Amperometric Detection of Alcohols and Carbohydrates Coupled with Their Electrocatalytic Oxidation by 2,2,6,6-Tetramethylpiperidinyl-1-oxy (TEMPO)

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Received December 13, 1995; accepted February 1, 1996

Application of the well-known electrocatalytic oxidation of alcohols by 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) as a novel tool for the amperometric detection of alcohols under basic conditions has been examined. Cyclic voltammograms of TEMPO in the absence and the presence of 1-pentanol, diethyleneglycol, and glucose demonstrated that these alcohols can electrochemically detected in terms of the anodic response of an aqueous 150 mM NaOH solution of TEMPO at a glassy carbon electrode. In flow injection analysis (FIA) utilizing an aqueous 150 mM NaOH solution of 100 μM TEMPO as a carrier solution with an applied potential of 0.4 V *vs.* saturated calomel electrode (SCE) and a flow rate of 0.5 ml/min, a good dependency of FIA responses upon the concentration was observed for 1-pentanol (20 μM—1 mM), diethyleneglycol (5 μM—1 mM), and glucose (5 μM—1 mM). Reproducible and stable responses have been obtained for these alcohols on repetitive injections of the sample solutions. The results on the FIAs of various alcohols have demonstrated that the amperometric detection of primary alcohols and especially polyhydroxylated compounds such as 1,ω-diols and carbohydrates can be achieved with a high sensitivity by the present methodology.

Key words electrochemical indirect detection; alcohol; carbohydrate; 2,2,6,6-tetramethylpiperidinyl-1-oxy; flow injection analysis

Organic nitrosonium salts, which can be easily generated from nitroxide radicals such as 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) by one-electron oxidation, are among the most versatile reagents to oxidize various functional groups.[1] Extensive studies have been reported, especially on the oxidation of alcohols into aldehydes or ketones, which has been shown to proceed smoothly in the presence of bases in organic or aqueous organic solvents[1–4] and even in aqueous media.[5] Furthermore, the transformation of alcohols into carbonyl compounds can be catalytically achieved in combination with an anode as a co-oxidizing reagent, that is, by controlled potential electrolysis at a potential as low as 0.4 V in the presence of a catalytic amount of TEMPO.[6,7] These results on the electrosynthetic applications seem to be of great interest in the field of electroanalytical chemistry as well. Namely, the presence of alcohols can be transformed into electrochemical signals as a result of their oxidation to induce rapid turnover in electrochemically formed redox cycles of nitroxide radicals such as TEMPO. In addition, it has been shown that electrodes modified with nitroxide radicals can constitute effective and simple systems for synthetic reactions based on the nitroxide radical-mediated oxidations.[8–12] We thought that these findings might lead to a novel analytical methodology based on the catalytic oxidation of alcohols by the redox cycles of nitroxide radicals, *i.e.*, it might be possible to develop an electrochemical detector with a novel modified electrode for the determination of alcohols and carbohydrates in high-performance liquid chromatography (HPLC).

Recently, electrochemical detectors have come into the limelight in chromatographic analyses of carbohydrates. This is because carbohydrates generally have no strongly absorbing chromophores available for direct ultraviolet detection, so it is necessary to utilize refractive index detectors, which suffer from inherent poor sensitivity. However, one difficulty with electrochemical detection is that carbohydrates are hard to oxidize at a common carbon electrode, leading to low sensitivity and selectivity. Thus, much attention has been directed to developing a novel electrochemical system to detect carbohydrates at a lower potential. Several useful electrochemical approaches have been explored, which can be conveniently divided into the following two groups: (1) pulsed-potential amperometry employing noble metallic electrodes, such as gold and platinum,[13] or cobalt(II) phthalocyanine-modified electrodes[14]; (2) constant-potential amperometric detection at oxide-covered metal-based electrodes.[15–22] However, simple organic mediators, such as TEMPO, capable of oxidizing alcohols effectively at a low potential, have never been included in such detection systems as far as we are aware.

In this paper, we describe an electrocatalytic oxidation with TEMPO as a novel tool to detect alcohols and carbohydrates by flow injection analysis (FIA) with a carrier solution containing the mediator in a convenient constant-potential mode.

