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Separation and simultaneous detection of anticancer drugs in a microfluidic device with an amperometric biosensor

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ABSTRACT

A simple and highly sensitive method for simultaneous detection of anticancer drugs is developed by integrating the preconcentration and separation steps in a microfluidic device with an amperometric biosensor. An amperometric detection with dsDNA and cardiolipin modified screen printed electrodes are used for the detection of anticancer drugs at the end of separation channel. The preconcentration capacity is enhanced thoroughly using field amplified sample stacking and field amplified sample injection techniques. The experimental parameters affecting the analytical performances, such as pH, temperature, buffer concentration, water plug length, and detection potential are optimized. A reproducible response is observed during multiple injections of samples with a RSD <5%. The calibration plots are linear with the correlation coefficient between 0.9913 and 0.9982 over the range of 2–60 pM. The detection limits of four drugs are determined to be between 1.2 (±0.05) and 5.5 (±0.3) fM. The applicability of the device to the direct analysis of anticancer drugs is successfully demonstrated in a real spiked urine sample. Device was also examined for interference effect of common chemicals present in real samples.

1. Introduction

Anthracyclines are the most potent anticancer drugs used to date. However, of several naturally occurring and synthetic anthracyclines, daunomycin (DAN), doxorubicin (DOX), idarubicin (IDA), and the derivative mitoxantrone (MIT) are the most prevalent anticancer drugs used clinically in the treatment of various types of cancers (Minotti et al., 2004; Peng et al., 2005). Although they are clinically efficient for the cancer patients, even though these drugs also bring some undesirable side effects such as meylosuppression, stomatitis, nausea, and vomiting. The most severe side effect is the cumulative dose related cardiotoxicity, and commonly attributed to a radical damage on the cardiac tissue (Hortobagyi, 1997). Furthermore, through excretion, these drugs can reach ground-water and may exert toxic effects on microbial populations and subsequently cause public health hazards (Alexy, 2003). Thus, it is desirable to develop a simple and highly sensitive method for the determination of these drugs. In addition, due to the structure similarity of these drugs simultaneous separation and detection is also required.

The most prevalent methods for anthracycline analysis are HPLC, coupled with UV absorption spectrometry, fluorescence spectrometry, or mass spectrometry (Badea et al., 2005; Hulhoven and Desager, 1976; Mahnik et al., 2006; Ricciarello et al., 1998). However, the complexity of mixtures, especially biomedical and environmental samples, has led to the development of more efficient analytical technique such as capillary electrophoresis (CE) that is now widely used as a routine analytical method in various biomedical applications (Shiddiky and Shim, 2007). In one study, zepto-molar amount of DOX had been determined using capillary electrophoresis–laser induced fluorescence (CE–LIF) and matrix-assisted laser desorption/ionization (MALDI) system in subcellular fraction; however, the technique is very cumbersome and was used for only DOX (Hempel et al., 2001). Thus, improvement in terms of simultaneous detection of anthracyline analogous is still desirable for the trace quantification. Recently, a microfluidic device consisted of both field amplified sample stacking (FASS) and field amplified sample injection (FASI) was developed and successfully implemented for the separation and detection of various biological analytes by our group (Shiddiky et al., 2006; Shiddiky and Shim, 2007). The second and most concerned step in the microfluidic device is the detection system. Various detection systems such as mass spectrometry, UV adsorption, and fluorescence detection method can apply for anthracycline detection but these techniques are multistage, complicated, tedious, lengthy, expensive, and less sensitive. The laser-induced fluorescence (LIF) is the most prevalent detection system used for anthracyclines as they possess native properties and therefore do not require derivatisation (Gillian et al., 2008). In general, electrochemical detection requires relatively simple, compact, low cost instrumentation, and shows

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low detection limits comparable to those of LIF detection. Thus, we tried to develop a very simple, robust, and sensitive method for simultaneous detection of anticancer drugs by combining the two most extensive strategies i.e., preconcentration of analytes and finally their electrochemical detection relying on the use of our previously developed robust microchip design (Shiddiky et al., 2006; Shiddiky and Shim, 2007).

