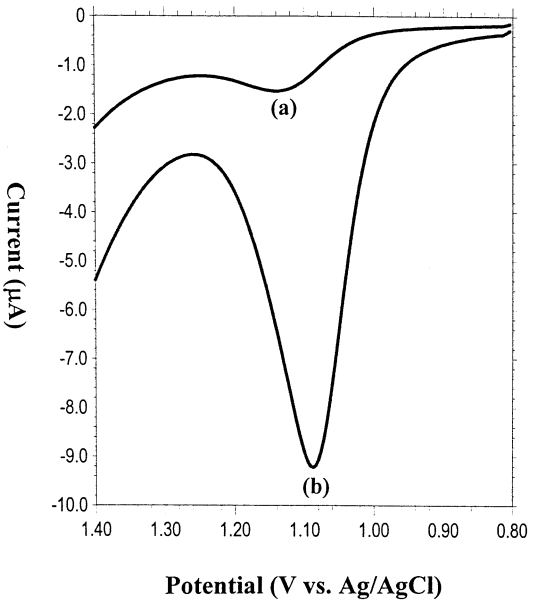

Fig. 1. SW voltammograms for 25 μM codeine in 0.05 M, pH 6 phosphate buffer solution on a bare SPE (a) and the CMSPE (b). SW amplitude, 45 mV; SW frequency, 15 Hz; step height, 4 mV.

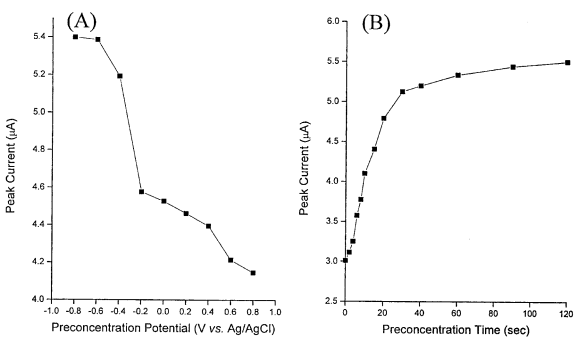

Fig. 2. Effects of preconcentration potential (A) and preconcentration time (B) on peak current for 25 μM codeine on the CMSPE. Preconcentration time = 30 s for (A) and preconcentration potential = −0.6 V for (B). Other conditions are as in Fig. 1.

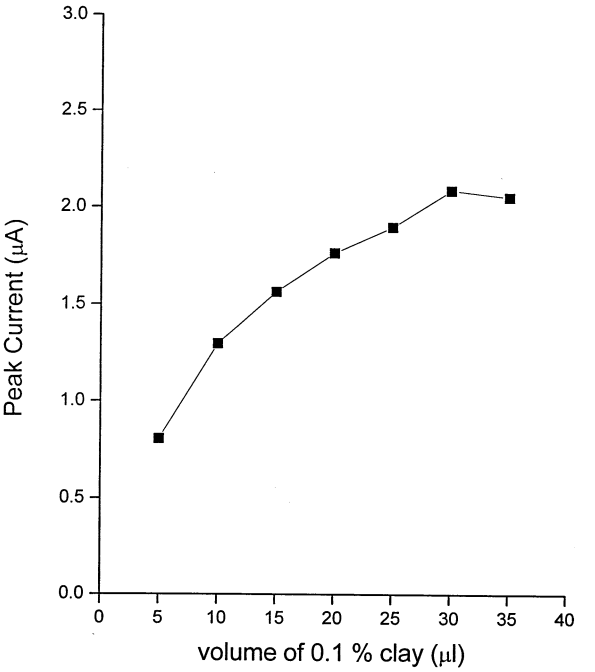

Fig. 3. Effect of clay composition on peak current for 25 μM codeine on the CMSPE. Other conditions are as in Fig. 1.

2.3. Analysis of tablets

A tablet was weighted accurately and dissolved in water (dilution factor 1/1000). The resulting stock solution was stirred magnetically for 15 min to ensure that tablet was dissolved completely. Without any treatments, the stock solution was diluted with supporting electrolyte (dilution factor 1/833). The solution was transferred to the voltammetric cell and the SW voltammograms were recorded in a similar way to the pure drug. The standard addition method was used to evaluate the content of codeine in the tablet.

2.4. Analysis of urine

A urine sample taken from a healthy person (female, age 25 years) was added to the voltammetric cell containing the supporting electrolyte (9:1). The voltammogram was recorded, then 10 μl spikes of the standard solution of codeine was introduced into the cell each time and the SW voltammograms were recorded after each addition.

