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Short communication

Determination of tranexamic acid in cosmetic products by high-performance liquid chromatography coupled with barrel plating nickel electrode

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ABSTRACT

Tranexamic acid (TA) is an important reagent in cosmetic skin-whitening formulation and a drug for the inhibition of plasminogen to plasmin in blood. Since there is no chromophore in tranexamic acid molecule to enable direct analysis by UV–visible absorption spectrophotometry, derivatization is thus required by excluding use of UV or fluorescence detection. We report here a relatively simple electrochemical TA detection method by using a barrel plating nickel electrode. Chromatographic separation was performed on a Hamilton PRP-X100 anion-exchange column (150 mm × 4.1 mm i.d., 10 μm particle size) with a (85:15, v/v) mixture of 0.1 mol l^{-1} NaOH and acetonitrile as mobile phase and pumped at a flow rate of 0.9 ml min^{-1}. By detecting at +0.55 V vs. Ag/AgCl, the calibration plot was linear in the concentration window of 3–1000 ppm with regression coefficient and detection limit (S/N = 3) of 0.9993 and 0.13 ppm (0.84 μmol l^{-1}), respectively. Successive injections (n = 10) of 50 ppm tranexamic acid showed a R.S.D. value of only 0.3% indicating good reproducibility of the proposed system. The method was successfully applied to the analysis of the content of tranexamic acid in cosmetic products and proved to be suitable for rapid and reliable quality control.

1. Introduction

Tranexamic acid (TA), *trans*-4-(aminomethyl) cyclohexanecarboxylic acid, is a synthetic derivative of lysine and marketed as Cyklokapron in USA and as Transamin in Asia. It is effective on the skin in healing of wounds and can prevent skin from roughening [1,2]. Examples for TA in cosmetics products are skin-whitening lotions, creams, milky lotions, facial packs and scalp care cosmetics. It is also an antifibrinolytic drug that competitively inhibits the activation of plasminogen to plasmin, a molecule responsible for the degradation of fibrin [3–6]. Sensitive detection and determination of TA is thus of interest not only in cosmetics but also in clinical and pharmaceutical samples. Since there is no chromophore in TA molecule to enable direct analysis by UV–visible absorption spectrophotometry, derivatization is thus required by excluding use of UV or fluorescence detection [7–13]. A more reliable HPLC–tandem MS-based TA assay [14], on the other hand, always requires expensive instrumentation and skilled technician.

We report here a simple, inexpensive and sensitive electrochemical method for TA detection using a barrel plating nickel electrode (Ni-BPE). The primary function of barrel plating is to provide an economical means to electroplate manufactured parts that meet specific finishing requirements [15,16]. Since the fabrication cost of the Ni-BPE is low, it can be disposable and as such manual polishing is not necessary. Our previous studies have successfully used this kind of disposable-type electrode for analytical applications [17–19]. In this study, we further demonstrate that the Ni-BPE coupled with a specifically designed electrochemical cell allows us to obtain an electrocatalytic profile towards the oxidation of TA. The user-friendly design of electrochemical cell is suitable for use in flow injection analysis (FIA) and is especially attractive in practical applications. Both the mechanism of electrocatalytic oxidation in FIA and the use as a detector in HPLC for the determination of TA at the Ni-BPE are discussed and investigated. It was finally applied to the determination of TA in cosmetics without the necessity of laborious sample preparation.

2. Experimental

TA (TCI), sodium hydroxide (Showa) and acetonitrile (ACN, ECHO) were used as received. All reagents were of analytical-reagent grade and prepared with de-ionized water. Cosmetic samples were purchased from a local supermarket and prepared by dissolving 1 g of the sample in mobile phase with suitable