Conventional electrochemical methods with bare electrodes for anthracycline detection show surface fouling of the electrode, unstable response, and low sensitivity. Thus, modification of the electrode surface is also important to attain a stable, sensitive, and reproducible response. Among many types of electrodes, the screen-printed electrode (SPE) is widely used in biosensor applications because of their low-cost, ease of mass production, flexible design, miniaturization, and disposability. SPE, however, has a relatively low sensitivity compared with conventional electrode. Thus, it is desirable to enhance the conductivity of the SPE with the treatment of gold nanoparticles (AuNPs). Conducting polymers are widely used in the fabrication of biosensors because biomolecules can be covalently immobilized onto the conjugated polymer layers as they contain functional groups such as –COOH or $–NH_2$ (Lee and Shim, 2001; Zhu et al., 2010). The modification of sensor probe was performed with double stranded DNA (dsDNA) and cardiolipin (CL) because the anthracyclines interact dsDNA in a stable manner through intercalation with the planar, aromatic group stacked between base pairs (Quigley et al., 1980). Additionally, anticancer drugs interact significantly with anionic lipid CL present on cancer cell surface during cancer therapy (Escriba et al., 1990). Thus, AuNPs deposited conducting polymer modified SPE was employed in this study, with dsDNA and CL as recognition elements for simultaneous detection of separated anticancer drugs in a microfluidic device.

In the present study, firstly the biosensor probe was fabricated by forming covalent bonds between amine terminated probe DNA (pDNA), and a newly synthesized carboxylic acid group-functionalized conducting polymer (polyTTBA) layer on the SPE/AuNPs surface followed by hybridization of complementary DNA (cDNA) and adsorption of CL. The biosensor surface was characterized using X-ray photon spectroscopy (XPS). Further, the fabricated biosensor was applied for the simultaneous detection of preconcentrated and separated anticancer drugs in a microfluidic device. Microfluidic device having both FASS and FASI channels for the preconcentration process and electrokinetic (EK) separation were used for electrochemical detection (ED) by chronoamperometry. Various experimental parameters affecting the separation process and detection such as, pH, temperature, buffer concentration, water plug length, and detection potential were optimized the detection limit for drugs was determined. The proposed method was tested in real human urine sample for the detection of spiked concentrations of anticancer drugs.

2. Experimental

2.1. Materials

A terthiophene monomer having a carboxylic acid group, 5,2′:5′,2″-terthiophene-3′-carboxylic acid (TTBA), was synthesized according to a previous method (Lee and Shim, 2001). Tetrabutylammonium perchlorate (TBAP, electrochemical grade) was purchased from Fluka (USA) and purified according to a general method, followed by drying under vacuum at 1.33×10^{-3} Pa (Shim and Park, 1997). 1-Ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC), *N*-Hydroxysuccinimide (NHS), dichloromethane (99.8%, anhydrous, and sealed under N_2 gas), and trisodium citrate were purchased from Sigma Co. (USA). Sodium tetrahydridoborate and $HAuCl_4 \cdot 3H_2O$ were obtained from Aldrich (USA). All aqueous solutions were prepared in ultra pure water obtained from a Milli-Q water purifying system (18 MΩ cm). The analytical standard mitoxantrone (MIT), idarubicin (IDA) doxorubicin (DOX), and daunomycin (DAN) were purchased from Aldrich (USA). Single stranded (ss) probe DNA and complementary DNA were received from Bioneer (South Korea). Stock solutions of MIT, IDA, DOX, and DAN were prepared in a 0.1 M PBS (pH 7.4) and diluted as required. Cardiolipin was purchased from Avanti Polar Lipid Inc. Phosphate buffer solutions (PBS) were prepared with 0.1 M of disodium hydrogen phosphate, 0.1 M of sodium dihydrogen phosphate, and 0.9% sodium chloride. The FASS buffer was a 20 mM borate solution pH of 9, and the FASI buffer was a 20 mM PBS of pH 5. A 20 mM borate buffer of pH 9 with 10% acetonitrile was used as a separation buffer. The detection buffer used was 100 mM PBS of pH 7.4. All solutions were prepared with ultra pure water (18 MΩ cm) and deoxygenated by bubbling nitrogen for 30 min and filtered through 0.45 mm millipore filters (Millipore, Billerica, MA, USA) before performing ED experiments. All other chemicals were of extra pure analytical grade and used without further purification.

2.2. Instruments

All electrochemical experiments were performed in a three electrode system consisting of a modified working SPE, Ag/AgCl reference electrode, and Pt-wire counter electrode with an EG&G Princeton Applied Research, Model 273 Potentiostat/Galvanostat. A Spelman CZE 1000 R (NY, USA) was used as the high-voltage supply. The microfluidic device consists of two parallel preconcentration channels (FASS and FASI channels) in conjunction with a separation channel as shown in Fig. 1(A). The separation channel (from R6 to R8, $l = 92$ mm) consisted of a 150 μm double-tee injector (Shiddiky and Shim, 2007). The FASS channel (from R2 to R3, $l = 60$ mm) includes a narrow sample channel tee injector of width 25 μm. The length of the FASI channel (from R4 to R5) was 80 mm. The widths of FASS, FASI, and separation channels were 150, 150, and 100 μm respectively. The depth of channels was ~23 μm. A platinum wire was inserted into the individual reservoir to establish electrical contact. The electrochemical detector consisted of an Ag/AgCl, Pt-wire, which were placed in the detection reservoir, where the working SPE was aligned horizontally with respect to the separation channel exit.

