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An Electrochemical Approach to Simultaneous Determination of Acetaminophen and Ofloxacin

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Abstract This study presents a simple electrochemical approach for preparing a poly(L-serine) film-modified glassy carbon electrode, which responds quickly and sensitively during the simultaneous determination of acetaminophen and ofloxacin in prepared, environmental, and pharmaceutical samples. The prepared electrode exhibited catalytic activities and promoted the oxidation of acetaminophen and ofloxacin. Acetaminophen and ofloxacin showed linear responses between 1.0×10^{-5} and 1.0×10^{-4} mol/L and their limits of detection were 1.2×10^{-7} and 1.6×10^{-7} mol/L, respectively. The average recoveries (±relative standard deviations) of acetaminophen and ofloxacin were 96.8 ± 3.5 % and 97.6 ± 3.2 %, respectively, indicating that the prepared electrode and detection method are very accurate and reproducible for the simultaneous determination of acetaminophen and ofloxacin.

Keywords Acetaminophen · Ofloxacin · Poly(L-serine) film-modified electrode · Voltammetry

Over the past few years, pharmaceuticals and personal care products have been of great concern, and many of them are considered to be emerging (environmental) contaminants. Among these contaminants, acetaminophen and ofloxacin have received much attention because they are the two most-used medicines in many countries (Lin et al. 2008; Choi et al. 2008) and are two of the most frequently detected compounds in sewage (Nakata et al. 2005; Brown et al. 2006; Lin et al. 2008). Acetaminophen is one of the most common medications in households and is the most consumed drug in Taiwan (Lin et al. 2008). Acetaminophen is frequently used for the relief of pain and fever; in addition, 5 % of therapeutic acetaminophen doses are excreted unchanged in urine (Dargan and Jones 2002). Ofloxacin, one of the most commonly used second-generation topical fluoroquinolones, has been used for years in human and veterinary medicine to prevent or treat bacterial infections throughout the world (Andreu et al. 2007). It is estimated that in 2002 the ofloxacin output in China was about 1,200 tons (Chan et al. 2006) and more than 70 % was excreted in the original form after dosing (Lode et al. 1987). Fluoroquinolones are the most frequently detected antibiotics above analytical quantitation limits in wastewater treatment plant (WWTP) effluents in Sweden (Lindberg et al. 2006), and has been detected in several other countries such as Australia, Canada, China, Taiwan, and USA (Miao et al. 2004; Costanzo et al. 2005; Karthikeyan and Meyer 2006; Lin et al. 2008; Zorita et al. 2009). In Taiwan, acetaminophen and ofloxacin detection frequencies in each potential pharmaceutical contamination source were over 90 % (Lin et al. 2008). The frequent presence of acetaminophen and ofloxacin in WWTP effluents poses a potential risk to aquatic and terrestrial organisms.

Therefore, efforts to develop new detection and degradation methods for acetaminophen and ofloxacin are very important. So far, different methods have been reported for the determination of acetaminophen and ofloxacin, such as high-performance liquid chromatography (Lee et al. 2007; Shao et al. 2008), and liquid chromatography–mass spectrometry (Lee et al. 2007; Lin et al. 2008). Although these methods are accurate, they rely on multi-step sample clean-up procedures and are therefore relatively expensive and

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time-consuming. Little attention has been paid to the simultaneous determination of acetaminophen and ofloxacin using electrochemical techniques that may be simple, rapid, and less expensive. It was reported that poly(L-serine) (PLS) film-modified electrodes may be used to decrease over potential, improve mass transfer, and enrich active substance during electrochemical reactions of interest (Song et al. 2008; Chitravathi et al. 2011). To the best of our knowledge, simultaneous electrochemical determination of acetaminophen and ofloxacin using PLS film-modified electrodes has not yet been addressed, although such techniques are important for the sensitive and rapid analysis of acetaminophen and ofloxacin. In this study, a cyclic voltammetric (CV) approach was used to achieve the electropolymerization of L-serine on a GCE surface and to prepare PLS film-modified GCE. CV and linear sweep voltammetry (LSV) analyses were performed to examine the electrode's characteristic and response. Differential pulse voltammetry (DPV) was used to obtain the linear concentration ranges and detection limits of acetaminophen and ofloxacin. The method developed in this study has great potential for practical use in the simultaneous determination of acetaminophen and ofloxacin residues in real environmental media and pharmaceutical samples.