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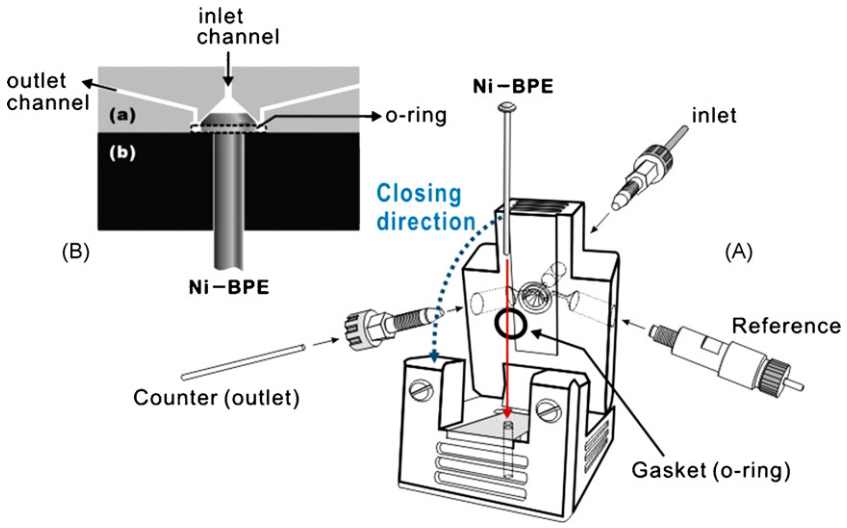

Fig. 1. Schematic representation of the Ni-BPE working system.

dilution. The two cosmetic products were labeled to contain 2% (w/w) and 1% (w/w) of TA, respectively. The excipients present are distilled water, POE-20 behenyl ether, glyceryl monostearate, methyl glucose sesquistearate and PEG-20 glyceryl stearate, PEG-100 stearate/glyceryl stearate, cetyl alcohol, myristal myristate, squalane, shea butter, ceteary octanoate, isohexadecane, lanolin, dimethicone, tocopherol acetate, propylene glycol, carbomer, 1,3-butylene glycol, disodium edetate, sodium pyrrolidone carboxylate and methyl paraben. The real samples were ultrasonically treated for 15 min; then centrifuged at 25 °C and 4500 rpm for 5 min. After filtered sequentially through 0.45 μm Critical syringe filters (Nashua, NH, USA), the final filtrates were analyzed by HPLC.

Cyclic voltammetric (CV) and chronoamperometric experiments were carried out using a CHI 900 electrochemical workstation (Austin, TX, USA). The Ni-BPE three-electrode system is the same as reported in our previous study and is activated by cycling from +0.6 to −0.2 V in 0.1 mol l^{-1} NaOH [18]. The Ni-BPE (1.25 mm diameter and 31 mm length) with an average weight of 392.2 ± 0.5 mg (n = 10) was a special order from Zensor R&D (Taichung, Taiwan). Working geometric surface area of the Ni-BPE is ~0.012 cm^2. Fig. 1 illustrates the schematic representation of the Ni-BPE working system used in this work. It consists of a Ni-BPE working electrode, a stainless tube counter electrode (outlet) and an Ag/AgCl reference electrode. The HPLC system consisted of a BAS-PM92E high-pressure microprocessor pump drive, a Rheodyne 7125 sample injection valve (20 μl loop) and the proposed electrochemical detector. The HPLC system with an anion-exchange column (Hamilton PRP-X100, 150 mm × 4.1 mm i.d., 10 μm particle size) was equilibrated in (15:85, v/v) ACN +0.1 mol l^{-1} NaOH carrier solution at +0.55 V vs. Ag/AgCl until the current became constant. It usually takes 5 min at room temperature. The TA oxidation peak signal was uniformly taken as a quantitative parameter.

3. Results and discussion

3.1. Electrochemical studies

Fig. 2 compares CV responses at a conventional glassy carbon electrode (GCE) and the activated Ni-BPE in the absence/presence of 60 ppm TA in 0.1 mol l^{-1} NaOH at a scan rate (v) of 1 mV s^{-1}. As can be seen, no faradic responses are observed at GCE in presence/absence of TA. In contrast, the bare Ni-BPE shows a profound redox response centered at +0.45 V vs. Ag/AgCl and there is an increase in the anodic peak current together with a decrease in the cathodic peak current upon 60 ppm TA addition. The redox potential is similar to those of Ni-based electrodes reported earlier [20–23]. The electrocatalytic oxidation mechanism of TA at the Ni-BPE surface represents a mediated catalytic oxidation:

$$Ni^{II}(OH)_2 + OH^- \rightleftharpoons Ni^{III}O(OH) + H_2O + e^- \quad (1)$$

$$Ni^{III}O(OH) + \text{(TA)} \rightarrow Ni^{II}(OH)_2 + \text{Oxidized product of TA} \quad (2)$$