2.3. Preparation of AuNPs

AuNPs were prepared according to a previously reported procedure (Shiddiky and Shim, 2007) as follows. A 1 wt% $HAuCl_4$, 38.8 mM trisodium citrate, and 0.0075 wt% $NaBH_4$ solutions were prepared in separate vials. Next, an empty flask was filled with 45 mL of distilled water. The prepared solutions were sequentially added to the water as follows: 0.5 mL of 1.0 wt% $HAuCl_4$, 1 mL of 38.8 mM trisodium citrate, and 0.5 mL $NaBH_4$ while stirring for 1 min, 1 min and 5 min respectively. The resulting solution turned pink, indicating the formation of particles.

2.4. Fabrication of screen-printed electrode (SPE)

The details of the printing process for the SPE were described previously (Shiddiky et al., 2006). The printing was applied on polyethylene-based films (10 mm × 30 mm). Each film consisted of 20 individual SPE. First, the Ag conducting layer was printed onto the film followed by printing of the carbon ink layer. Finally, an insulating ink layer was printed to cover the junction between the working area and the Ag conducing layer, giving a definite shape to the working electrode ($d = 1.25$ mm).

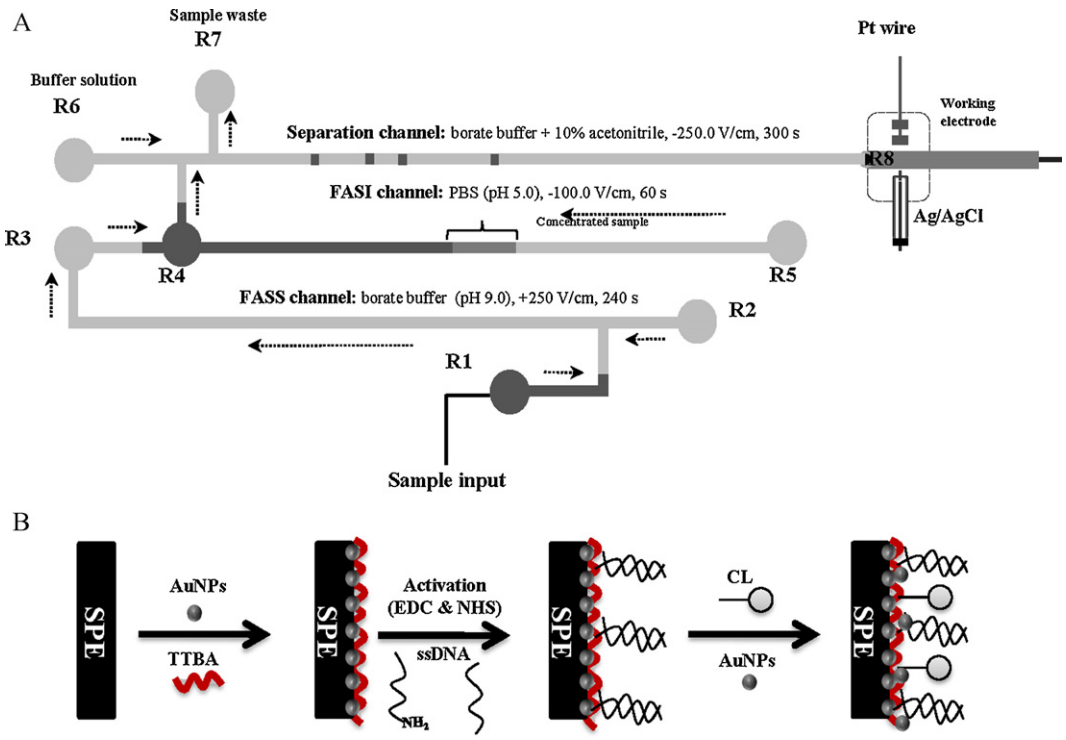

Fig. 1. (A) Schematic diagram of the microfluidic device. (B) Schematic representation of the biosensor fabrication.

