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Disposable in-field electrochemical potable sensor system for free available chlorine (FAC) detection

Seung-Cheol Chang and Deog-Su Park†

Abstract

The work described in this study concerns the development of a disposable amperometric sensor for the electrochemical detection of a well-known aqueous pollutant, free available chlorine (FAC). The FAC sensor developed used screen printed carbon electrodes (SPCEs) coupled with immobilised syringaldazine, commonly used as an indicator in photometric FAC detection, which was directly immobilised on the surface of SPCEs using a photopolymer PVA-SbQ. To enable in-field analysis of FAC, a prototype hand-held electrochemical analyzer has been developed to withstand the environment with its rugged design and environmentally sealed connections; it operates from two PP3 (9 volt) batteries and is comparable in accuracy and sensitivity to commercial bench top systems. The sensitivity of the FAC sensor developed was 3.5 $nA\mu M^{-1}cm^{-2}$ and the detection limit for FAC was found to be 2.0 μM.

Key Words : disposable, free available chlorine, in-field, screen printing

1. Introduction

Environmental monitoring of aqueous pollutants in water resources is a fundamental part of the public health and the protection of environment and free available chlorine (FAC), an aqueous pollutant, is one of the major parameters in establishing water quality[1]. The term *chlorine in water* refers not only to the elemental chlorine species, Cl_2, but also to a variety of other species including hypochlorous acid, HOCl, hypochlorite ion, OCl^-, and several chloramine species such as monochloramine, NH_2Cl, and dichloramine, $NHCl_2$[2]. Usually, hypochlorous acid and hypochlorite ion are given the term *free available chlorine*, FAC[3]. FAC is understood to have the following hazardous adverse effects; intensification of taste and odour characteristics of phenolic and other organic compounds in the water supply, and FAC may form carcinogenic chloro-organic compounds such as chloroform[4]. In spite of the problems, FAC is widely used in industry, agriculture and water treatments as an oxidising agent or a disinfectant[5]. This is because chlorine may destroy and deactivate disease-producing microorganisms effectively and may improve overall water quality resulting from the reaction with ammonia, iron, manganese, sulphide and several organic substances[6].

Classically, numerous analytical methods have been reported for FAC detection in water such as colorimetric, titrimetric and spectrophotometric methods[3]. However, these methods may result in a considerable time lag between taking samples and receiving the results, which may present difficulties in the handling of coloured and turbid samples. In order to overcome these problems, electrochemical analysis based on more flexible methods such as amperometric or voltammetric sensor systems have been selectively used for estimating chlorine in water[7-10]. To date, despite considerable advantages, few sensor-based systems are available due to difficulties in electrode production and the expenses.

In 1971, Bauer and Rupe introduced FACTS (the Free Available Chlorine Test with Syringaldazine). Thereafter, syringaldazine (3,5-dimethoxy-4-hydroxybenzaldazine) has been used as a conventional reagent for FAC detection using spectrophotometry. In FACTS, syringaldazine is used for measuring FAC over the concentration range of 70 nM to 14.1 μM. Free available chlorine will oxidise syringaldazine on a 1:1 molar basis to produce a coloured product with an absorption max-

Center for Innovative Bio-Physio Sensor Technology, Pusan National University

†Corresponding author: dsupark@pusan.ac.kr
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imum at 530 nm, the absorbance measured from the coloured product is proportional to FAC concentration. FACTS is also unaffected by significant concentrations of monochloramine, dichloramine, nitrate, nitrite and oxidised form of manganese in water[11].

The work described in this paper aims to demonstrate that it is possible using conventional FAC detecting methods to utilise the development of a simple sensor-based system. In order to develop a new sensor system, electrochemical changes in the redox response of syringaldazine-FAC were characterised and optimised. To demonstrate in-field FAC detection, a new disposable screen printed carbon printed electrodes were prepared and modified with immobilised syringaldazine. In this system, syringaldazine was directly immobilised on the surface of SPCEs using a photo-polymer[17,18], poly (vinyl alcohol) bearing stryrylpyridinium groups (PVA-SbQ) matrix.