(TA)

As per the above scheme, the higher oxidation state ($Ni^{III}O(OH)$) appearing at +0.45 V vs. Ag/AgCl is responsible for the mediation. The electrochemical observation clearly indicates the electrocatalytic behavior of TA at the Ni-BPE. Nevertheless, the electrooxidation of TA on the oxide metals can be very complex and may involve strong adsorption steps of reactants and/or reaction products [24–27]. In this study, the interaction between NiOOH and the terminal $-NH_2$ functional groups of TA was believed to be essential for the detection. Most importantly, when applying a potential around the oxidation peak (i.e., +0.45 V) to the Ni-BPE, the increase in the oxidation current can be used for the amperometric determination of TA.

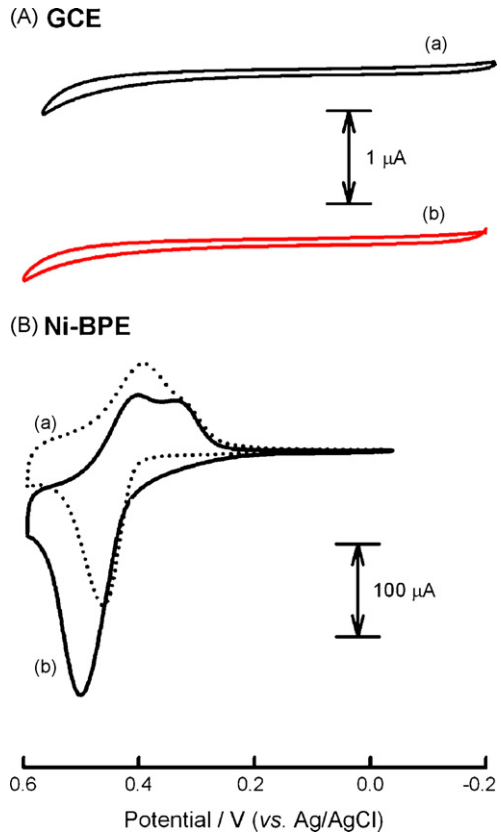

Fig. 2. Cyclic voltammetric responses at (A) GCE and (B) Ni-BPE in 0.1 mol l^{-1} NaOH without (a)/with (b) the addition of 60 ppm TA at $v = 1$ mV s^{-1}.

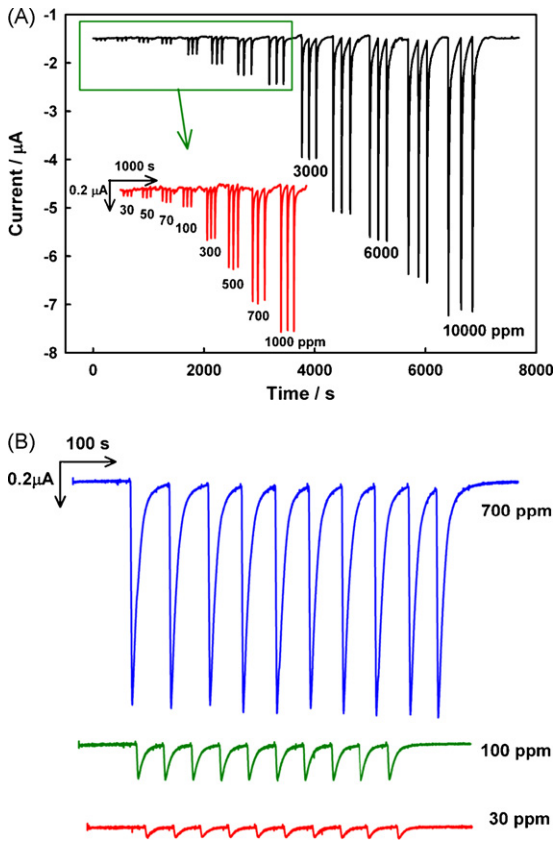

Fig. 4. (A) Typical FIA responses of Ni-BPE with increasing concentration of TA in 0.1 mol l^{-1} NaOH carrier solution. (B) FIA responses of different [TA] under repeated injections ($n = 10$). Conditions: E_{app} = +0.55 V vs. Ag/AgCl and H_f = 0.3 ml min^{-1}.