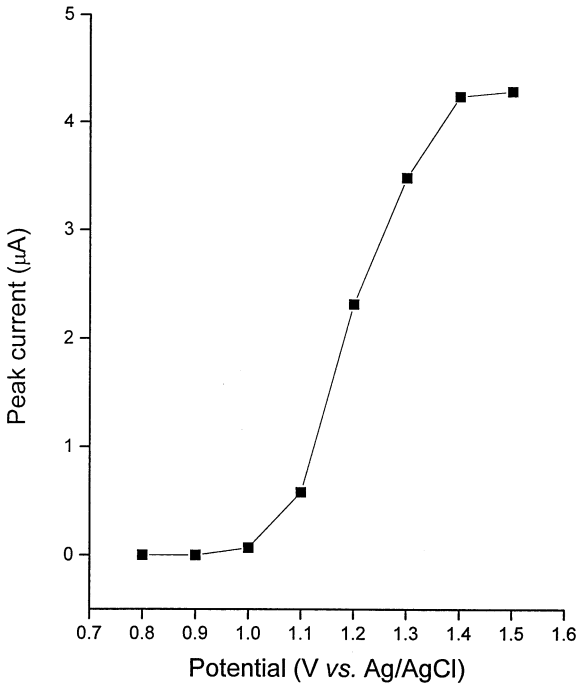

Fig. 4. Hydrodynamic voltammograms for 5 ppm (12.5 μM) codeine on the CMSPE. Supporting electrolyte, 0.05 M; pH 6 phosphate buffer solution. Flow rate, 0.6 ml/min.

3. Results and discussion

3.1. Voltammetric behavior

Fig. 1 demonstrates the accumulation effect of the CMSPE in the determination of codeine by SWV. On scanning from +0.8 V toward a positive potential on a clean SPE, only a much smaller anodic peak at +1.14 V was observed for 25 μM codeine (curve a). Whereas, a large increase in the peak current at +1.08 V was observed when the CMSPE was used (curve b). Ogorevc et al. reported the ability of clay to efficiently preconcentrate trace copper(II) prior to its voltammetric determination [17]. Therefore, the enhanced current response of codeine in anodic direction is possible due to the accumulation effect of the CMSPE toward codeine oxidation.

The effect of preconcentration potential on the SW response for the oxidation of codeine is shown in Fig. 2A. The peak current increased as the potential of the electrode shifted negatively between +0.8 and −0.8 V. A preconcentration potential of −0.6 V was, therefore, chosen in all subsequent work. As to the effect of the preconcentration time, for 25 μM of codeine, the peak current increased as the preconcentration time

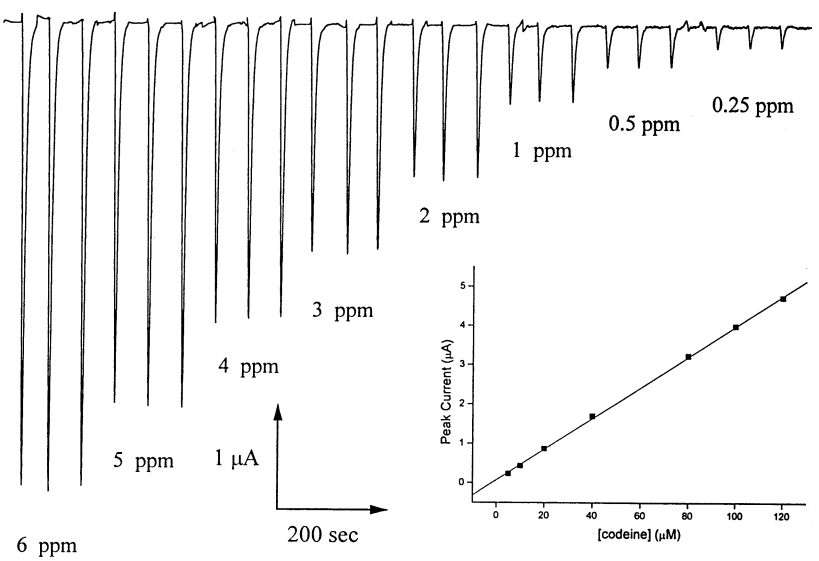

Fig. 5. Calibration curve in FIA for 0.25–6 ppm (0.625–15 μM) codeine on the CMSPE. Applied potential = +1.4 V and other conditions are as in Fig. 4.