2. Experiment

2.1. Materials and Reagent

The basic disposable screen-printed carbon electrodes (SPCEs) were fabricated automatically using an 'in-house' procedure from carbon ink with Ag/AgCl reference and counter electrodes printed onto a 1 mm thick PVC substrate. Syringaldazine (4-hydroxy-3,5-dimethoxy benzaldehyde azine), sodium hypochlorite solution and sodium chloride (NaCl) were obtained from Aldrich. 2-Propanol, potassium chloride (KCl) and potassium phosphates (KH_2PO_4, K_2HPO_4) were obtained from Sigma. Chlorine demand free (CDF) water was prepared in the Department of Chemistry in Pusan National University. All chemicals were of analytical grade and were used as received. PVA-SbQ matrix (SPP-H-13) was from Toyo Gosei Kogyo Co. (Tokyo, Japan).

2.2. Buffers and Stock Solutions

All buffers and stock solutions were prepared using chlorine-demand-free water to remove residual chlorine demand. CDF water was prepared using deionised water to which sufficient chlorine was added to give 5.0 mgL^{-1} free chlorine (FAC) and was stored for 2 days at room temperature. To remove remaining chlorine, the above solution was placed in UV-lamp container for 6 hours. All glassware was also rinsed with this solution before use. 320 μM syringaldazine solution was prepared by dissolving 1.15 mg of 3,5-dimethoxy-4-hydroxybenzaldazine in 10 ml 2-propanol. This solution was used as an indicator in FACTS procedure. FAC solutions of concentration between 0 and 30 μM were prepared from stock sodium hypochlorite solution by dilution with CDF water. The FAC concentration in chlorine stock solutions was established by the thiosulfate method[3].

2.3. Apparatus

Cyclic voltammetry (CV) was performed using Kosentech KST-P1 (Korea) and BAS-50 (Bioanalytical Systems, West Lafayette, IN, USA) electrochemical analyzers equipped with a Perspex 10-well template for accommodation of the screen-printed electrodes was built by the Workshop of the Chemistry Department. All real-time amperometric responses were monitored using a low-noise multi-channel potentiostat interfaced to a PC coupled with data acquisition software from Kosentech Ltd. (Pusan, Korea) and in-field analysis were carried out using the potable electrochemical analyser developed. A Spectroline® UV lamp was obtained from Spectronics Co. (New York, USA) and was used for PVA-SbQ photo-polymerisation.

2.4. Assessment of Syringaldazine Modified SPCEs

640 μM syringaldazine solution was prepared by dissolving 2.3 mg of syringaldazine in 10 ml of 2-propanol. This solution was diluted to 20 ml with buffer. A 200 μl aliquot of the solution was thoroughly mixed with 300 μl of PVA-SbQ prepolymer for 5 minutes. A 5 μl aliquot of the mixture was placed on the surface of the working electrode of the SPCEs. The electrodes were exposed to long wave UV light (365 nm, at 10 cm) for 2 h to allow polymerisation, and dried in a dark place for 12 h at room temperature. Some 0.23 μg of syringaldazine was modified on each SPCE.

2.5. Electrochemical Cell Set-Up

An electrochemical cell was set up using a Perspex 10-well template with SPCEs. Each electrode was inserted into a slot cut in the Perspex template such that the working area was directly located under the 7 mm diameter aperture at the bottom of the well. An O-ring gasket located in a recess and placed between the electrode and the Perspex well formed a tight seal when the

