Colloid Polym Sci (2012) 290:1451–1456
DOI 10.1007/s00396-012-2714-y

SHORT COMMUNICATION

Temperature responsive carbon nanotubes/poly (*N*-isopropylacrylamide)-modified electrodes for electrochemical selective determination of dopamine, uric acid, and ascorbic acid

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Received: 21 January 2012 / Revised: 29 April 2012 / Accepted: 14 June 2012 / Published online: 28 June 2012

Abstract Poly(*N*-isopropylacrylamide)(PNIPAAm) with a lower critical solution temperature of about 32 °C was used as matrix to prepare temperature responsive carbon nanotubes (CNT) and functionalized CNT (fCNT) composites to modify glassy carbon electrode (GCE) as working electrode for electrochemical selective detection of dopamine, uric acid, and ascorbic acid. The GCE modification temperature (25 and 37 °C, denoted as 25f and 37f), working temperature (25 and 37 °C, denoted as 25aq and 37aq), and the type of CNT (CNT and fCNT) were found to significantly affect the electrocatalytic activity of the composites toward redox reactions of $Fe(CN)_6^{3-/4-}$ as a probe and the selective detection ability for the three analytes. The fCNT/PNIPAAm composite with the 25f–37aq temperature combination exhibited strong electrocatalytic activity and highly selective detection ability for the three analytes. In contrast, the same composite with the other three combinations (25f–25aq, 37f–25aq, and 37f–37aq) and the CNT/PNIPAAm composite with all four combinations exhibited insignificant electrocatalytic activity and no selective detection ability.

Keywords Poly(*N*-isopropylacrylamide) · Carbon nanotube · Temperature-sensitive polymer · Lower critical solution temperature · Electrocatalytic activity · Simultaneous detection

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Introduction

There have been intense investigations in the development of methods for the selective detections of dopamine (DA), uric acid (UA), and ascorbic acid (AA) [1–9]. Although the simultaneous determination of DA, AA, and UA at polymer-modified electrodes has been reported [5–9], it was not reported at a temperature responsive carbon nanotubes (CNT)/poly(*N*-isopropylacrylamide)(PNIPAAm) composite-modified electrode. PNIPAAm is one of the most studied thermosensitive polymers with a lower critical solution temperature (LCST) of around 32 °C. As the external temperature passes through the LCST, a thermoreversible phase separation takes place in the aqueous solution due to the temperature dependence of the interactions of polymer chains with water molecules. CNT, possessing graphene structure, good conductivity, and high mechanical strength, could facilitate electron transfer between the electroactive species and electrode [10]. The oxidation of DA, UA, or AA was found to be electrocatalyzed at several CNT-modified electrodes [4, 9–13], because CNT provided a lot of active sites with electrocatalytic activity.

In this work, CNT/PNIPAAm and fCNT/PNIPAAm composite films are prepared by casting from aqueous solutions at 25 °C (<LCST, denoted by 25f) or 37 °C (>LCST, denoted by 37f). The dispersion of CNT and fCNT in the composites as well as the surface properties (hydrophilicity or hydrophobicity) of the films differ at the two casting temperatures because of the LCST. Therefore, CNT/PNIPAAm- and fCNT/PNIPAAm-modified electrodes have different electrocatalytic activities toward redox reactions of ferricyanide ions ($Fe(CN)_6^{3-/4-}$) as a probe in 25 and 37 °C solutions (25aq and 37aq). The current research investigates the dependence of the electrocatalytic activity to the probe on the functionalization of CNT, casting temperature, and working temperature. The effect of this electrocatalytic activity on the selective detection of three analytes important to the human body (i.e., DA, UA, and AA) that are difficult to be simultaneously detected is also examined.