3.2. Flow injection analysis

The FIA parameters were first optimized for the purpose of analytical application. Initial experiments are concerned about the optimization of hydrodynamic flow rate (H_f) and applied potential (E_{app}) in the determination of TA by FIA. Fig. 3 shows typical FIA responses for 100 ppm of TA under various H_f and E_{app}. As can be seen in the H_f variation, the FIA peak currents were unaltered in the flow rate window of 0.1–0.3 ml min^{-1}. Beyond that, a sharp decrease in the peak current response was noticed. The peak current value at H_f = 0.3 ml min^{-1} is about twice higher than that at H_f = 0.5 ml min^{-1}. The decrease in peak current response at higher flow rates is possibly due to some kinetic restriction at the electrode/solution interphase. Virtually the same voltammograms observed by a simple cyclic voltammetry before and after high flow rates ruled out the possibility of this behavior be attributed to the leaching of electroactive material. We thus choose H_f = 0.3 ml min^{-1} as an optimal value for further experiments. Effect of the E_{app} on the TA detection response yields a peak-like current behavior with a maximum peak current response appears at +0.55 V vs. Ag/AgCl. This observation is in accordance with the cyclic voltammetric behavior of $Ni^{III}O(OH)$ species on the electrode surface as shown in Fig. 2. Hence, we have chosen E_{app} = +0.55 V for further experiments.

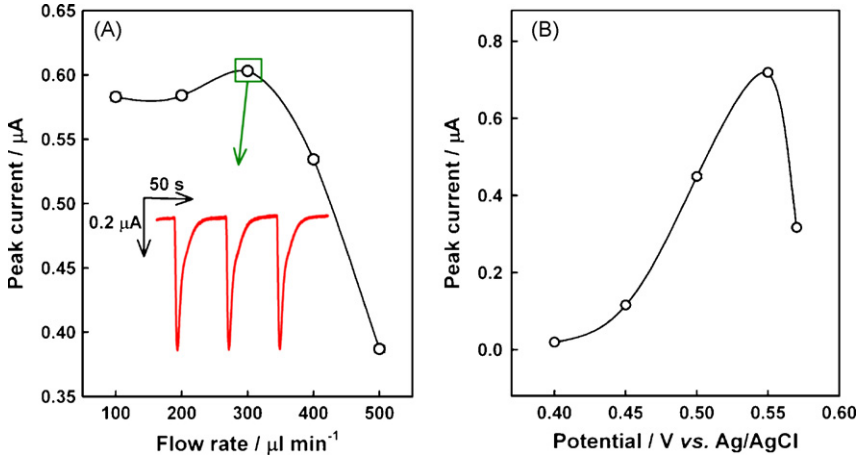

Fig. 3. Effects of (A) H_f and (B) E_{app} on the detection of 100 ppm TA at the Ni-BPE in 0.1 mol l^{-1} NaOH carrier solution.

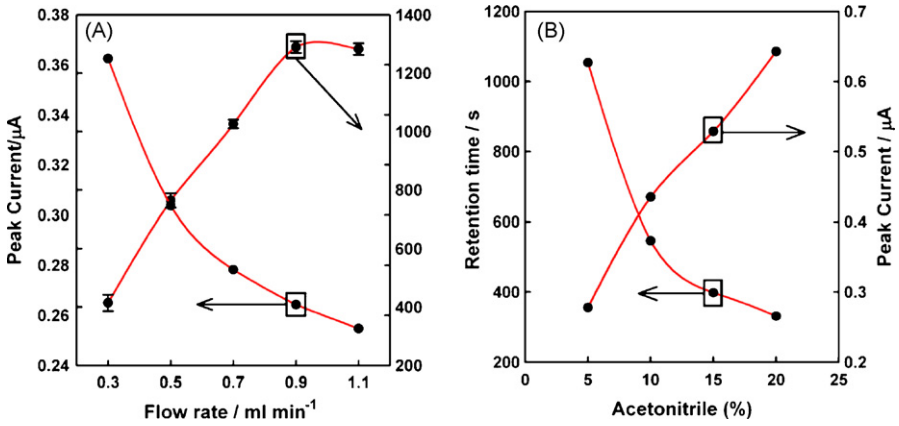

Fig. 5. HPLC responses on the effect of (A) H_f and (B) NaOH/ACN ratio on the detection of 300 ppm of TA at E_{app} = +0.55 V vs. Ag/AgCl. (A) 15% ACN + 0.1 mol l^{-1} NaOH as the eluent and (B) H_f = 0.9 ml min^{-1}.