Materials and Methods

Ofloxacin and acetaminophen chemicals were purchased from Sigma (USA) while L-serine was obtained from Alfa Aesar (UK). Sodium phosphate dibasic dehydrate ($Na_2HPO_4{\cdot}2H_2O$) and phosphoric acid (H_3PO_4) used for the preparation of phosphate buffer solutions were supplied by Merck. All the reagents (analytical grade) were used as received without further purification. The stock solutions (1.0×10^{-3} mol/L) of acetaminophen and ofloxacin were prepared by dissolving the two chemicals into 0.1 M phosphate buffer solution (pH 4.0), and then stored at 4°C. Double distilled water was used throughout the experimental work.

The determination of the acetaminophen and ofloxacin and their electrochemical behaviors in prepared solutions was performed in a conventional three-electrode system based on CV, LSV, and DPV analyses. A PLS film-modified GCE and a platinum wire were used as the working and counter electrodes, respectively. The reference electrode was an Ag/AgCl electrode. A 660B electrochemical workstation (CH Instruments Inc., USA) was used to record experimental data.

The PLS film-modified GCE was prepared as follows. A GCE was polished with 0.05 μm alumina slurry, then rinsed with redistilled water, and finally sonicated in redistilled water to give a clean and mirror surface. The electropolymerization of L-serine on GCE surface was carried out using cyclic sweeps between −0.6 and 2.0 V versus Ag/AgCl in a 0.1 mol/L phosphate buffer (pH 5.0) containing 1.0×10^{-2} mol/L L-serine. During the electrochemical polymerization process, an oxidation peak was observed at 1.41 V versus Ag/AgCl in the anodic scan and a reduction peak appeared at −0.30 V versus Ag/AgCl in the reverse (cathodic) scan due to the formation of PLS (Fig. 1).

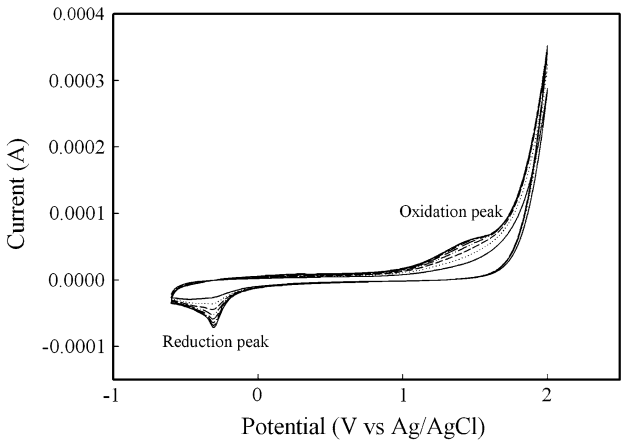

Fig. 1 CVs for the electropolymerization of L-serine on the tested glassy carbon electrode (GCE). Supporting electrolyte: 0.1 M phosphate buffer (pH 5.0); scan rate: 100 mV/s. L-Serine concentration: 1.0×10^{-2} mol/L

A phosphate buffer solution (pH = 4.0, 0.1 mol/L) was used as the supporting electrolyte for electrochemical measurements of acetaminophen and ofloxacin. The potential scan ranges were $0 \leftrightarrow 1.2$ V (starting/ending at 0 V, scan rate 100 mV/s), 0–1.2 V (anodic, scan rate 10 mV/s), and 0–1.2 V (anodic, scan rate 5 mV/s, pulse amplitude of 50 mV and a pulse width of 20 ms) versus Ag/AgCl for the CV, LSV, and DPV measurements, respectively. All electrochemical experiments were performed at room temperature.