Results and Discussion

The possibility of an electrocatalytic system based on TEMPO (*cf.* Chart 1) as a tool to detect alcohols was first examined by cyclic voltammetry (CV) at a glassy carbon (GC) electrode. As model compounds, 1-pentanol, diethyleneglycol, and glucose were chosen. Aqueous NaOH of around 150 mM has been used as a mobile phase for common anion-exchange chromatography of various carbohydrates.[14–16,19–22] The catalytic oxidation with TEMPO is known to require a base.[1–5] Thus, an aqueous 150 mM NaOH solution was utilized as the medium. Voltammograms for an aqueous 150 mM NaOH solution of TEMPO in the absence and the presence of 1-pentanol

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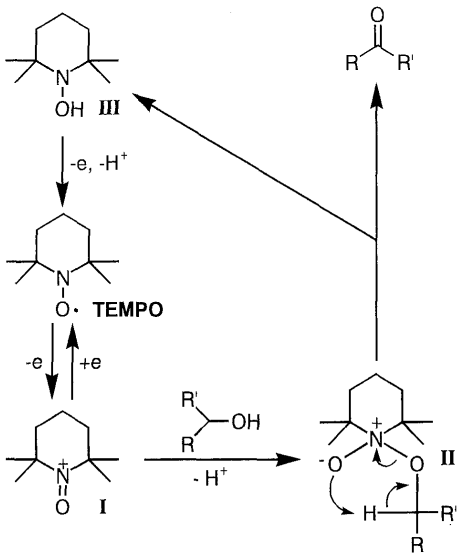

Chart 1

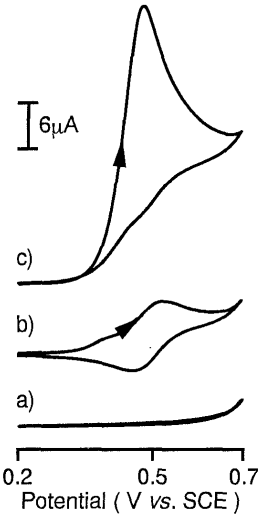

Fig. 1. Cyclic Voltammograms for TEMPO (1 mM) in Aqueous 150 mM NaOH Solution at a GC Electrode

a) For a blank solution; b), c) for TEMPO in the absence and the presence of 1-pentanol (1 mM), respectively; sweep rate, 10 mVs^{-1}.

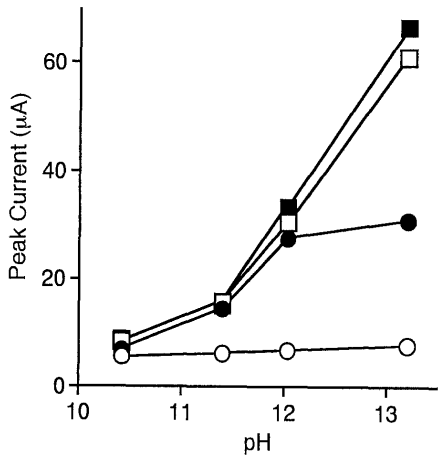

Fig. 2. Effects of pH of the Medium upon the Peak Currents Observed on Cyclic Voltammograms for Only TEMPO (1 mM) (—○—) and for TEMPO in the Presence of 1-Pentanol (—●—), Diethyleneglycol (—□—), or Glucose (—■—) (Each 1 mM)

A GC electrode was used, with a sweep rate of 10 mVs^{-1}.

are compared in Fig. 1. In the medium, TEMPO exhibited a redox wave with a good reversibility: an anodic peak at 0.52 V *vs.* saturated calomel electrode (SCE) due to the formation of the corresponding nitrosonium ion I (Chart 1) from TEMPO, and a cathodic peak at 0.45 V on a reverse scan corresponding to the reduction of I to TEMPO (Fig. 1b). On addition of 1-pentanol, the anodic peak enlarged more than 5 times and the cathodic peak disappeared (Fig. 1c). The observed behavior can be explained by a generally accepted mechanism as depicted in Chart 1.[5,7] Namely, 1-pentanol will be oxidized through its addition to anodically generated I, followed by intramolecular proton-transfer in the adduct II. The oxidation process can establish an electrochemical redox cycle between the hydroxylamine III, TEMPO, and I, resulting in the enhanced anodic response. In the basic medium, the formation of II will proceed smoothly, that is, I will be totally consumed as soon as it is anodically generated and will not reside at the electrode surface to afford the cathodic response in the presence of a sufficient amount of the alcohol. A similar voltammetric behavior was observed in the presence of diethyleneglycol or glucose. The results indicate that the increment in the anodic response of TEMPO, the so-called catalytic current, can be used for the electrochemical detection of these alcohols in aqueous alkaline solutions, as we expected. None of the alcohols themselves exhibited any anodic response under the basic conditions, and the voltammograms were indistinguishable from that for the background (Fig. 1a).