2.5. Fabrication of biosensor

The fabrication steps of biosensor are shown in Fig. 1(B). Firstly, AuNPs were electrodeposited onto SPE in a 0.5 M H_2SO_4 solution containing 0.001% $HAuCl_4$ using the potential sweep from +1.4 to +0.0 V. The electrodeposition conditions were as follows: deposition time, 60 s; deposition potential, −1 V; scan rate, 0.1 V/s. The poly-TTBA (pTTBA) layer was formed on SPE/AuNPs. Briefly, 1 mM TTBA monomer containing solution prepared in 1:1 of di (propylene glycol) methyl ether and tri (propylene glycol) methyl ether was dropped on SPE/AuNPs surface and incubated the electrode until dried, and then transferred for polymerization. A polymer film was formed on the electrode through potential cycling twice from 0.0 to +1.4 V (vs. Ag/AgCl) at a scan rate of 0.1 V/s in a 0.1 M PBS (pH 7). After polymerization, the SPE/AuNPs/pTTBA electrode was immersed for 12 h in a 10 mM PBS (pH 7) containing 10 mM EDC and 10 mM NHS to activate the carboxylic acid groups of pTTBA layer. An amine terminated pDNA was immobilized to form the SPE/AuNPs/pTTBA/ssDNA and further cDNA was hybridized to form SPE/AuNPs/pTTBA/dsDNA electrode. After washing, electrode was treated with CL solution prepared in chloroform for 30 s. The AuNPs were then formed on the probe surface by dipping the electrode into a colloidal AuNPs solution (Chandra et al., 2011), forming SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs as the final sensing probe.

2.6. Fabrication of microfluidic device

The channel pattern of microfluidic device was fabricated using the previously reported method (Shiddiky et al., 2006; Shiddiky and Shim, 2007). Slide glasses (2.5 cm × 7.5 cm) were washed chemically before proceeding. Briefly, slide glasses were cleaned ultrasonically in detergent, ethanol, acetone, and Milli-Q water in an ultrasonic bath. Piranha (safety note: always add peroxide to sulfuric acid, never vice versa) and NH_3/H_2O_2 (3:1) solutions were also used for cleaning. A positive type photomask with the channel structure was designed using computer-aided design (CAD), transferred onto a patterned stencil, and finally printed onto a polystyrene based film using a screen printer (Model BS-450HT, Bando Industrial, South Korea). The Hexamethyldisilazane (HMDS) was spin coated using a home-made spin coater at 3000 rpm for 5 s onto the surface of the glass plates as reported earlier (Shiddiky et al., 2005). The Microposit S1813 PR positive type photoresist was then spin-coated at 3000 rpm for 25 s over the surface of the HMDS onto glass wafers. After a 3 min prebaking at 110 °C, the photomask was placed onto the photoresist- coated glass slides and exposed to UV light (365 nm, 115 V, 60 Hz, 0.20 A) with a UV flood source (Model ENF-240C, Spectronics, Westbury, NY, USA) for 7 min. The glass slides were developed by immersing into the developer solution for 6 min and cleaned using distilled water for 10 min. A post baking step was carried out at 110 °C for 5 min. All exposed glasses were etched in a slowly stirred mixture solution of $HF:H_2O:NH_4$ (14 mL:85 mL:56.5 g) for 20 min. The photoresist from the etched glass wafers was removed using distilled water and acetone and dried in an oven at 105 °C overnight. All Reservoirs were made by punching holes in the cover of the glass plates. The etched plates and cover plates were then cleaned as described above and thermally bonded in a furnace placed for 10 h over the cover glass. Finally, the channel outlet side was cut off, leaving it at the end side of the chip. After cutting and smoothening, we checked the channel exit under a microscope and found it to be a flat end.

2.7. Preconcentration, separation, and electrochemical detection procedure

The 100 mM stock solutions of test compounds were prepared in 0.1 M PBS (pH 7.4). Before the experiment, all test solutions were filtered using 0.45 μm millipore filters. The interior of the channel was washed with 0.1 M NaOH and distilled water for 30 min. The final step of washing was performed five times with a running buffer. In total, 20 mM borate buffer solution (pH 9) and 20 mM PBS (pH 5) were used to fill the FASS and FASI channels respectively by pumping. Then, the separation channel