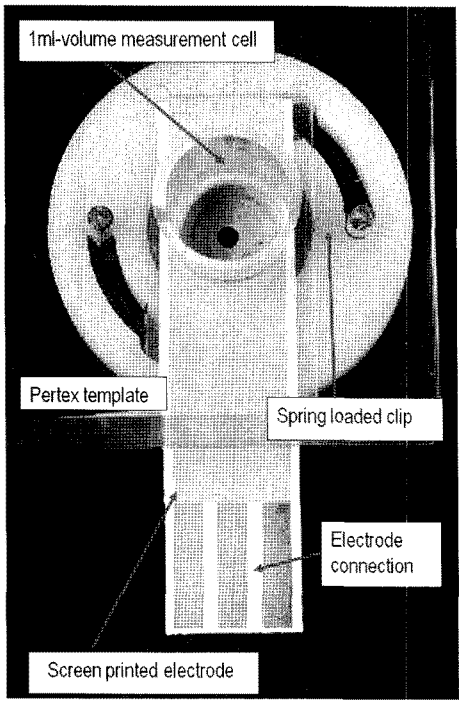

Fig. 1. Electrochemical cell set up incorporating Perspex template and SPCEs.

electrode was held in place using a spring-loaded clip. Once in place the electrode comprised the bottom of a 1 ml-volume electrochemical measurement cell. The set up is shown in Fig. 1.

2.6. Measurement Procedure

Syringaldazine (FACT) method was carried out using a standard method[3]. CV of FAC-Syringaldazine reaction using SPCEs was performed as flowing protocol: In each measurement, a 1.6 ml aliquot of a FAC sample was placed in a 5 ml test tube to which 0.4 ml of 0.32 mM syringaldazine was added. The test-tube was inverted 5 times. After inversion, 1.0 ml of the mixed solution was placed in a well of the template and electrochemical scanning was initiated after 2 minutes. Amperometric FAC Detection using syringaldazine modified SPCEs was performed as follow: A syringaldazine modified SPCE (PVA-SbQ(Syring.)-SPCE) was inserted into an electrochemical cell containing 450 μl of phosphate buffer containing 20 % ($^{v}/_{v}$) 2-propanol and connected to a potentiostat as described, then the electrode was polarised at a potential of +50 mV vs. Ag/AgCl. This was repeated to construct FAC calibration curves for a range of FAC concentrations from 0 to 128 μM using an unused modified electrode for each measurement. Intra-electrode reproducibility and storage stability were also investigated.

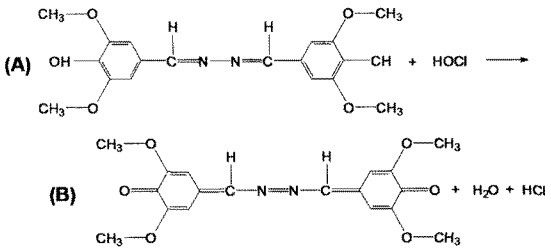

Fig. 2. Schematic diagram of syringaldazine-FAC reaction. The azine-form-syringaldazine (A) is oxidised by FAC, and changes to azo-form-syringaldazine (B) on a 1 : 1 molar basis at pH 6.5.

3. Results and Discussion

3.1. Characterisation and Optimisation

The schematic diagram of the syringaldazine-FAC reaction was presented in Fig. 2. The azine-form-syringaldazine (A in Fig. 2) is oxidised by FAC, and changes to azo-form-syringaldazine (B in Fig. 2) on a 1 : 1 molar basis. The coloured product (azo-form) produced is slightly soluble in water. Therefore, at FAC concentrations higher than 1 mgL^{-1}, the final reaction mixture must contain 2-propanol to prevent product precipitation and colour fading[11]. The optimum colour and minimum fading were obtained in a solution containing a minimum amount of 20 % ($^{v}/_{v}$) 2-propanol. As reported previously, the FACTS method is sensitively affected by pH. Therefore, the pH had to be fixed at 6.5 during the procedure. These experimental conditions were also demonstrated in previous studies[12,13].

3.2. Cyclic Voltammetry of Azine-Form Syringaldazine

Cyclic voltammograms (CVgrams) with SPCEs were obtained in buffer and in 64 μM syringaldazine solution. Potential sweeping was initiated in an anodic direction from −150 mV in all experiments. No specific oxidation and reduction current peaks were observed in the absence of syringaldazine over the total sweep range from −150 mV to +650 mV. By contrast, clear current peaks were observed in the presence of 64 μM syringaldazine; 3.8 μA for the anodic sweep at +290 mV

and 4.2 μA for the cathodic sweep at +234 mV.