Fig. 4A shows typical FIA responses for increasing [TA] with respect to peak currents under the optimized working conditions. The FIA peak currents were systematically increased with the increase in [TA]. A linear detection range up to 10^4 ppm of [TA] with a regression coefficient of 0.998 was obtained. Repeatability of the measurements was studied by analyzing the [TA] with 30, 100 and 700 ppm repeatedly for 10 continuous injections and the obtained relative standard deviation (R.S.D.) values are 3.2%, 0.3% and 0.3%, respectively (Fig. 4B). Inter electrode assay of different electrodes (n = 10) is further evaluated to prove the applicability of the Ni-BPE. There is good reproducibility with a R.S.D. of 1.94% (n = 10) for measurements carried out with a 100 ppm TA solution. A detection limit (D_L) of 4.44 ppm is obtained as a signal-to-noise ratio value of 3 from the lowest injected concentration (30 ppm).

3.3. Chromatographic characteristics

Good chromatographic separation can allow a direct determination of the TA content in real samples. A further experiment for the detection of TA by HPLC using the Ni-BPE as detector is studied. In this work, ACN +0.1 mol l^{-1} NaOH is used as the mobile phase. The effect of H_f and solvent ratio to the retention time and peak responses on the detection of 300 ppm TA are shown in Fig. 5. As can be seen, the chromatographic peaks systematically increase with the increase in H_f up to 0.9 ml min^{-1}; after that a plateau is noticed. In contrast, the retention time was decreased with the increase in H_f. Overall, H_f = 0.9 ml min^{-1} was chosen as good retention time and chromatographic resolution can be obtained at this flow rate.

The percentage of ACN in the carrier solvent also affects the retention time and peak response when employing an anion-exchange column for the separation of TA. Fig. 5B displays typical variation in the percentage of ACN against retention time and response peak current of TA. As can be seen, the chromatographic peaks were shifted to short retention time with large peak current value at higher percentage of ACN. Meanwhile, since organic solvent may change the electrochemical behavior of TA on the Ni-BPE, the mobile phase was used as the electrolyte to examine the electrochemical behavior of TA. Virtually the same cyclic voltammogram