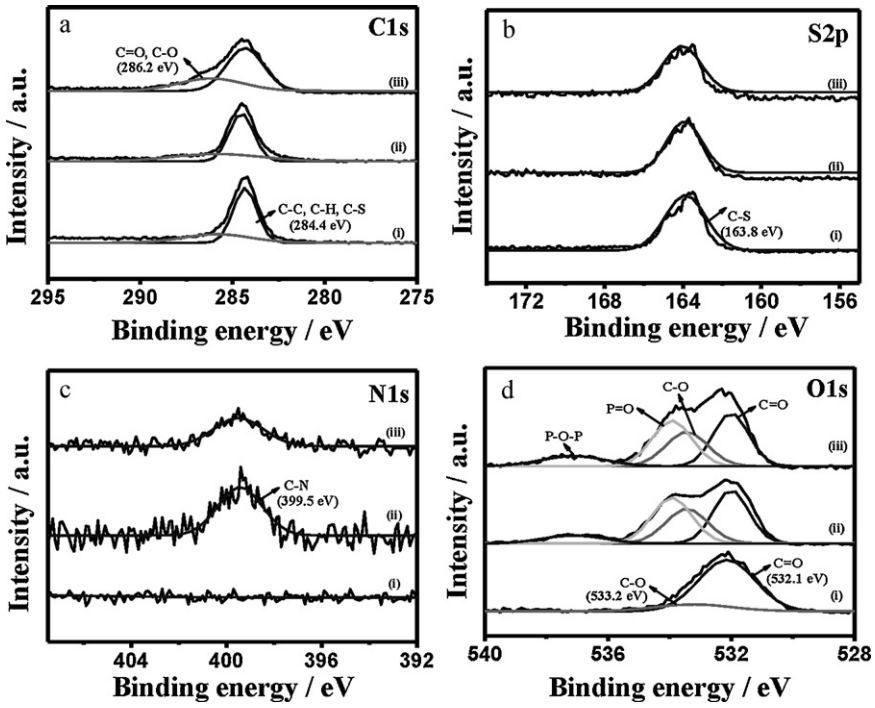

Fig. 2. XPS surveys of (a) C1s (b) S2p, (c) N1s, and (d) O1s for (i) SPE/AuNPs/pTTBA, (ii) SPE/AuNPs/pTTBA/dsDNA/CL, and (iii) SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs modified electrode.

was filled with the buffer solution, and 100 μL of the analytes was injected by applying 300 V/cm to R1 for 60 s. Subsequently, a potential of 250 V/cm was applied to R2 for 240 s to start the sample stacking. After that, 20 mM PBS was injected for 90 s, and a potential of −100 V/cm was applied to R3 for 60 s, in order to move the preconcentrated analytes to the FASI channel. After the FASI step, the preconcentrated analytes zone reached R4, and then EK separation was initiated for the detection of these analytes. In this step, 20 mM PBS was filled at R6 and R7, and the detection buffer consisting of 0.1 M PBS buffer (pH 7.4) was filled at R8. A voltage of −250 V/cm was applied to R6, with R8 being grounded during the separation.

3. Results and discussion

3.1. Characterization of biosensor probe

The biosensor probe was fabricated as presented in Fig. 1(B). During the electrodeposition of AuNPs onto SPE, the peak currents increased (a → c) as the number of sweep increased indicating the deposition of AuNPs and increase in the conductivity of SPE/AuNPs surface (Fig. SI(A(a))). Scanning electron microscope image shows the presence of AuNPs which confirm the successful electrodeposition ((Fig. SI(A)(a))inset) (Chandra et al., 2011). After AuNPs deposition the pTTBA layer was formed. The obtained cyclic voltammogram is shown in Fig. SI(A(b)). During cathodic scan a peak at around 470 mV appeared which is due to the electrodeposited AuNPs (Lee et al., 2009). It is interesting to note that during second scan the AuNPs peak is reduced which shows that the pTTBA layer is formed on to the SPE/AuNPs surface. The fabricated biosensor was further characterized using XPS. All XPS spectra were taken after 50 s of Ar ion gas etching and calibrated with a C1s peak at 284.6 eV as an internal standard. Fig. 2 exhibits the XPS spectra of the (a) C1s (b) S2p, (c) N1s, and (d) O1s peaks for the surfaces of (i) SPE/AuNPs/pTTBA, (ii) SPE/AuNPs/pTTBA/dsDNA/CL, and (iii) SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs. After electrodeposition of AuNPs on SPE two sharp peaks at 86.5 eV and 84.6 eV was observed (Lee et al., 2010) (data not shown). This proves the successful deposition of AuNPs onto the SPE surface. The C1s spectrum for pTTBA exhibited two peaks at 284.4 and 286.2 eV that correspond to the C–C, C–H, or C–S bonds, and a C=O, C–O bonds, respectively (Fig. 2(a(i))) (Chandra et al., 2011). The S2p spectrum exhibited a peak at 163.8 eV corresponding to the C–S bond (Fig. 2(b(i))), which corresponds to the sulfur component of pTTBA. This proves the successful coating of pTTBA on SPE/AuNPs. Fig. 2(c (ii)) shows the N1s peak for pDNA immobilized surface at 399.5 eV corresponding to C–N bond formation between –COOH group of pTTBA and $–NH_2$ of pDNA. The spectral deconvolution of O1s of the DNA immobilized electrode yielded peaks at 531.3 and 534.8 eV that corresponds to P=O and P–O–P bonding, respectively (Fig. 2(d(ii))). These peaks represent the phosphorus atom present in the DNA molecule which further demonstrates the successful immobilization of DNA. The P=O peaks were observed after adsorption of CL (Fig. 2(d(iii))) corresponding to phosphate in the CL molecule. The P=O and P–O–P peaks, however, have not been observed in the spectrum for the SPE/AuNPs/pTTBA electrode (Fig. 2(d(i))).