However, the CVgrams of azine-form of syringaldazine were sensitively dependent on the change of pH. The peak potentials, E_{pa} and E_{pc}, of azine forms shifted as a function of pH and plots of this shift yield a straight line. The slope for the line was calculated to be $\Delta E_p / \Delta pH = -0.06$ V/pH. It is indicated that the entire reaction mechanism involves H^+, and also means that the electrochemical reaction mechanism of azine-form syringaldazine involves a chemical reaction step.

3.3. Cyclic Voltammetry of Azine/Azo-Form Syringaldazine

A series of repeatable CV was performed in 64 μM syringaldazine solution and also in the same solution containing 32 μM FAC. The same CV was repeated in plain buffer without syringaldazine to investigate the background response. CVgrams without syringaldazine in buffer and in 32 μM FAC, a small difference observed which was probably due to differences in intra-electrode reproducibility. The difference was not significant. After mixing a FAC sample and syringaldazine as described, a dense purple colour was developed which lasted for about 5 minutes during the potential sweeping. In the presence of FAC, the change in CVgrams of the azine-form is showed in Fig. 3. As can be seen, E_{pa} of azine-form was shifted from +290 mV to +400 mV and the magnitude of i_{pa} is decreased to one third of its initial level. However, the cathodic peak (i_{pc}, E_{pc}) of the azine-form almost disappeared and another redox couple appeared in the potential range from 0 to 200 mV. The cathodic peak current was 3.2 μA for cathodic sweep at +62 mV and the anodic peak current was 1.3 μA for anodic sweep at +138 mV.

From the results obtained, it was suggested that the new redox peak couple arisen from the azo-form of syringaldazine that was oxidised from the azine-form by FAC. This might be due to a limited scan range. In order to overcome this problem, the experiments were repeated in FAC over the concentration range of 0-128 μM with a wider range of potential sweeping, from −1.2 to +1.0 V. This new set of experiments was also repeated in FAC samples without addition of syringaldazine. A set of represented cyclic voltammograms in

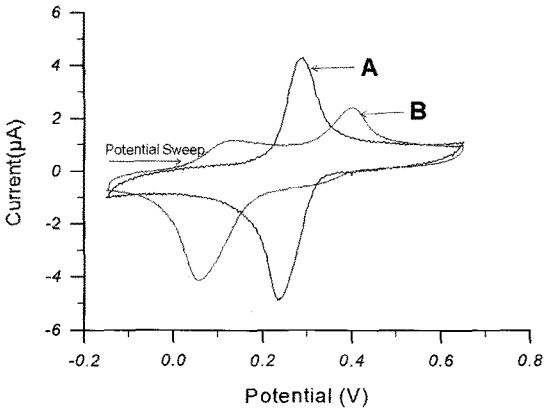

Fig. 3. Cyclic voltammograms for SPCE in 64 μM syringaldazine in 100 mM phosphate buffer (pH 6.5, 100 mM KCl, Curve A) and in 32 μM FAC (Curve B). Experimental conditions; Scan range was from −150 mV to +650 mV at a scan rate of 100 mVs^{-1} and scan started at −150 mV.