The effects of pH upon the voltammetric responses of TEMPO in the absence and presence of the alcohols were also examined. The CV was performed in media of pH 10.4, 11.4 12.0 and 13.2, which were prepared by adding HCl to aqueous 150 mM NaOH solution. In these media, TEMPO showed essentially the same voltammograms as that depicted in Fig. 1b, but the catalytic currents induced by the addition of the alcohols were remarkably affected by the pH of the sample solutions. As shown in Fig. 2, the catalytic current observed in the presence of each alcohol was negligible at pH 10.4, while it increased as the medium became more basic. This is quite reasonable since the catalytic oxidation will not be effectively achieved by the redox cycle shown in Chart 1 unless enough base is present to facilitate the addition of the alcohol to anodically generated I. The response observed at pH 13.2 (150 mM NaOH) can be regarded as maximal, since the catalytic current in the presence of each of the alcohols was essentially unchanged in aqueous solution containing 50—200 mM NaOH.

The voltammetric results described above suggest that FIA of aliphatic alcohols and carbohydrates can be achieved by using the amperometric response of TEMPO in a constant-potential mode at a potential as low as 0.4 V. The FIA seems attractive from an analytical viewpoint, since such mild electrochemical conditions should afford a high sensitivity and selectivity, as has been claimed in the amperometric detection of carbohydrates at oxide-covered metal-based electrodes with applied potentials of around 0.5 V.[15-22] Figure 3 shows hydro-

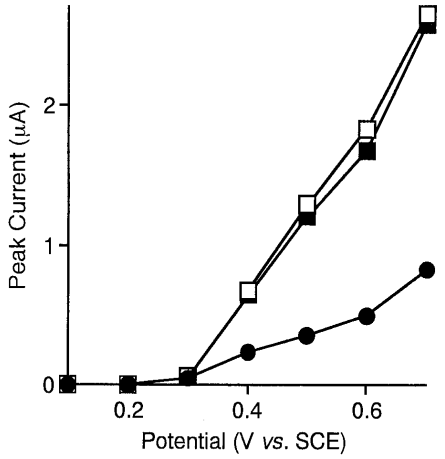

Fig. 3. Hydrodynamic Voltammograms for 1-Pentanol (—●—), Diethyleneglycol (—□—), and Glucose (—■—) (1 mM Each)

Flow conditions: carrier solution, aqueous 150 mM NaOH solution of 100 μM TEMPO; flow rate, 0.5 ml/min; injection volume, 20 μl.

Table 1. Effect of the Applied Potential in FIA for 1-Pentanol, Diethyleneglycol, and Glucose (each 1 mM) upon the Ratio between Peak and Background Currents (I_p, I_{bg})[a]

Potential (V *vs.* SCE)	I_p/I_{bg} for			I_{bg} (μA)
	1-Pentanol	DEG[b]	Glucose	
0.1	—	—	—	0.056
0.2	—	—	—	0.056
0.3	0.107	0.161	0.161	0.384
0.4	0.123	0.349	0.338	1.93
0.5	0.043	0.158	0.147	8.25
0.6	0.029	0.108	0.099	16.9
0.7	0.033	0.106	0.103	25.0

a) Flow conditions are the same as in Fig. 3. *b*) DEG stands for diethyleneglycol.

dynamic voltammograms (HDV) obtained for 1-pentanol, diethyleneglycol, and glucose under FIA conditions, where an aqueous 150 mM NaOH solution of 100 μM TEMPO was utilized as a carrier solution with a flow rate of 0.5 ml/min. The injection amount of sample solutions was 20 μl throughout the present study. When the detection was conducted at a potential below 0.3 V, FIA responses for these alcohols were zero or negligible. At a higher potential than 0.4 V, their electrochemical responses were observed as peaks, and the peak currents became larger proportionally as the applied potential was increased from 0.4 to 0.7 V without reaching a maximum value: the background current also increased with the applied potential.