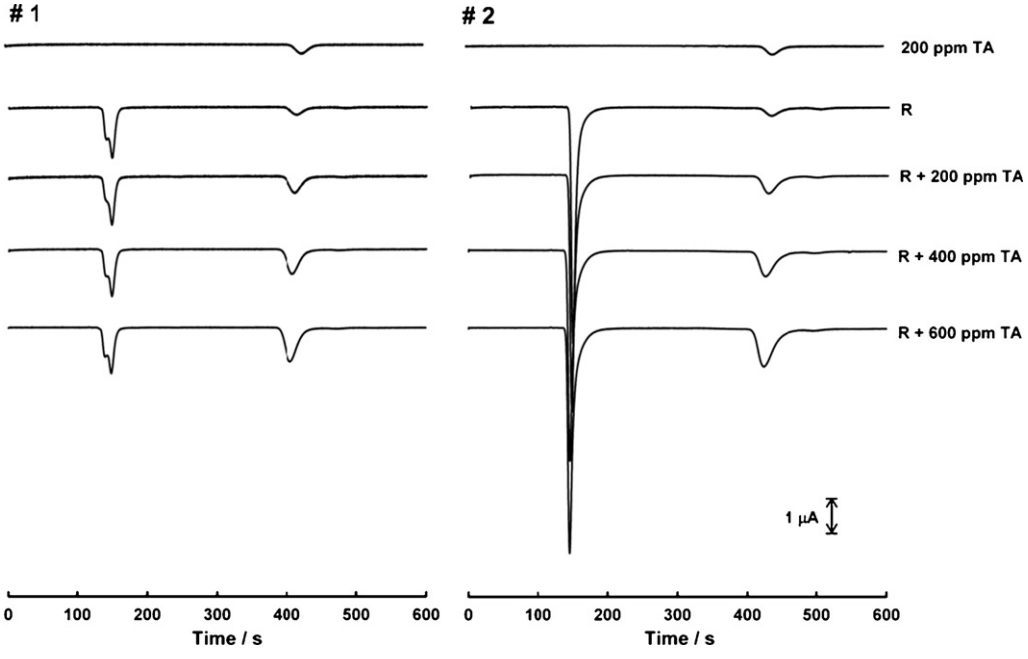

Fig. 6. Real sample analysis of cosmetic skin-whitening lotions by HPLC coupled with a Ni-BPE detector. Eluent = 15% ACN + 0.1 mol l^{-1} NaOH, E_{app} = +0.55 V vs. Ag/AgCl and H_f = 0.9 ml min^{-1}.

Table 1
Determination of TA containing cosmetic skin-whitening products using HPLC with Ni-BPE detector.

Sample[a]	Original detected value (ppm)[b]	Spike (ppm)	Detected value after spike (ppm)	Recovery (%)
#1	181.7	200	384.1 ± 1.3	101.2 ± 0.6
	± 1.2	400	591.7 ± 3.6	102.5 ± 0.8
		600	804.0 ± 1.5	103.7 ± 0.2
#2	183.0	200	388.2 ± 4.9	102.6 ± 2.4
	± 1.9	400	573.8 ± 2.5	97.7 ± 0.6
		600	778.5 ± 4.5	99.3 ± 0.7

[a] Dilution factor 1/110 and 1/54 for #1 and #2, respectively.
[b] Labeled value (after dilution) 182.3 and 184.9 ppm for #1 and #2, respectively.

was observed as compared to that in aqueous solution. Good stability with R.S.D. = 2.34% (n = 10) in the detection of TA indicates that the mobile phase is suitable in electrochemical detection. Overall, considering peak resolution, electrode stability and peak current values, an eluent ratio of 15% ACN/0.1 mol l^{-1} NaOH was therefore chosen as an optimal solvent condition for the HPLC experiments. A linear response for TA in the window of 3–1000 ppm with a slope and regression coefficient of 0.0013 μA ppm^{-1} and 0.9993, respectively, was observed. Reproducibility was tested by injecting 50 and 500 ppm of TA repeatedly for 10 measurements and the obtained R.S.D. values are 0.30% and 1.44%, respectively, for the above samples. Note that the chemical analytical techniques for TA determination published to date are summarized for pharmaceutical preparations and buffered solutions recently [12]. The calculated D_L (S/N = 3) of 0.13 ppm (i.e., 0.84 μmol l^{-1}) is better than most derivatization-based UV or fluorescence detection methods.

3.4. Analytical applications

Two different real samples of cosmetic skin-whitening #1 lotion (2% (w/w) as labeled) and #2 serum (1% (w/w) as labeled) were tested by HPLC with Ni-BPE as detector. Fig. 6 shows the chromatograms of the samples by standard addition method. Table 1 provides results of the analysis. As can be seen, the measured values of 181.7 ± 1.2 ppm in #1 lotion and 183.0 ± 1.9 ppm in #2 serum by this method are very close to the labeled values (after dilution) of 182.3 and 184.9 ppm for the respective samples. The recoveries of the cosmetic samples spiked with 200–600 ppm were also good and are in the range of 97.7–103.7%. These results demonstrate that the method is quite suitable for simple and sensitive detection of TA in cosmetics.

4. Conclusions

A cheap Ni-BPE coupled with HPLC was developed for the quantification of tranexamic acid in cosmetic lotions. Specific redox couple corresponding to the $Ni^{II}(OH)_2/Ni^{III}O(OH)$ species electrogenerated at +0.45 V vs. Ag/AgCl was proposed to involve in the mediated oxidation of TA in 0.1 mol l^{-1} NaOH. Both hydrodynamic FIA and HPLC parameters were systematically optimized in this study. The obtained analytical performance without any tedious off-line pretreatment procedures was better than the derivatization-based spectroscopic approaches. It can be applied to the determination of TA not only in cosmetics but also in clinical and pharmaceutical samples.

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