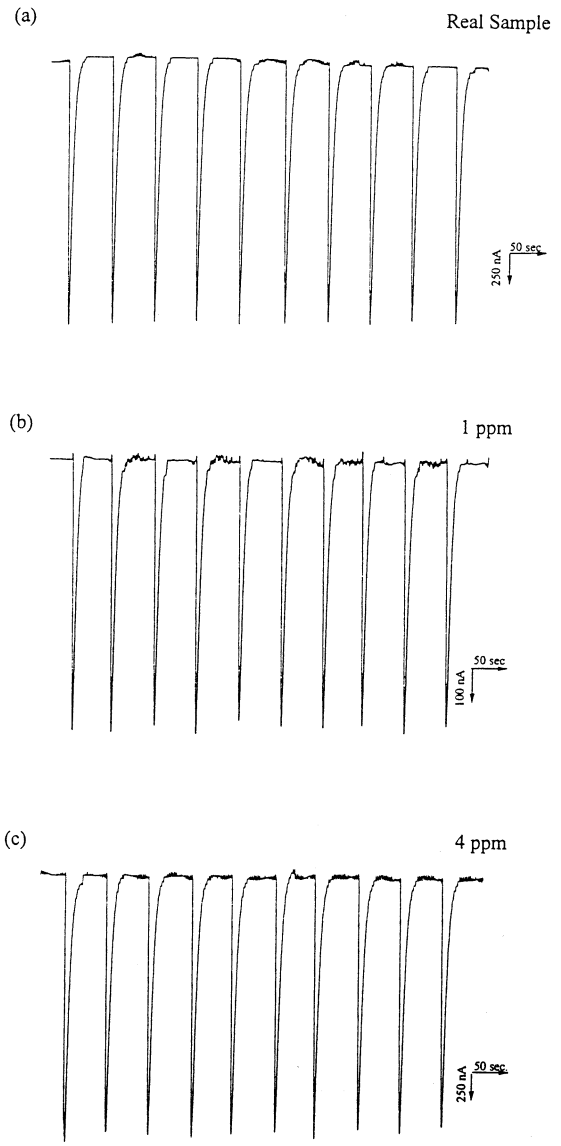

Fig. 6. Reproducibility in FIA for (a) real sample (b) 1 ppm (2.5 μM) standard solution (c) 4 ppm (10 μM) standard solution.

increased and started to level off at around 30 s as shown in Fig. 2B. Note that it took longer time for the peak current to level off for a lower concentration of codeine (data not shown).

A further investigation was made to the transportation characteristics of codeine on the CMSPE. The LSV current response obtained on the CMSPE was found linearly proportional to the scan rate, which illustrated that the process was adsorption-controlled. Further evidence for the adsorptive behavior of codeine was demonstrated by the following experiment. When the CMSPE was switched to a medium containing only supporting electrolyte after being used in measuring a codeine solution, a voltammetric peak signal was still observed. Thus, by the combination of polarity adsorption and preconcentration features of nontronite clay, the oxidation current of codeine showed enhanced response.

Fig. 3 shows the effect of the clay composition in preparing the CMSPE for the detection of codeine. As can be seen, 30 μl of the clay colloid (0.1 wt.% in H_2O) was found to be an optimum volume for the coating process, this amount was used in all subsequent study.

3.2. Analytical characterization

Both the electrode and the detection aspects should be considered to arrive at the optimum conditions for codeine determination. The voltammetric oxidation of codeine on the activated CMSPE was investigated in the pH range of 2–8 in different buffer systems of Britton–Robinson, citrate, and phosphate. The experimental results showed that the shapes of curves were nearly the same in all cases, however, the current intensity in phosphate buffer (4.87 μA) was higher than that in Britton–Robinson (2.72 μA) and citrate (2.58 μA) buffers. A phosphate buffer (pH 6) was chosen with respect to sharper response for analytical application.

The peak current obtained in SW voltammetry depends on various instrumental parameters such as SW amplitude, SW frequency, and step height. These parameters have inter-related effects on the response, but here only the general trends were examined. It was found that these parameters had little effect on the peak potential. By changing the SW amplitude in the range of 10–100 mV, the peak currents increased with increasing amplitude until 45 mV. However, when the amplitude was greater than 45 mV the peak width increased at the same time. Hence, 45 mV was chosen as the SW amplitude for the following experiments. The

step height together with the frequency defines the effective scan rate. Hence, an increase with either the frequency or the step height results in an increase in the effective scan rate. The response for codeine increased with the increase of SW frequency; however, above 15 Hz the peak current was unstable and obscured by a large residual current. By maintaining the frequency as 15 Hz, the effect of step height was studied. However, the reproducibility of the detection was affected as the step heights become larger than 5 mV due to too few points are sampled. In order to accurately record the response, the step height of 4 mV is chosen. Overall, the optimized parameters can be summarized as follows: preconcentration potential = -0.6 V; preconcentration time = 30 s; SW amplitude = 45 mV; SW frequency = 15 Hz; step height = 4 mV.