Since in an amperometric detection the highest sensitivity with the lowest noise level will be achieved at a potential giving the largest signals-to-background ratio, the peak and the background currents observed on the HDV (I_p and I_{bg}, respectively) were compared to determine the best applied potential in the present FIA. The results are summarized in Table 1. The I_{bg} value was remarkably enhanced at a potential more positive than 0.5 V, rendering the ratio of I_p/I_{bg} rather small. Thus, 0.4 V, at which the largest values of I_p/I_{bg} were noted for all of the alcohols, seems to be the applied potential of choice in the present FIA.

Table 2. Effect of the Concentration of TEMPO in FIA for 1-Pentanol, Diethyleneglycol, and Glucose (each 1 mM) upon the Ratio between Peak and Background Currents (I_p, I_{bg})[a]

Concentration of TEMPO	I_p/I_{bg} for			I_{bg} (μA)
	1-Pentanol	DEG[b]	Glucose	
20 μM	0.071	0.243	0.241	0.750
50 μM	0.123	0.355	0.350	1.553
100 μM	0.123	0.349	0.338	1.930
200 μM	0.120	0.329	0.308	2.740
500 μM	0.111	0.275	0.257	4.590
1 mM	0.074	0.188	0.177	7.270

a) Flow conditions: carrier solution, an aqueous 150 mM NaOH solution of TEMPO; applied potential, 0.4 V *vs.* SCE; flow rate, 0.5 ml/min; injection volume, 20 μl. *b*) DEG stands for diethyleneglycol.

Table 3. Effect of the Flow Rate in FIA for 1-Pentanol, Diethyleneglycol, and Glucose (each 1 mM) upon the Ratio between Peak and Background Currents (I_p, I_{bg})[a]

Flow rate (ml/min)	I_p/I_{bg} for			I_{bg} (μA)
	1-Pentanol	DEG[b]	Glucose	
0.1	0.257	0.551	0.497	1.84
0.3	0.178	0.457	0.430	1.81
0.5	0.143	0.382	0.358	1.82
0.7	0.126	0.329	0.308	1.82
1.0	0.098	0.250	0.239	1.90

a) Flow conditions: carrier solution, an aqueous 150 mM NaOH solution of 100 μM TEMPO; applied potential, 0.4 V *vs.* SCE; injection volume, 20 μl. *b*) DEG stands for diethyleneglycol.

The effects of concentration of TEMPO and flow rate upon the responses in FIA for 1-pentanol, diethyleneglycol, and glucose were also examined in a similar way, using the I_p/I_{bg} value as a probe. As shown in Table 2, a larger baseline response, I_{bg}, was observed in a carrier solution containing a higher concentration of TEMPO, and a concentration range between 50—200 μM seems to afford a maximal value of I_p/I_{bg}, namely a satisfactory sensitivity in the present FIA to detect each of the alcohols. Accordingly, an aqueous 150 mM NaOH solution containing 100 μM TEMPO was employed as the carrier solution for further examination.

Table 3 shows the effects of flow rate upon the FIA responses for 1-pentanol, diethyleneglycol, and glucose. Within the range of flow rate examined here, I_{bg} was almost unchanged, while a slower flow rate induced a larger I_p/I_{bg}, indicating that the alcohols can be detected with a high sensitivity by the present system with a slow flow rate. The results are reasonable, since a slow flow rate can be expected to allow sufficient time for an alcohol to induce an effective turnover of the redox cycle of TEMPO at the electrode surface. Although the largest I_p/I_{bg}, that is, the highest sensitivity was recognized at a flow rate of 0.1 ml/min for all of the alcohols, 0.5 ml/min was considered to be more practical, and enough to obtain a satisfactory sensitivity, taking future application of the present methodology to HPLC analysis into consideration.

Based on the satisfactory conditions obtained above,

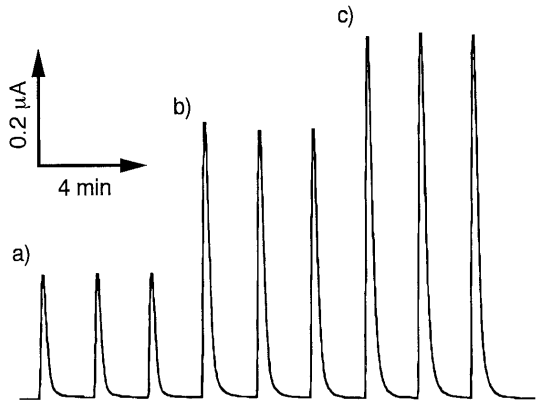

Fig. 4. Typical FIA Responses for a) 0.1 mM, b) 0.5 mM, and c) 1.0 mM Glucose

Flow conditions: carrier solution, aqueous 150 mM NaOH solution of 100 μM TEMPO; applied potential, 0.4 V *vs.* SCE; flow rate, 0.5 ml/min; injection volume, 20 μl.