3.2. Voltammetric characterization of anticancer drugs

The LSVs were recorded for the test solution containing 10 μM MIT, IDA, DOX, and DAN with SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs as shown in Fig. 3. The LSVs recorded only for SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs without drugs showed no reduction peak (Fig. 3(a)) in 0.1 M PBS of pH 7.4. However, the LSVs in Fig. 3(b–e) show peaks for the SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs in the buffer solution containing 10 μM MIT, IDA, DOX, and DAN respectively. The reduction peaks of MIT, IDA, DOX, and DAN appeared at −620, −600, −615, and −650 mV versus the Ag/AgCl electrode. In order to completely reduce the test compounds we selected −50 mV more negative potential. Thus, an applied potential of −700 mV was used to obtain the chronoamperometric response.

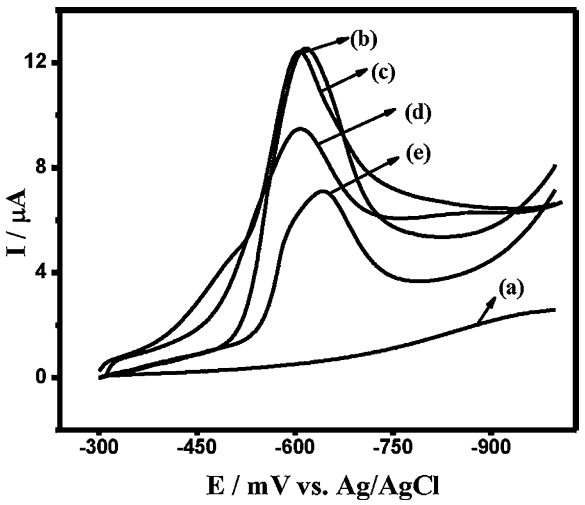

Fig. 3. Linear sweep voltammograms for the SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs in (a) 0.1 M PBS (pH 7.4) containing 10 μM each of (b) MIT, (c) IDA, (d) DOX, and (e) DAN, respectively. Scan rate: 50 mV/s.

3.3. Optimization of electrochemical detection

To optimize the experimental parameters for the electrochemical detection of test compounds, the response currents were monitored as a function of pH of the medium and temperature. The optimization plots are given in Supplementary information (SI). The current increased as the pH increased from 5.4 to 7.4 and decreased when the pH s further increased from 7.4 to 9.4 (Fig. SI(B(a))). Thus, 7.4 was selected as the optimum pH. The temperature dependency of the current response was investigated in the range from 10 to 40 °C (Fig. SI(B(b)). The current response increased with increasing temperature up to 30 °C and further did not increase significantly when the temperature increased beyond 30 °C. Thus, the subsequent experiments were carried out at 30 °C.

3.4. Separation efficiency of the microchannel

The behavior of the developed biosensor (SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs) to the test solution containing 10 nM of MIT, IDA, DOX, and DAN was examined in the separation channel using a 20 mM borate buffer (pH 9) as the separation buffer. The response current of signals for monitoring the separated drugs increased more than 10 times using the SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs probe compared with that of the bare SPE. The separation is finished within 100.0 s for all drugs. The migration times of MIT, IDA, DOX, and DAN are 50.3 (±1.8), 66.5 (±2.2), 88.0 (±2.5), and 98.3 (± .8) s, respectively (Fig. 4(A)). The half-peak widths ($W_{1/2}$, the peak width at maximum half point), resolution ($R_S = 2tR/(W_1 + W_2)$), and corresponding separation efficiency (expressed as the number of the theoretical plates, $N = 5.54\ (tR/W_{1/2})^2$) of MIT, IDA, DOX, and DAN are ~4350, ~18,200, ~29,800, and ~109,300, respectively.