Under optimum conditions, the SWV current response was linearly dependent on the concentration of codeine between 2.5 and 42.5 μM in 0.05 M, pH 6 phosphate buffer solution with the slope (μA/μM), intercept (μA), and correlation coefficient of 0.239 ± 0.001, -0.110 ± 0.034, and 0.999, respectively. The detection limit (3σ) is 20 nM.

To characterize the reproducibility of the CMSPE, repetitive measurement–regeneration cycles as described in the Section 2 were carried out. The results of ten successive measurements showed a relative S.D. of 2.40% (2.5 μM codeine), 0.65% (20 μM codeine), and 0.35% (40 μM codeine), respectively. Since one of the advantages of SPEs is disposable, the reproducibility of the different CMSPEs was investigated instead of the inter-day reproducibility. The results of 12 measurements showed a relative S.D. of 2.13% (20 μM codeine).

3.3. FIA amperometric detection

To optimize the amperometric response, parameters affecting the amperometric response to codeine such as the applied potential and the flow rate were studied. Fig. 4 shows the hydrodynamic voltammograms for codeine obtained under flow injection condition conditions with 0.05 M, pH 6 phosphate buffer solution as a carrier, at 100 mV intervals between $+0.9$ and $+1.5$ V. As can be seen, the maximum response for codeine occurred at about $+1.4$ V, which is in good agreement with the SW voltammetric result. An applied potential of $+1.4$ V was, therefore, selected for the subsequent work.

The FIA current increased with the increase of the flow rate from 0.4 to 0.6 ml/min and it decreased as the flow rate was higher than 0.6 ml/min. Moreover, the detection signals were very broad at the flow rate higher or lower than 0.6 ml/min, this flow rate was, thus, chosen as optimized flow rate value.

FIA responses (at a working potential of 1.4 V) for increasing levels of codeine concentration are shown in Fig. 5. The CMSPE responded favorably to the concentration increments over the 0.25–6 ppm (0.625–15 μM) with well-defined peaks increased linearly with the codeine concen-

Table 1
Determination of codeine in commercial available tablets with the CMSPE

Labeled value after dilution (μM)	Codeine tablet	Original detected value (μM)	Spike (μM)	Detected value after spike (μM)	Recovery (%)
4.0	#1	3.83 ± 0.03	5	8.67 ± 0.07	96.8 ± 1.52
			10	13.75 ± 0.04	99.2 ± 0.50
			15	18.46 ± 0.01	97.5 ± 0.21
			20	24.09 ± 0.23	101.3 ± 1.15
4.0	#2	4.06 ± 0.11	5	8.98 ± 0.05	98.4 ± 2.41
			10	14.65 ± 0.08	105.9 ± 1.36
			15	18.86 ± 0.21	98.6 ± 1.58
			20	24.17 ± 0.16	100.5 ± 0.97

Dilution factor, 1/833000.

tration. The resulting calibration plot is characterized with the slope (μA/μM), intercept (μA), and correlation coefficient of 0.313 ± 0.003, 0.068 ± 0.024, and 0.999, respectively. The detection limit ($S/N = 3$) was 0.05 ppm (0.125 μM).

The stability of the CMSPE was also studied in combination with the flow injection system. Analytical assay data for both of the commercial samples and standard mixtures were shown in Fig. 6. Excellent reproducibility was obtained. The coefficient of variation ($n = 10$) for the real samples, 1 ppm (0.25 μM), and 4 ppm (10 μM) codeine standard solution was 2.98, 1.39, and 2.14%, respectively.

3.4. Application to a pharmaceutical formulation tablets

The CMSPC was applied to the measurement of codeine in commercially available drugs by SWV. The accuracy of the method was determined by its recovery during spiked experiments. Two commercial drugs, which contain codeine, were spiked with codeine standard solution at a concentration of 5, 10, 15, and 20 μM. As shown in Table 1, the recoveries of codeine from the pharmaceutical matrices were satisfactory with values ranging from 96.8 to 105.9%. Further, the detected value was also comparable with the labeled value. These results confirm that the quantitative and reproducible results can be obtained with this method.

3.5. Application to a urine sample

The SWV was used for the determination of codeine in a urine sample. The peak current increased with increasing drug concentration from 40 to 80 μM. The recoveries range from 98.42 to 102.76%, confirming the good selectivity of this method.

4. Conclusions

A rapid, inexpensive, sensitive and selective method was shown for the determination of codeine with the CMSPE. The method was proved to be suitable for the selective measurements of codeine in urine and tablets without any preliminary treatment. Furthermore, due to CMSPE's stability, precision, and low cost, it offers a good possibility as a substitute for the previous approaches used in routine analysis of codeine.

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