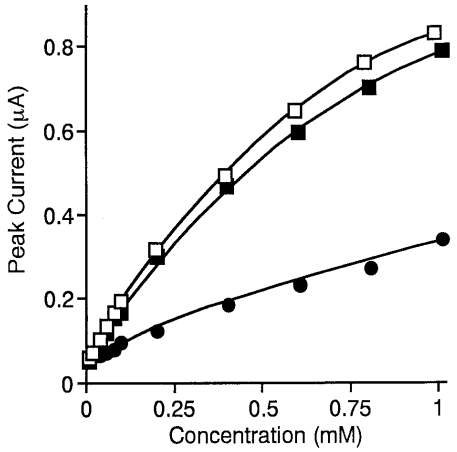

Fig. 5. Calibration Curves Obtained by FIA for 1-Pentanol (—●—), Diethyleneglycol (—□—), and Glucose (—■—)

Flow conditions are the same as in Fig. 4.

the relationship between the FIA responses and the concentration of alcohols was examined. Representative FIA responses for glucose are illustrated in Fig. 4: applied potential, 0.4 V *vs.* SCE; carrier solution, an aqueous 150 mM NaOH solution containing 100 μM TEMPO; flow rate, 0.5 ml/min. A good dependency of the response on the concentration of glucose was observed, as shown in Fig. 5. Using the present system, the carbohydrate can be detected even at 5 μM. Similar concentration–FIA response relationships were recognized for 1-pentanol and diethyleneglycol over the concentration ranges of 20 μM—1 mM and 5 μM—1 mM, respectively, and these results are also included in Fig. 5.

The present method has proved to be a reproducible tool to detect alcohols, as can be seen from the results presented in Fig. 6. Thus, when a sample solution of 1-pentanol, diethyleneglycol, or glucose was successively injected 100 times, the relative FIA responses observed at injection number 100 against those for the first injection were 95, 94, and 98%, respectively. The relative standard deviations ($n=100$) were 2.7% for 1-pentanol, 2.2% for diethyleneglycol, and 2.4% for glucose. The results demonstrate that the strong basic conditions employed

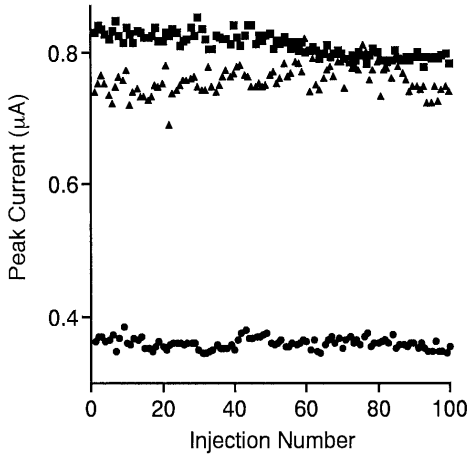

Fig. 6. FIA Responses for 100 Injections of 1-Pentanol (—●—), Diethyleneglycol (—■—), and Glucose (—▲—) (Each 1 mM)

Flow conditions are the same as in Fig. 4.

Table 4. FIA Responses for Various Alcohols[a]

Alcohol	Relative response[b]	Alcohol	Relative response[b]
1-Pentanol	0.45	Glucose	1.00
2-Pentanol	0.17	Galactose	1.10
2-Methyl-2-butanol	—	Rhamnose	1.05
1,5-Pentanediol	0.74	Mannose	1.04
Ethyleneglycol	0.97	Arabinose	0.95
Diethyleneglycol	1.06	Xylose	0.79
Triethyleneglycol	1.04	Fructose	0.41
Tetraethyleneglycol	1.06	Sucrose	1.08
DEG monomethyl ether[c]	0.81	γ-Cyclodextrin	1.33
Polyethyleneglycol #400	1.07		

a) Flow conditions are the same as in Fig. 4. *b*) Against the FIA response for glucose. *c*) DEG stands for diethyleneglycol.

here do not cause passivation of the electrode, and imply the availability of the present detection system for a long-term, repetitive operation.