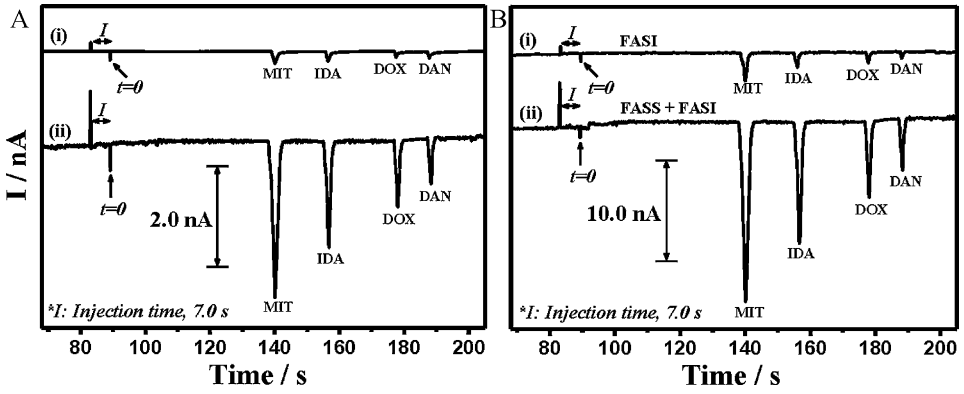

Fig. 4. (A) Electropherograms of 10 μM MIT, IDA, DOX, and DAN with (i) bare and (ii) SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs sensor probe. Detection potential, −0.7 V. Separation field strength, −2 kV; injection time, 7.0 s at −2 kV; running buffer, borate buffer +10% acetonitrile, and detection buffer 100 mM PBS (pH 7.4). (B) Electropherograms of MIT, IDA, DOX, and DAN obtained in (i) FASI step and (ii) FASS and FASI together.

Table 1
Analytical performance of microfluidic device.

Analytes	C[a]	Response precision (%RSD, $n = 5$)	Dynamic range (pM)	Detection limit[b] (fM)
(A) Analytical performance of the method				
MIT	17,050	2.0	2.0–60.0	1.2 ± 0.05
IDA	15,520	1.2	5.0–55.0	2.2 ± 0.1
DOX	13,116	3.6	7.5–50.0	3.6 ± 0.2
DAN	10,121	1.2	8.0–50.0	5.5 ± 0.3

Analytes	Level added (pM)	Level found ± S.D (pM)	R.S.D. (%)	Recovery (%)
(B) Recoveries of anticancer drugs from spiked real urine samples ($n = 3$)				
MIT	25.0	24.6 ± 0.9	3.6	98.4
IDA	25.0	23.9 ± 1.0	4.1	95.6
DOX	25.0	24.3 ± 0.9	3.7	97.2
DAN	25.0	24.5 ± 1.1	4.4	98.0

[a] C, concentration enhancement factor.
[b] Estimated detection limits, based on S/N = 3.

3.5. Preconcentration of anthracyclines

To concentrate the trace anticancer drugs in the microchannel, the sample stacking method was used as reported earlier (Shiddiky et al., 2006). The conventional electrokinetic chromatography with electrochemical detection (EKC–EC) was carried out as earlier (Shiddiky et al., 2006) without performing the preconcentration steps using the SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs sensor probe. The concentration experiment was carried out using two more channels that included field amplified sample stacking (FASS) and field amplified sample injection (FASI) steps. Fig. 4(B) shows the electropherograms obtained using (i) FASI only and (ii) FASS and FASI together. The concentrations of the test compounds required to preconcentrate the samples with FASS only and with FASS and FASI together were 10 nM and 3.1 nM, respectively. The test compounds preconcentrated using only FASS and FASS and FASI together, and were detected within the same migration time. The final enhancement of the sensitivity using FASI and FASS was ~1093 times by considering the dilution ratio of the sample solution and the enhancement of the current response. In order to optimize the preconcentration conditions, we examined the current response according to the buffer concentration and water plug length, because the enhancement of the sensitivity is affected by the stacking efficiency (Es) (Shiddiky et al., 2006). Fig. SI(B(c)) shows the effect of the buffer concentration on the signal enhancement using the FASS step. As the buffer concentration increased from 5 to 20 mM, the peak height of the signal increased up to 20 mM, but decreased again at the concentration above 20 mM. This might be due to the increased joule heating resulting from the increase in the ionic strength. Fig. SI(B(d)) shows the effect of the length of the water plug on the peak height of the signal in the range of lengths of 10–60 mm. As the length of the water plug increased, the peak area increased up to a length of 40 mm and then did not increase over 40 mm. At a length of 40 mm, the best separation efficiency and S/N were observed. Thus, the subsequent experiments were carried out at the buffer concentration of 20 mM and the water plug length of 40 mm.

3.6. Calibration curve and real sample analysis

Fig. 5(A) shows the electropherogram recorded for the SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs probe at various concentrations of anticancer drugs. The calibration curve was obtained by plotting the peak area according to the sample concentration, as shown in Fig. 5(B). Under the optimized conditions, concentration enhancement factor, response precision, dynamic range, and detection limit were calculated and are summarized in Table 1(A). Dynamic ranges of anticancer drugs are determined to be from 2 to 60 pM with the detection limits between 1.2 (±0.05) and 5.5 (±0.3) fM.