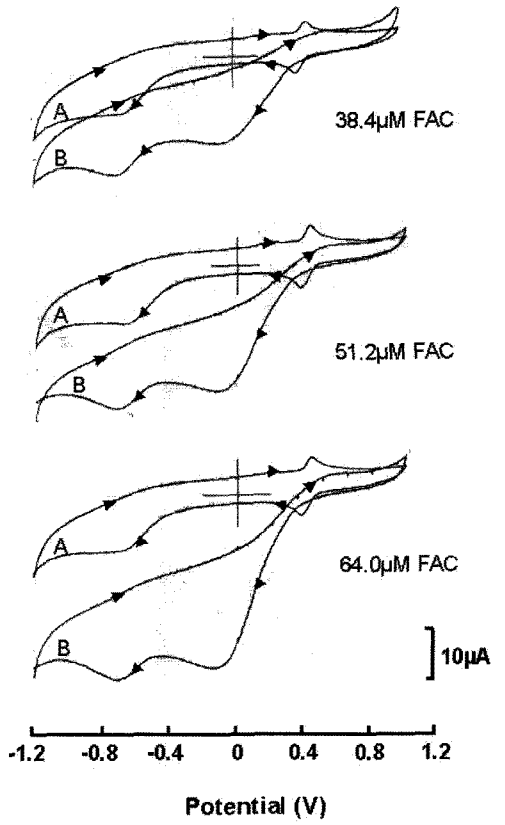

Fig. 4. Cyclic voltammograms for SPCE of 64 μM syringaldazine in 100 mM phosphate buffer (pH 6.5 and 100 mM KCl, Curve A) and in 38.4, 51.2 and 64.0 μM FAC (Curve B). Experimental conditions were the same as in Fig. 3.

buffer and FAC in the absence of syringaldazine with a scan range from −1.2 to +1.0 V are shown in Fig. 4. As can be seen in Curve A, a cathodic current peak was produced at potentials lower than −250 mV in plain buffer. This might be caused by reduction of dissolved oxygen at the surface of SPCE and similar results were reported in previous studies[14-16]. Although the cathodic peak of cyclic voltammogram in 32 μM FAC solution, increased to almost double the magnitude of the results obtained in PPB alone, no significant anodic and cathodic peaks were observed over the potential range of −0.2−+1.0 V (Curve B in Fig. 4). In the same range of potentials, cyclic voltammograms obtained from syringaldazine in the absence of FAC showed clear redox peaks of the azine-form. In the presence of FAC, by contrast, the redox peaks disappeared but a significant reduction peak was observed. A cathodic current from the syringaldazine/FAC reaction, i.e. azo-form syringaldazine, starts at +400 mV with a cathodic peak potential, E_{pc}, at −130 mV. During potential sweeping, it was observed that E_{pc}, determined from the voltammograms of the azo-form of syringaldazine, continuously decreased to near the background level instead of reaching a steady state. After 18 scans, 13 minutes-potential sweeping, E_{pc} showed only 9 % of its initial level on the first scan. The suggested reason for this is that the reaction product, azo-form, slightly decomposed in aqueous solution and showed insufficient stability as discussed.

3.4. Hand held Electrochemical Immunoassay System

In order to achieve in-field analysis, the authors developed and built a rugged and environmentally sealed, battery operated electrochemical measuring system capable of the same accuracy and sensitivity as a commercial bench-top system which being able to be held in the palm of the hand. The portable electrochemical measuring system was self contained and operated continuously for weeks powered by only two PP3 batteries, only requiring the presence of a laptop loaded with the supplied software for data recording. The protocol for use of the portable instrument, shown in Fig. 5, was briefly as follows: connect the USB (Universal Serial Bus) connector to the ADC and the Laptop Computer. Power-up the computer and start a developed USB interface logger program. The digital display of

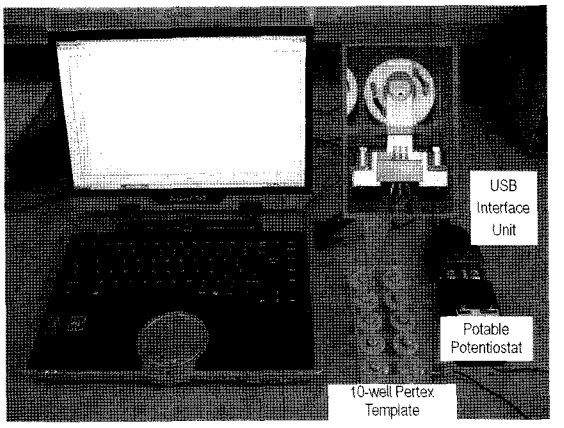

Fig. 5. A prototype portable in-field electrochemical analyzer.