Experimental

Preparations of CNT, fCNT, and PNIPAAm

CNT was synthesized by the thermal chemical vapor deposition method in our laboratories [14]. The COOH-functionalized CNT (fCNT) was prepared by acid treatment of CNT at 60 °C for 3 h in a mixture of sulfuric acid and nitric acid at a ratio of 3/2 by volume. The COOH groups formed on the fCNT were verified by a Fourier transform infrared spectrophotometer (Perkin Elmer, Spectrum One) [14]. Reagent-grade *N*-isopropylacrylamide was supplied by Aldrich and used as received. PNIPAAm was synthesized by free radical polymerization of *N*-isopropylacrylamide (1.257 M) using ammonium persulfate (0.063 M) as an initiator in deionized water purged with N_2 at 25 °C for 6 h. The reaction mixture was then heated to 60 °C to precipitate the polymer. After removing the top clear portion, 20 mL of deionized water was added to the precipitate to dissolve the polymer, followed by heating to 60 °C to precipitate the polymer. This precipitation–dissolution–precipitation process was repeated two more times to obtain pure polymer, followed by drying at 60 °C in a vacuum oven. The weight average molecular weight of the synthesized PNIPAAm was about 550,000, which was measured in methanol at 25 °C using an Ubbelohde viscometer as described in literature [15].

Preparations of CNT/PNIPAAm- and fCNT/PNIPAAm-modified GCE

PNIPAAm was first dissolved in deionized water at 25 °C. The CNT (or fCNT) was then added to the polymer solution to obtain 2/10 by weight aqueous solutions of CNT/PNIPAAm (or fCNT/PNIPAAm). About 20 μL of each solution was cast on a prepolished glassy carbon electrode (GCE) with a 3-mm-diameter circular active area. The GCE was then placed in an oven at 25 or 37 °C (25f or 37f, respectively) until the cast film on the GCE dried.

Electrochemical analyses of CNT/PNIPAAm- and fCNT/PNIPAAm-modified GCE

A potentiostat (CH611D, CH Instruments) was used to conduct cyclic voltammetry (CV) at 25 or 37 °C (25aq or 37aq, respectively) in a conventional three-electrode system with a GCE as working electrode, a platinum wire as auxiliary electrode, and an Ag/AgCl/3 M KCl as reference electrode. The CNT/PNIPAAm- and fCNT/PNIPAAm-modified GCE (via casting at 25 or 37 °C) was then immersed in 5 mM potassium ferricyanide [$K_3Fe(CN)_6$] (Nihon Shiyaku Industries) in 0.1-M KCl aqueous solution at 25 or 37 °C. A pH 7.4 phosphate buffer solution (0.1 M) served as a background electrolyte in all experiments. CV was conducted between −0.4 and 0.8 V at a scan rate of 100 mV/s. The second CV cycle was used to investigate the electrocatalytic activity of the composite films. To investigate the ability for simultaneous detecting DA, UA, and AA (Sigma–Aldrich), the CNT/PNIPAAm- and fCNT/PNIPAAm-modified GCE was immersed in the aqueous solution containing the mixture of the three analytes. CV analysis was then performed at 25 or 37 °C between −0.4 and 0.6 V at a scan rate of 25 mV/s.

Field emission scanning electron microscopy characterizations of the CNT/PNIPAAm and fCNT/PNIPAAm composites

A field emission scanning electron microscopy (FESEM) system (Hitachi S-4800) operated at 3 KeV was used to investigate the surface morphology of CNT/PNIPAAm and fCNT/PNIPAAm composites cast at 25 or 37 °C.

Results and discussion

Dispersion of CNT and fCNT in PNIPAAm

Figure 1 shows the FESEM images for CNT and fCNT dispersions in PNIPAAm matrix as cast at 25 and 37 °C. CNT aggregation was found exposed on the film surface from casting at 25 °C, which was lower than the LCST (32 °C) of PNIPAAm (Fig. 1a). In contrast, CNT aggregation was buried in PNIPAAm from casting at 37 °C, which was higher than the LCST (32 °C) of PNIPAAm (Fig. 1b). The aggregation of CNT indicated that unfunctionalized CNT did not uniformly disperse in PNIPAAm matrix. The exposure of CNT in Fig. 1a was associated with the dissolution of PNIPAAm in water during casting at 25 °C so that CNT was uncovered. The buried CNT in Fig. 1b was associated with the precipitation of PNIPAAm from the solution during casting at 37 °C so that CNT aggregates were covered by PNIPAAm. Figure 1c, d show FESEM images for fCNT dispersion in PNIPAAm matrix as cast at 25 and 37 °C, respectively. The dispersion of fCNT appeared to be more uniform than that of CNT. The fCNT in Fig. 1c appeared to be more uncovered than that in Fig. 1d. Visual observations reveal that the samples in Fig. 1b and d were white and opaque, whereas the samples in Fig. 1a and c were black and translucent, consistent with FESEM images. Evidently, the casting temperature and CNT functionalization both affected the surface morphologies of the composite films.