The present FIA was also applied for the detection of various type of alcohols (1 mM). The dependency of the FIA responses upon the structure of alcohols is listed in Table 4, where the response obtained for each alcohol was normalized against that for glucose. Previous synthetic studies have documented that oxidation by the nitrosonium ions generated from TEMPO and its derivatives is slower for secondary alcohols than for primary ones, leading to a pronounced preference for oxidation of the latter, that is, a high chemoselectivity.[2,4,6] The origin of the sluggishness in TEMPO-mediated oxidation of secondary alcohols has been ascribed to slow intramolecular proton-transfer in the adduct II, shown in Chart 1, due to severe steric interaction encountered in the proper conformation for the process.[5,7] In the present analytical system, a similar trend was noted. Thus, 2-pentanol exhibited an FIA response corresponding to nearly one-third of that for 1-pentanol. In the case of a tertiary alcohol, 2-methyl-2-butanol, no FIA response was recognized. The dependence of the FIA responses upon the structure of the alcohols can be explained as mentioned above. Namely, slower oxidation of the secondary alcohol by the nitrosonium ion II generated from TEMPO

will prevent the redox cycle from being effectively formed, leading to a lower electrochemical response, while the tertiary alcohol with no α-hydrogen could not be oxidized by II and hence no FIA response resulting from the catalytic oxidation was observed at all. 1,ω-Diols and carbohydrates except for fructose were detected by the present system with sensitivities more than twice that for 1-pentanol. This is because the diols have two primary hydroxyl groups, and the carbohydrates have one primary hydroxyl group and an anomeric hydroxyl group, namely a lactol moiety, which is also known to be oxidized to a lactone by the nitrosonium ions derived from TEMPO and its derivatives.[2,3,6]

The results described so far demonstrate that the electrocatalytic oxidation of alcohols by TEMPO represents a useful tool for electrochemical detection of primary and secondary alcohols, especially polyhydroxylated compounds such as carbohydrates, and is applicable for an FIA with a constant-potential mode. As far as practical application is concerned, the effects of coexisting compounds upon the FIA response in the present method remain to be investigated since the nitrosonium ion generated from TEMPO is known to oxidize various compounds other than alcohols.[1] However, combination with chromatographic separation should be a practical application for the present system. Thus, it is expected that a practical application of the present methodology to an electrochemical detection system in HPLC will be realized by post-mixing of a TEMPO carrier solution or immobilization of TEMPO chemically on the surface of a carbon electrode, although in the latter case some problems might arise with respect to the modification method, stability of the TEMPO-modified electrode under the strong basic conditions, and so on. Further studies on these points are under way.

Experimental

Reagents Deionized and distilled water was used throughout the present study. All other chemicals were of reagent grade and were used without further purification.

CV GC disks (GC 30, 3 mm i.d.) were obtained from Tokai Carbon and fabricated as previously described.[23] Before the measurements, a GC electrode was polished mechanically by a polishing system (Maruto, ML-150P) with a polishing paper (#1200) followed by alumina powder (0.05 μm) on a polishing cloth, then sonicated in deionized water for 5 min, washed with water and MeOH, and dried with a stream of nitrogen. Cyclic voltammograms were recorded with a potentiostat (Huso, Model 315A) equipped with an X–Y recorder (Riken Denshi, Model F-5C). A three-electrode configuration was employed: a GC electrode as the working electrode, an SCE as the reference electrode, and a platinum wire electrode as the counter electrode. All voltammetric measurements were carried out at room temperature with a sweep rate of $10\,mV\,s^{-1}$ over the potential range between 0.2 and 0.7 V *vs.* SCE.

FIA All measurements were performed at room temperature with an FIA system consisting of a plunger pump (Shimadzu, LC-5A), an injector with a 20 μl sample loop (Shimadzu, SIL-1A), a high-sensitivity potentiostat equipped with a low-pass filter (Huso, HECS 318 and 974), a thin-layer electrochemical cell (a flow cell from a Shimadzu L-ECD-6A electrochemical detector), and a recorder (Rikadenki, R-50). The working electrode for the electrochemical cell was a GC plate (GC 30, 15 × 30 mm), obtained from Tokai Carbon, and was polished as described above for a GC disk electrode, before being placed in the electrochemical cell. The reference electrode was an SCE. All sample solutions were prepared from aqueous 150 mM NaOH.

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