Bias Voltage and WE Current, located near the top of the program display screen, continuously monitors the output from the potentiosatat. The backbone of the hand held Electrochemical Immunoassay System is the DS2200C (CRL, UK). The DS2200C is a two channel Digital Sampling Oscilloscope with 12-bit ADC resolution and up to 200,000 samples/sec conversion rate. The DS2200C came complete with the EasyScope software package (CRL, UK) which offered an attractive Windows based oscilloscope display, with single, dual and XY modes, and the capability of displaying the frequency spectrum of the input signal using its inbuilt F.F.T. function.

3.5. Amperometric FAC Detection using Syringaldazine Modified SPCEs

Amperometric measurements were performed with PVA-SbQ(Syring.)-SPCEs as described. After addition of a 50 μl aliquot of FAC samples into the cell, the PVA-SbQ-(Syring.) polymer layer, which was directly polymerised on the surface of working electrodes, produced intense purple colour instantly. Simultaneously, a reduction peak was observed and recorded. However, the PVA-SbQ(Syring.)-SPCEs were not suitable for repeated use. After a rapid colour producing reaction, the azo-form-syringaldazine did not return to its initial form. Therefore, unused fresh PVA-SbQ(Syring.)-SPCEs had to be used for each measurement. A clear reduction current peak was produced within 1 minute at all concentrations of FAC. However, as observed in the results of CV, the amperometric current response pro-

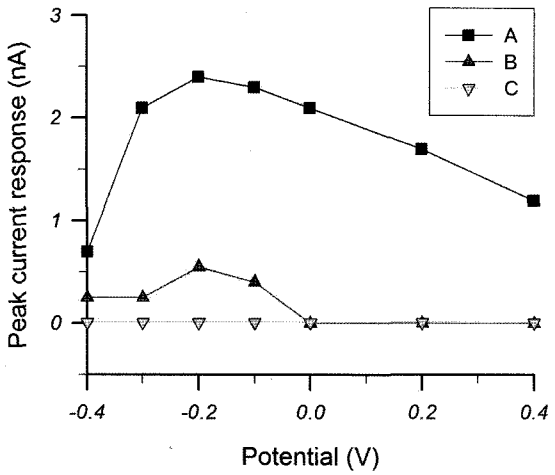

Fig. 6. Hydrodynamic voltammograms for FAC detection using PVA-SbQ(Syring.)-SPCEs in 32 μM FAC (A), using PVA-SbQ-SPCEs in 32 μM FAC (B) and using both electrodes in phosphate buffer (C).

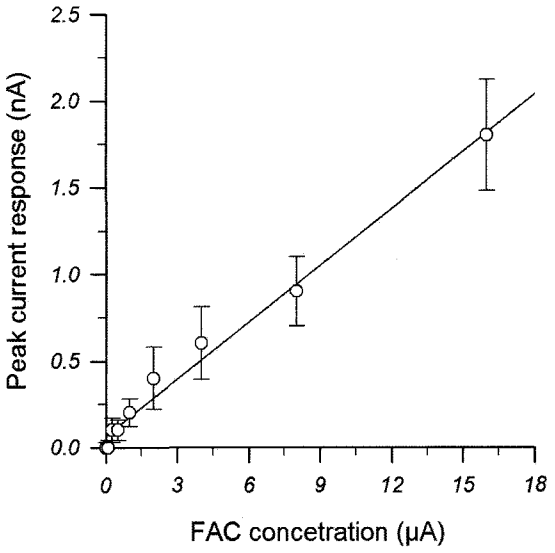

Fig. 7. Calibration curve for FAC using PVA-SbQ(Syring.) -SPCEs.

duced also gradually decreased after reaching the peak level at a recording time of about 1 minute. Consequently, the current responses decreased to near the baseline current in 5 - 10 minutes. The optimum applied potential was determined in buffer containing 32 μM FAC using PVA-SbQ(Syring.)-SPCEs. The same experiments were repeated in plain buffer to assess the background responses of the electrodes. PVA-SbQ-SPCEs without modified syringaldazine were also used in the same experiments to assess the interaction between bare SPCEs and FAC.