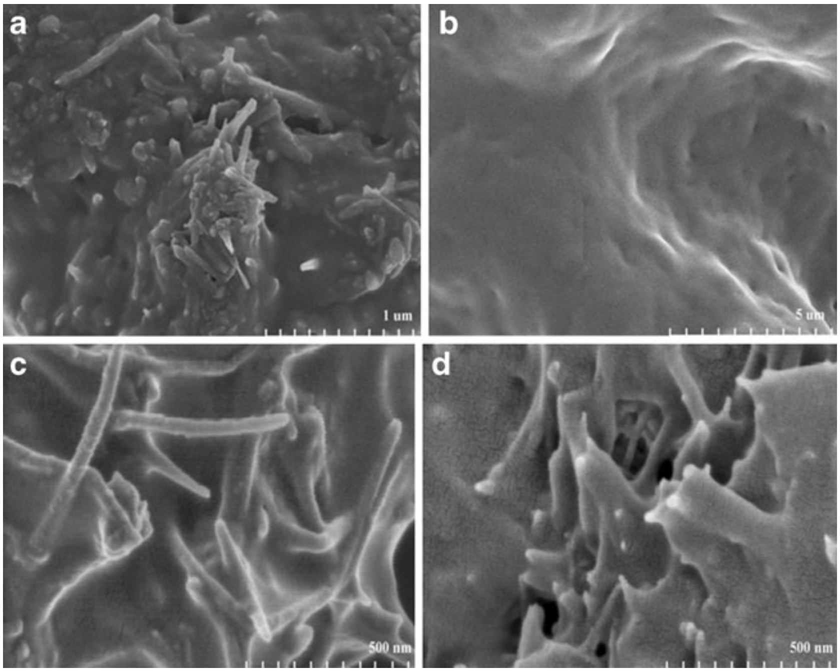

Fig. 1 FESEM images for the composites cast from CNT/PNIPAAm 2/10 in deionized water at **a** 25 °C and **b** 37 °C, and from fCNT/PNIPAAm 2/10 at **c** 25 °C and **d** 37 °C

CV analyses for CNT/PNIPAAm and fCNT/PNIPAAm films

Figure 2 shows the CV curves recorded at 25 and 37 °C (25aq and 37aq) using $Fe(CN)_6^{3-/4-}$ as a probe for the CNT/PNIPAAm 2/10 (Fig. 2a) and the fCNT/PNIPAAm 2/10 (Fig. 2b) films that were cast at 25 and 37 °C (25f and 37f) on GCE. From Fig. 2a, the 25f–25aq temperature combination exhibited slightly enhanced current density with a decreased oxidation potential compared with bare GCE. This observation indicated that the CNT/PNIPAAm with the 25f–25aq combination exhibited a slight electrocatalytic activity toward redox reactions of the $Fe(CN)_6^{3-/4-}$ probe. The other three combinations showed much lowered current densities compared with bare GCE, indicating that the CNT/PNIPAAm with the three temperature combinations exhibited no electrocatalytic activity. This suggests that CNT without functionalization resulting in aggregation (Fig. 1) exhibited no electrocatalytic activity to the redox reactions of the probe although the 25f–25aq combination that led to the exposure of the CNT on the film surface could provide interactions of CNT and the probe, leading to low electrocatalytic activity to the probe. The 25f–37aq and 37f–37aq combinations that exhibited no redox signals could be attributed to the formation of hydrophobic surface in aqueous solution at 37 °C (>LCST) at which the polar linkage (i.e., the hydrophilic linkage) in the cast CNT/PNIPAAm films could possibly orientate inward the film and the hydrophobic group outward the film, resulting in a hydrophobic surface and therefore much decreased interactions between the film and the probe (anions), leading to negligible detection ability of the film. It was thus evident that the interactions of CNT and the probe were important to provide electrocatalytic activity for the composite. For the composite with the 37f–25aq, the exposure of CNT on the film surface and thus the CNT interactions with the probe can be expected to be at a level between the composite with the 25f–25aq condition and those with the other two conditions. The current density for the composite with the 37f–25aq condition (curve c in Fig. 2a) was consistently between the other three conditions (curves a, b, and c in Fig. 2a).