Results and Discussion

The electrochemical behaviors of acetaminophen and ofloxacin on the PLS film-modified GCE and bare GCE were investigated using CV. Figure 2 shows the cyclic voltammograms of 1.0×10^{-4} mol/L acetaminophen and ofloxacin in 0.1 mol/L phosphate buffer (pH = 4.0). On the bare GCE, the oxidation of acetaminophen and ofloxacin peaked at 0.654 and 1.076 V versus Ag/AgCl, respectively. When using the PLS film-modified GCE, the remarkable increase in peak current and negative shift of peak potential for acetaminophen/ofloxacin oxidation indicate that the electrocatalytic activity for acetaminophen/ofloxacin oxidation was much better on the modified GCE than on the bare GCE. The peak currents of acetaminophen and ofloxacin oxidation on the PLS film-

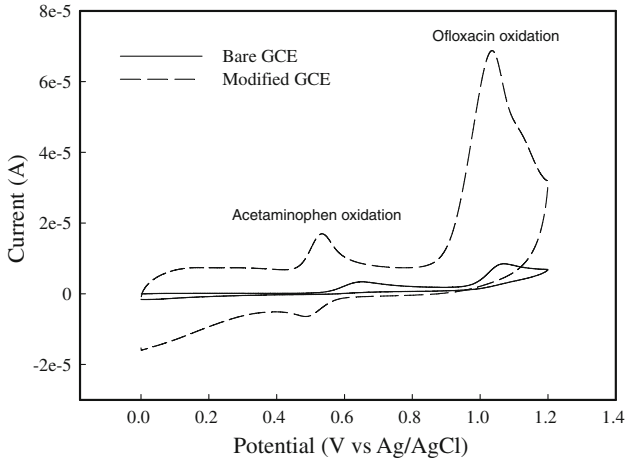

Fig. 2 CVs (scan rate = 100 mV/s) of 1.0×10^{-4} mol/L AP and OFL mixture in 0.1 M phosphate buffer (pH 4.0) on the PLS film-modified and bare GCEs

modified GCE were much higher than that of the bare GCE, and the oxidation peak potentials also negatively shifted by 132 and 67 mV, respectively. This phenomenon is similar to that observed by Song et al. (2008) for electrochemical determination of estradiol using a PLS film-modified GCE. The reasons for promoting the oxidation of acetaminophen and ofloxacin mixture in PLS film-modified GCE can be explained as follows. The ring-nitrogen atoms and carbonyl groups of ofloxacin and the carbonyl and hydroxyl groups of acetaminophen formed hydrogen bonds with the hydroxyl and amino groups of PLS units. Such bonding increased the adsorption capacities of acetaminophen/ofloxacin on the surface of PLS film-modified GCE and thus effectively improved their detection sensitivity.

Electrochemical determination of acetaminophen and ofloxacin may be affected by the pH of the electrolyte solution. The electrochemical oxidation of acetaminophen and ofloxacin in 0.1 mol/L phosphate buffer with different pH values was studied using LSV. Figure 3a shows the oxidation peak currents of 1.0×10^{-5} mol/L acetaminophen and ofloxacin as a function of pH. When pH gradually increased from 4.0 to 8.0, the oxidation peak currents of acetaminophen at the PLS film-coated GCE remained similar while the oxidation peak current of ofloxacin gradually decreased. Thus, the pH 4.0 phosphate buffer was used as a supporting electrolyte suitable for the simultaneous electrochemical determination of acetaminophen and ofloxacin. The linear shift in oxidation peak potential (E_{pa}) towards negative potential with an increase in pH indicated that protons are directly involved in the oxidation of acetaminophen and ofloxacin. Moreover, such potential shifts were used to obtain the equations E_{pa} (V) = –0.057 pH + 0.755 ($r^2 = 0.998$) and E_{pa} (V) = −0.052 pH + 1.159 ($r^2 = 0.990$) for acetaminophen and ofloxacin, respectively (Fig. 3b). The slopes of 0.057 and 0.052 V/pH in these two equations are in agreement with the theoretical slope (2.303 mRT/2F) of 0.059 V/pH, so 2 protons (m) were involved in both reactions (equal to the corresponding numbers of electron-transfer). Nematollahi et al. (2009) also found $m = 2$ for the initial oxidation of acetaminophen on a bare GCE in acidic solutions.