Hydrodynamic voltammograms were constructed at potentials over the range of −0.4 V to +0.4 V and are represented in Fig. 6. As shown in Curve A in Fig. 6, the maximum response of PVA-SbQ(Syring.)-SPCEs were observed at a potential of −200 mV. Although FAC showed current responses with bare PVA-SbQ-SPCEs at negative potentials, no responses were observed at potentials more positive than 0 V (Curve B). In the absence of FAC, Curve C, no current changes were observed in the voltammograms obtained using PVA-SbQ modified electrodes in the presence and absence of syringaldazine. However, the time taken to achieve a stable baseline response decreased at zero or positive potentials. Furthermore, at these potentials, the direct reduction of FAC at the electrode surface could be prevented. Because of these considerations, 50 mV was used as the operating potential in all experiments.

Table 1. PVA-SbQ(Syring.)-SPCEs Characteristics

Detection Limit	2.0 μM
Linear Range	2 μM − 20 μM
Sensitivity	3.5 nA μM^{-1} cm^{-2}
Inter-electrode reproducibility (CV)	15 %

3.6. Electrode Calibration

PVA-SbQ(Syring.)-SPCEs were calibrated using FAC standards. Calibration curves for FAC were constructed measuring current peaks and are shown in Fig. 7. Each point represents the mean value of 5 measurements and the error bars represent one standard deviation. In Fig. 7, the equation of the regression line was calculated to be $y=0.11x+0.07$ and the regression coefficient was calculated to be 0.987. The detection limit, sensitivity and linear range of the electrode were calculated using the results obtained. These experimental results are summarised in Table 1. The inter-electrode reproducibility for FAC measurement was assessed. The inter-electrode performance (n=20) showed a coefficient of variation is less than 15 % over the FAC concentration range from 2 μM to 18 μM.

3.7. Electrode Storage Stability

An assessment of the electrode storage stability was carried out by comparison of standard curves constructed over an interval of six months. Even though

electrodes were kept at room temperature under dark and dry conditions, over 85 % of the initial response was observed after six months.

4. Conclusion

The work described in this study has successfully demonstrated that the application of a conventional FAC detection method (FACTS) for disposable amperometric FAC sensor manufacture. The electrochemical aspect of the sensor allows a system which is disposable, rapid, simple, low cost, and which can be incorporated into a hand held device; while the use of syringaldazine allows for high degree of specificity for FAC. The simple screen-printing technique and the effective syringaldazine modification using direct polymerisation on electrodes allow inexpensive FAC sensor fabrication.

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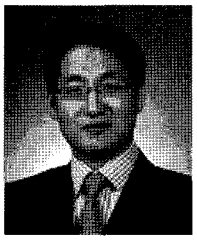

장 승 철

- 1994년 2월 동국대학교 화학과 졸업 (이학사)
- 2000년 5월 뉴캐슬대학교 의생화학과 (이학박사)
- 1999년 6월~2005년 12월 뉴캐슬대학교 의생화학과 연구원
- 2006년 1월~2007년 2월 뉴캐슬대학교 의생화학과 책임연구원
- 2007년 3월~현재 부산대학교 신개념바이오피지오센서기술연구센터 조교수 재직
- 주관심분야 : cell-chip 기반의 바이오센서 및 진단디바이스 연구, 마이크로센서 및 bioMEMS 시스템 개발

박 덕 수

- 1985년 2월 부산대학교 화학과 졸업 (이학사)
- 1987년 2월 부산대학교 화학과 졸업 (이학석사)
- 1994년 2월 부산대학교 화학과 졸업 (이학박사)
- 1995년 10월~1996년 10월 미국 뉴멕시코주립대학교 연구원
- 2004년 3월~현재 부산대학교 신개념바이오피지오센서기술연구센터 조교수 재직
- 주관심분야 : 화학 및 바이오센서, 전기전도성고분자 응용, 전기화학센서시스템