From Fig. 2b, the fCNT/PNIPAAm composite with the 25f–37aq combination exhibited much enhanced current density and a decreased oxidation potential compared with bare GCE, indicating that the fCNT/PNIPAAm composite with the 25f–37aq combination had high electrocatalytic activity to the redox reactions of the probe. The other three combinations showed much lowered current densities compared with bare GCE, indicating that the composite with the three combinations exhibited no electrocatalytic activity to the probe. This might be associated with the observation that the 25 °C-cast fCNT/PNIPAAm film resulted in uniform coating with PNIPAAm via hydrogen bonding between the COOH of fCNT and the amide group of PNIPAAm, and the CV analyses at 37 °C (>LCST) resulted in the fixation of the good dispersion (Fig. 1c). The 25f–25aq and 37f–25aq combinations provided poor fixation for the dispersion because the CV analysis was conducted at 25 °C which was lower than the LCST of PNIPAAm, leading to no electrocatalytic activity. For the 25f–25aq combination, the exposed anionic fCNT from casting at 25 °C without being later covered or fixed by PNIPAAm during CV analyses at

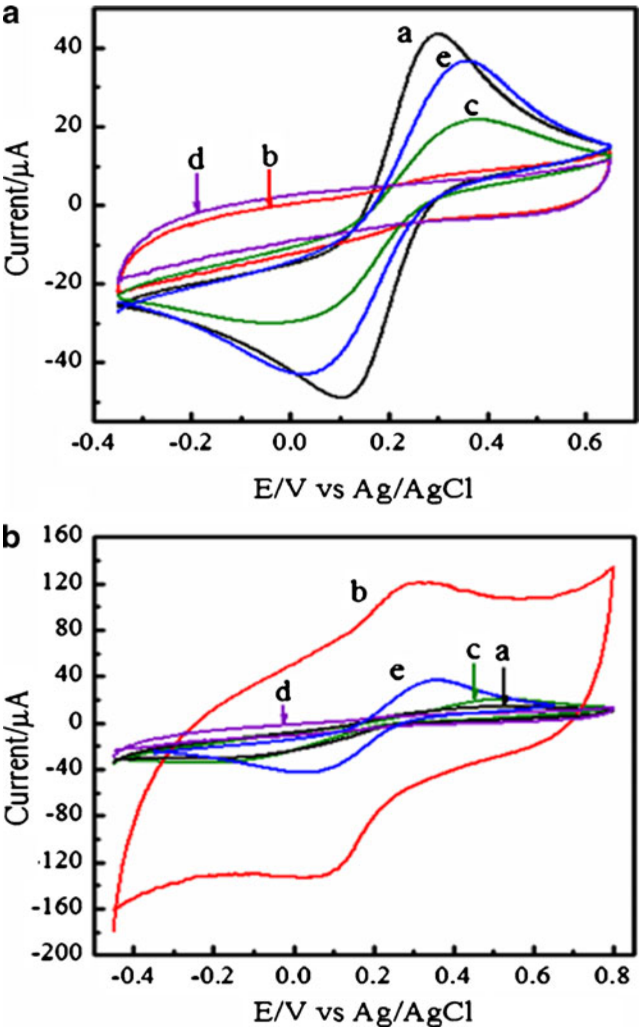

Fig. 2 CV recorded at **a** CNT/PNIPAAm 2/10 film- and **b** fCNT/PNIPAAm 2/10 film-modified GCE electrode in 5 mM $Fe(CN)_6^{3-/4-}$ aqueous solution for (*a*) 25f–25aq, (*b*) 25f–37aq, (*c*) 37f–25aq, (*d*) 37f–37aq, and (*e*) bare GCE. Scan rate 100 mV/s