The calibration curve was obtained in pH 4.0 phosphate buffer using DPV (Fig. 4a). The oxidation peak current of acetaminophen or ofloxacin was proportional to its concentration over the range from 1.0×10^{-5} to 1.0×10^{-4} mol/L (correlation coefficient $R > 0.996$) (Fig. 4b). At the signal to noise (S/N) ratio of 3, the determined detection limits (DLs) of acetaminophen and ofloxacin were 1.2×10^{-7} and 1.6×10^{-7} mol/L, respectively. The DL value of acetaminophen (1.2×10^{-7} mol/L) was higher than that on a graphite oxide modified GCE (4×10^{-8} mol/L) (Song et al. 2011), but lower than those on a multiwall carbon nanotube modified GCE (6.0×10^{-7} mol/L) (Alothman et al. 2010) and a Nafion/TiO_2-graphene/GCE (2.1×10^{-7} mol/L) (Fan et al. 2011). The DL of ofloxacin (1.6×10^{-7} mol/L) in this study was lower than that (3×10^{-7} mol/L) reported by Rizk et al. (1998) using a dropping mercury electrode. The accuracy and reproducibility of acetaminophen and ofloxacin detection were evaluated by measuring a 3.0×10^{-5} mol/L acetaminophen and ofloxacin mixture standard solution 5 times. The average recovery and relative standard deviation (RSD) were 97.6 and 3.2 %, respectively, for acetaminophen, and 96.8 and 3.5 %, respectively, for ofloxacin, indicating excellent accuracy and reproducibility for acetaminophen and ofloxacin detection.

Commercial pharmaceutical formulations of acetaminophen and ofloxacin and hospital wastewater (the hospital located in southern Taiwan) were analyzed to evaluate the use of PLS film-modified GCE for practical purposes. Panadol (Cold and Flu, Day and Night) (GSK, Taiwan) and Tarivid (or Sinflo F.C. Tab.) (ofloxacin 200 mg) (Taiwan Biotech. Co., LTD.) tablets were adopted for the tests. These tablets were accurately weighed, then transferred to a 200 mL flask, and finally dissolved in 200 mL 0.1 M phosphate buffer (pH = 4.0). The prepared solution and hospital wastewater were examined using the PLS film modified GCE through DPV. The determined amounts of acetaminophen and ofloxacin of Panadol and ofloxacin tablets were 493.2 and 194.8 mg/tablet, respectively (n = 3), similar to their corresponding label claims (Table 1). The determined average concentration of acetaminophen in hospital wastewater samples was 1.8×10^{-7} mol/L (27.2 μg/L), while the ofloxacin was not detected (ND) (below method detection limit). The data based on the wastewater samples of a southern Taiwan hospital in this study are lower than those (acetaminophen

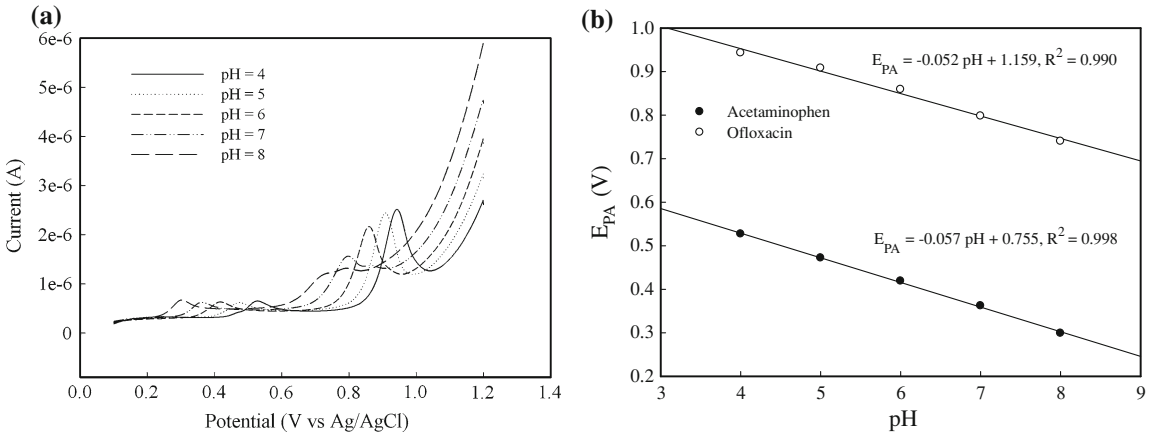

Fig. 3 **a** LSVs (scan rate = 10 mV/s) of 1.0×10^{-5} mol/L AP and OFL mixture solutions with different pH values. **b** Variation of anodic peak potential versus pH