The practical applicability of the developed method was investigated by detecting drugs in a human urine sample. Midstream urine from a healthy individual was collected and filtered through a membrane filter. Drugs-spiked urine solutions were prepared by adding drugs to the final concentration of 25 pM in five times diluted urine samples. Fig. 5(C) shows the electropherogram of drugs when prepared in urine sample. Interestingly in the urine sample the migration time of drugs was similar as found in the buffer solution. This shows that the urine salts does not have any impact on the conductivity. The recoveries of drugs from the urine sample was calculated based on the calibration curve and are presented in Table 1(B). The concentration recovery for MIT, IDA, DOX, and DAN was 98.4, 95.6, 97.2, and 98% respectively with relative standard deviations <5%. These results clearly indicate the applicability of developed device for simultaneous anticancer drugs detection in urine sample. We examined the interference of

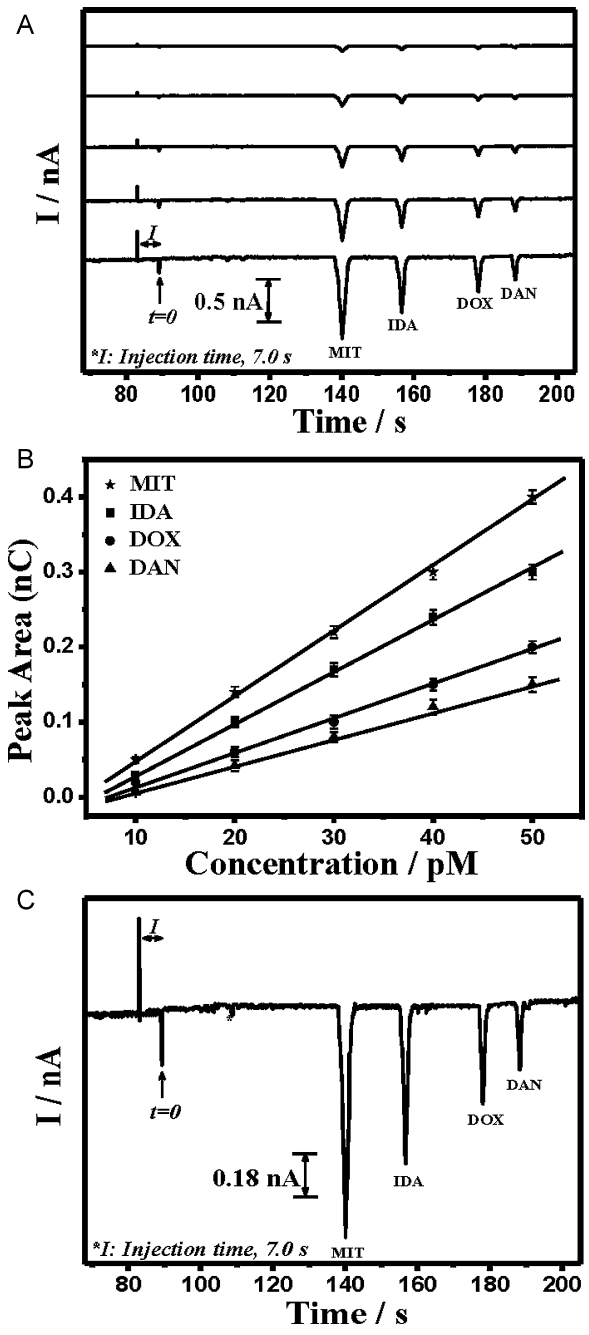

Fig. 5. (A) Electropherograms obtained for varying concentrations anticancer drugs, (B) calibration curves obtained by plotting the signals obtained in (A) and (C) electropherogram obtained for drugs in urine sample. Experimental conditions same as in Fig. 4(A).

the electroactive compounds commonly present in the real samples. For this purpose, ascorbic acid, uric acid, acetaminophen, and dopamine were examined under the optimized experimental conditions. No foreign peak in the electropherograms was observed which shows that the device has no interference with other chemicals. The absence of the additional peaks, however, suggested that unknown samples were not preconcentrated with the drugs as reported earlier (Lee et al., 2008; Shiddiky et al., 2009). Another explanation could be possibly due to the fixed applied potential at sensor probe. Furthermore, in case of urine sample examination, no foreign peak was observed, which also shows that urine components even donot interfere in the detection of these drugs.

4. Conclusion

The anticancer drugs, MIT, IDA, DOX, and DAN in trace quantities are preconcentrated, separated, and detected by employing electrokinetic separation and electrochemical detection method using an SPE/AuNPs/pTTBA/dsDNA/CL/AuNPs amperometric biosensor. The separation is performed within 100 s: the migration times of MIT, IDA, DOX, and DAN are 50.3 (±1.8), 66.5 (±2.2), 88.0 (±2.5), and 98.3 (±3.8) s, respectively. The detection limits of the analytes are between 1.2 (±0.05) and 5.5 (±0.3) fM and the proposed method is successfully performed with real human urine sample. The strategy described here has many attractive features: simplicity, rapidity, no requirement for specific labels, and inexpensive. In future, this method can be used for simultaneous detection of anticancer drugs in hospital wastes and body fluids of cancer patients undergoing chemotherapy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.07.038.

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