25 °C could lead to expulsion interactions between the anionic fCNT and the anionic probe, resulting in weak redox signals (curve a in Fig. 2b). For the 37f–25aq combination, the anionic fCNT, however, was covered by PNIPAAm after casting at 37 °C and screened by PNIPAAm during CV analyses at 25 °C and this lowered the expulsions between the anionic fCNT and the anionic probe resulting in slightly enhanced redox signals compared with the 25f–25aq (curves a and c in Fig. 2b). The 37f–37aq combination provided much covered fCNT by PNIPAAm after casting at 37 °C (Fig. 1d) and the later CV analysis at 37 °C (>LCST) resulted in the fixation of the poor dispersion; this led to no electrocatalytic activity (curve d in Fig. 2b). In addition, being responsive to the 37 °C during casting the film, the polar linkage (i.e., the hydrophilic linkage) in the polymer orientated inward the film and the hydrophobic group outward the film, resulting in a hydrophobic surface and

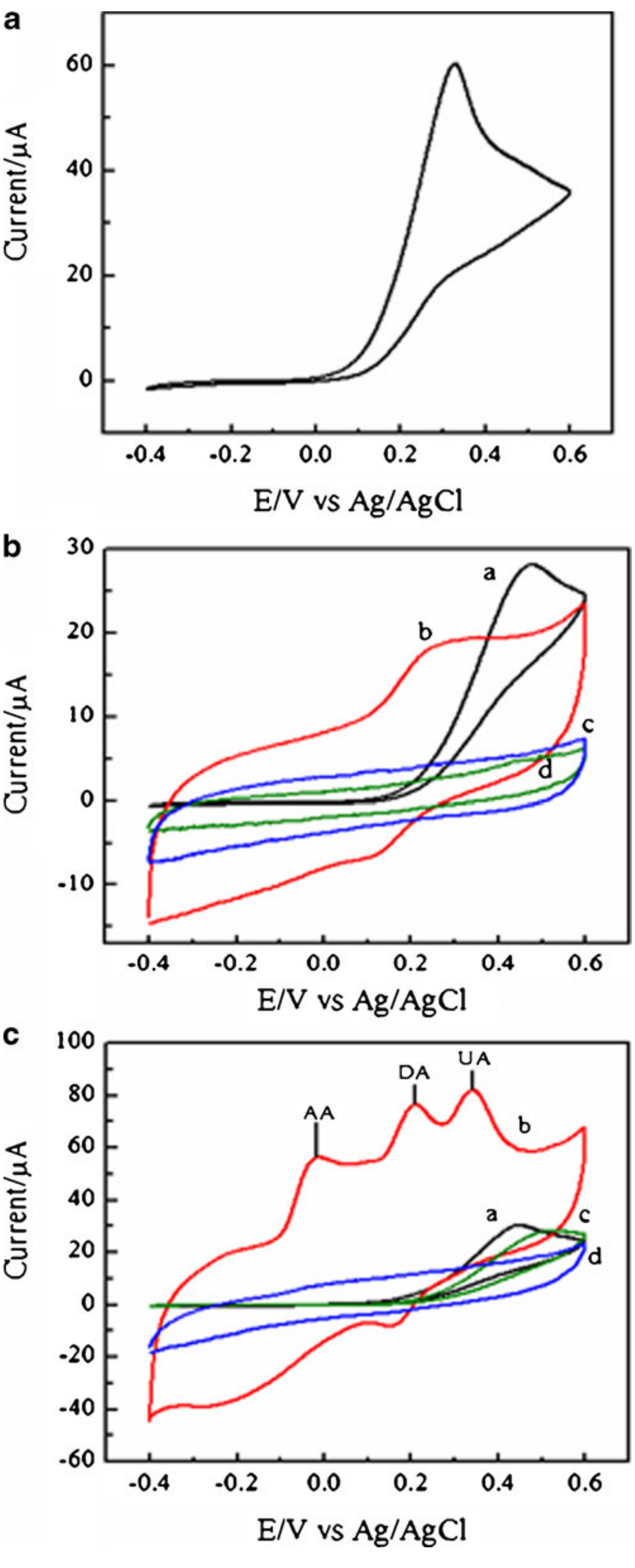

Fig. 3 CV recorded at **a** bare GCE electrode, **b** CNT/PNIPAAm 2/10 film-, and **c** fCNT/PNIPAAm 2/10 film-modified GCE electrode in mixture of AA (4 mM), DA (400 μM), and UA (800 μM) in 0.1 M PBS (pH 7.4) aqueous solution containing 0.1 M KCl. CV curves in (**b** and **c**) were for (*a*) 25f–25aq, (*b*) 25f–37aq, (*c*) 37f–25aq, (*d*) 37f–37aq. Scan rate 25 mV/s

therefore a decreased swelling ability in aqueous solution, leading to lowered interactions of the film and the probe.