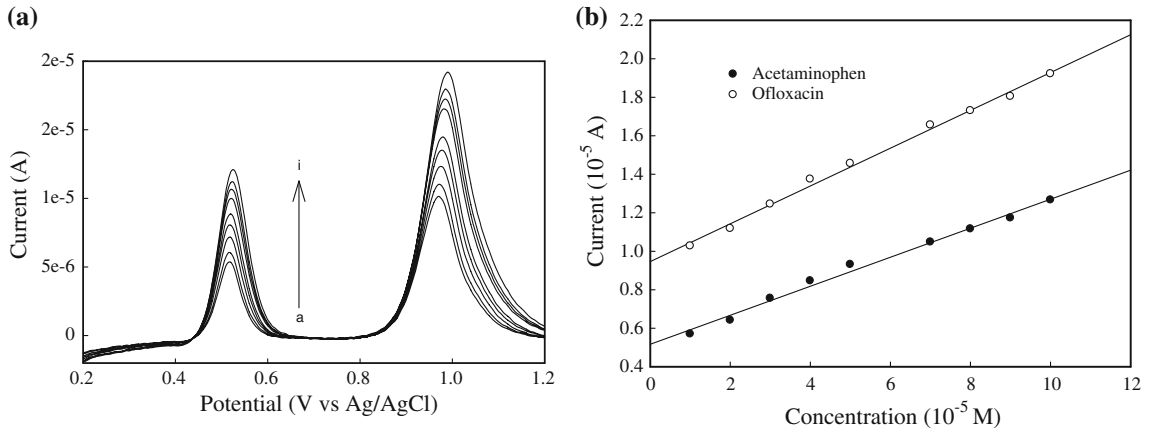

Fig. 4 **a** DPVs of AP and OFL mixtures with different concentrations (in 0.1 M phosphate buffer (pH 4.0)) on the PLS film-modified GCE. Scan rate, 5 mV/s; pulse amplitude, 50 mV; pulse width, 20 ms. AP and OFL concentrations: (a) 1×10^{-5}, (b) 2×10^{-5}, (c) 3×10^{-5}, (d) 4×10^{-5}, (e) 5×10^{-5}, (f) 7×10^{-5}, (g) 8×10^{-5}, (h) 9×10^{-5}, (i) 10×10^{-5} mol/L. **b** Variation in peak current versus concentration

Table 1 Determination of acetaminophen (AP) and ofloxacin (OFL) in real environmental samples and commercial pharmaceutical tablets (n = 3)

Sample	Concentration (μM)		Spiked		Determined (μM)		Recovery		RSD	
	Mean ± SD		(μM)		Mean ± SD		Mean (%)		(%)	
	AP	OFL	AP	OFL	AP	OFL	AP	OFL	AP	OFL
Hospital wastewater	0.18 ± 0.01	ND	50	50	50.9 ± 1.47	48.6 ± 1.65	101.4	97.2	2.9	3.4

Tablet	Labeled (mg)		Spiked (μM)		Determined (mg) Mean ± SD		Recovery Mean (%)		RSD (%)	
	AP	OFL	AP	OFL	AP	OFL	AP	OFL	AP	OFL
Panadol	500		–	–	493.2 ± 10.3		98.6		2.1	
Tarivid		200	–	–		194.8 ± 5.4		97.4		2.8

ND not detected (below method detection limit), *SD* standard deviation, and *RSD* relative standard deviation

and ofloxacin = 36.95 and 1.09 μg/L, respectively) reported by Lin et al. (2008) who used HPLC–MS/MS to analyze wastewater samples from six hospitals (mostly in northern Taiwan). To further validate the PLS film-modified GCE electrode for the determination of acetaminophen and ofloxacin in real environmental (hospital) samples, we spiked 5×10^{-5} mol/L acetaminophen and ofloxacin into the hospital wastewater samples. The recoveries of spiked wastewater samples for acetaminophen and ofloxacin were 97.2 and 101.4 %, respectively, with RSDs both smaller than 3.5 %, revealing that the PLS film modified GCE prepared in this study is accurate enough for practical application.

The PLS film-modified GCE exhibited excellent performance for the determination of acetaminophen and ofloxacin in real environmental samples (hospital wastewater) and commercial pharmaceutical (Panadol and Tarivid) tablets. The developed method has great potential for use in the simultaneous determination of acetaminophen and ofloxacin in practical (e.g., environmental, pharmaceutical, and medical) samples.

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