Simultaneous detection of DA, UA, and AA with CNT/PNIPAAm films

The insignificant electrocatalytic activity of the CNT/PNIPAAm film to the probe, as shown in Fig. 2a, led to very low or no electrocatalytic activity to the redox reactions of the mixture of DA, UA, and AA compared with bare GCE (Fig. 3a, b). Figure 3b shows that the 25f–25aq combination exhibited the strongest current density among the four temperature combinations, consistent with Fig. 2a. In conclusion, the insignificant electrocatalytic activity of the CNT/PNIPAAm 2/10 film resulted in inability to selectively detect the mixture of DA, UA, and AA as can be seen in Fig. 3b. However, the 25f–37aq and 37f–25aq combinations exhibited different results from those in Fig. 2a. The discrepancy can be attributed to the different levels of interactions between the composite films and probe from those between the films and mixture of DA, UA, and AA. The insignificant electrocatalytic activity of the CNT/PNIPAAm 2/10 film resulted in the inability to carry out selective detection in the mixture of DA, UA, and AA, as shown in Fig. 3b.

Simultaneous detection of DA, UA, and AA with fCNT/PNIPAAm films

The negligible insignificant electrocatalytic activity of the fCNT/PNIPAAm 2/10 film with the 25f–25aq, 37f–25aq, and 37f–37aq combinations to the probe, as shown in Fig. 2b, also led to negligible electrocatalytic activity to the redox reactions of the mixture of DA, UA, and AA. This resulted in inability for selective detection in the mixture, as shown in Fig. 3c (curves a, c, and d). The 25f–37aq combination had a much stronger anodic peak than the other three combinations (Fig. 2b), exhibiting strong anodic peaks and highly selective detection ability for the mixture of the three analytes (curve b in Fig. 3c). The anodic peaks in curve b of Fig. 3c at near 0, 0.2, and 0.35 V can be assigned to oxidations of AA, DA, and UA, respectively, via preliminary CV analyses of the three analytes individually. As previously discussed, the 25 °C-cast fCNT/PNIPAAm film could be uniformly coated by PNIPAAm (Fig. 1c) via hydrogen bonding between the COOH of fCNT and the amide group of PNIPAAm. Subsequent CV analyses at 37 °C (>LCST) also results in the formation of a staunch thin film on fCNT. The composite with this staunch film on fCNT can thus efficiently electrocatalyze the oxidation of DA, UA, and AA and distinguish between the three analytes via different interactions between the composite and each of

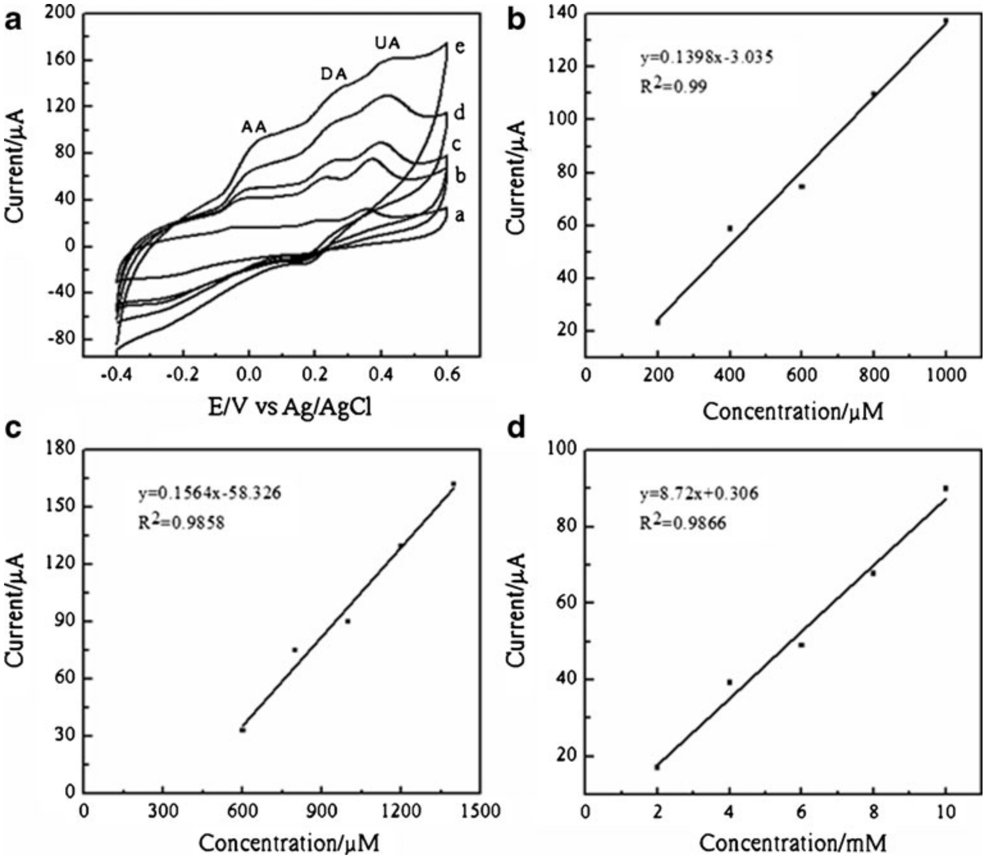

Fig. 4 **a** CV curves recorded at 37 °C at GCE electrode, modified by fCNT/PNIPAAm 2/10 film at 25 °C, in AA/DA/UA mixture-containing solution with concentration ratios of (*a*) 2 mM/200 μM/600 μM, (*b*) 4 mM/400 μM/800 μM, (*c*) 6 mM/600 μM/1,000 μM, (*d*) 8 mM/800 μM/1,200 μM, and (*e*) 10 mM/1,000 μM/1,400 μM. Scan rate 25 mV/s. Anodic peak currents were plotted versus concentrations of **b** DA, **c** UA, and **d** AA

the three analytes. After the CV scan at 37 °C, the modified GCE was brought to 25 °C in the same solution for an additional CV scan. The anodic current of the CV curve was found to be significantly decreased and the selective detection ability was lost. This finding indicated that the staunch coating of the fCNT by the PNIPAAm film was necessary for the fCNT/PNIPAAm composite film to exhibit strong electrocatalytic activity and selective detection ability for the three analytes.

Amperometric analysis of the fCNT/PNIPAAm film in mixtures of DA, UA, and AA

Figure 4 shows results of amperometric analysis for the fCNT/PNIPAAm film of the 25f–37aq condition, which exhibited strong electrocatalytic activity, for simultaneously detecting DA, UA, and AA. The CV curves in Fig. 4a show that the three analytes can be simultaneously detected. The anodic peaks at near 0, 0.2, and 0.35 V corresponded to oxidations of AA, DA, and UA, respectively. The peak currents were increased with increasing concentrations of the analytes. Figure 4b–d shows that the peak currents were linearly related to the concentrations of the analytes up to 1,000 μM, 1,400 μM, and 10 mM for DA, UA, and AA, respectively. The detection sensitivities are 1.98, 2.22, and 0.12 μA/(cm^2 μM) for DA, UA, and AA, respectively, given that the active area on GCE is 0.071 cm^2. The sensitivity of the simultaneous detection from high to low is UA >DA>AA.

Conclusions

The electrocatalytic activity of CNT/PNIPAAm and fCNT/PNIPAAm composites toward redox reactions of $Fe(CN)_6^{3-/4-}$ as a probe was found to be significantly temperature dependent. CV data revealed that the CNT/PNIPAAm composite was not electroactive at all at 37 °C (>LCST) and was slightly electroactive at 25 °C (<LCST) when the composite was prepared at 25 and 37 °C. The fCNT/PNIPAAm composite was slightly electroactive at 25 °C if the composite was prepared at 25 and 37 °C. The composite was not electroactive at 37 °C if the composite was prepared at 37 °C but was highly electroactive at 37 °C if the composite was prepared at 25 °C. The highly electroactive composite has led to highly selective detection ability for the mixture of DA, UA, and AA. The composites with insignificant electrocatalytic activities exhibited no selective detection ability for the three analytes.

Acknowledgments We gratefully acknowledge the financial support of the National Science Council of Taiwan under the NSC 98-2221-E-390-002-MY3 contract.

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