Study on the indirect electrochemical detection of ammonium ion with *in-situ* electrogenerated hypobromous acid

Miki Takahashi, Kenta Nakamura, Jiye Jin*

Department of Chemistry, Faculty of Science, Shinshu University

3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

*Correspondence:

E-mail: jin@shinshu-u.ac.jp; Fax : (+81)263-37-2559 ; Tel : (+81)263-37-2475

Abstract

The mechanism on the electrochemical oxidation of bromide in the presence of ammonium ion was studied by cyclic voltammetry and *in-situ* UV-Vis spectroscopy. The results indicated that the anodically generated bromine (Br_2) would be hydrolyzed to hypobromous acid (HBrO) at the pH range of 5 – 7 and was further disproportionate to hypobromite anion (BrO^-) when pH was large than 7. Both HBrO and BrO^- were confirmed to be participated in the following homogeneous chemical reaction with the ammonium ion co-existed in the electrolytic solution. However, HBrO is electroactive whereas BrO^- is electroinactive at carbon electrode. Based upon the reaction of HBrO with ammonium ion, an indirect electrochemical method was proposed for determination of ammonium ion at pH 7 with dual-electrode configuration, where HBrO was produced at a generator electrode and the excess HBrO was subsequently detected at a collector electrode after a reaction with ammonium ion. It was demonstrated that ammonium ion concentrations were proportional to the decreasing of reduction current at the collector electrode either in a batch solution or in a micro flow injection analytical (micro-FIA) system using an interdigitated array (IDA) Pt microelectrode and a carbon film ring-disk electrode (CFRDE), with the detection limit below 3 M. This approach shows the advantage of highly selectivity even in presence of a large amount of co-existed cations.

Keywords: indirect electrochemical detection**,** ammonium ion**,** hypobromous acid, hypobromite, interdigitated microelectrode array

1. Introduction

Ammonium ion is known as an important micronutrient and intermediate of the nitrogen cycle in aquatic ecosystem [1-3]. The development of a sensitive and selective analytical method for the determination of ammonium ion is very important in the environmental evaluation of water pollution and fundamental to the understanding of nitrogen cycling in aquatic ecosystems. Indophenol blue spectrophotometry has been widely used for the determined in environmental waters because of the ease in operation and inexpensive equipment [4]. It involves the formation of the deep blue color of indophenol by reaction of ammonium ion with hypochlorite and phenol under alkaline conditions. This method, however, suffered from the problems of low sensitivity and selectivity because some substances, such as copper, zinc, iron, bromide were found to interfere with the determination of ammonium ion by the indophenol reaction [5]. Ion chromatography with conductivity detection is the practical analytical tool for the simultaneous determination of anions and cations [6, 7]. But high concentrations of co-existing inorganic cations in environmental waters often interfere with the ammonium ion determination [8]. Electrochemical techniques are known to provide the advantages such as low equipment costs, easy of use and the possibility of monitoring the analyte in real time. Although ammonium ion is an electroinactive species, various electroanalytical methods, including the potentiometric sensor [9], catalytic cathodic stripping voltammetry [10], and the indirect voltammetric measurement based on the reaction of ammonium ion with *N*,*N'*-diphenyl-1,4-phenylenediamine in nonaqueous solution [11], have been reported for ammonium ion quantitation. Nevertheless, a limitation to widespread application of these techniques is their relatively slow response and inherent poor sensitivity.

The oxidation of ammonium ion with hypohalites, for example hypochlorite (ClO^-) or hypobromite (BrO^-) has been studied extensively in relation to the synthesis of hydrazine and the water disinfection [12, 13]. The hypohalites were reported to be as the chemiluminescence reagents for the determination of ammonium ion in rainwater samples [14, 15]. In this study, it was found that the voltammetric behavior in the oxidation of bromide was greatly affected by the co-existed ammonium ion. The experimental results from cyclic voltammetry and *in-situ* UV-Vis spectroscopy indicated that the following chemical reaction between the electrogenerated bromine and ammonium ion was pH dependent; only when bromine was hydrolyzed to hypobromous acid (HBrO) and hypobromite anion (BrO^-) in the relatively higher pH region, these species participated in the following chemical reaction with ammonium ion. Based upon the reaction of HBrO with ammonium ion, an indirect electrochemical method was proposed for determination of ammonium ion in phosphate buffer solution at the pH 7 with dual-electrode configuration, where HBrO was produced at a generator electrode and the excess HBrO was subsequently detected at a collector electrode after a reaction with ammonium ion. *In-situ* generation of such oxidation reagent can avoid the problems associated with the reagent stability and handing in analytical usage [16, 17]. It was demonstrated that ammonium ion concentrations were proportional to the decreasing of reduction current at the collector electrode either in a batch solution or in a micro flow injection analytical (micro-FIA) system with an interdigitated array (IDA) Pt microelectrode and a carbon film ring-disk electrode (CFRDE), with the detection limit below 3 M. The electrochemical indirect detection method shows the advantage of

highly selectivity even in presence of a large amount of co-existed cations, and can be used as an on line flow through detector in HPLC or capillary electrophoresis.

2. Experimental

2.1. Reagents

Potassium bromide (KBr) and ammonium chloride were of reagent grade, and were purchased from Wako (Osaka, Japan), and used without further purification. Stock solution of ammonium ion was prepared with distilled water. Prior to voltammetric or indirect detection, the required sample solutions were made up fresh by dilution with distilled water. The phosphate buffer solution (PBS) was prepared from 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4. The pH was adjusted with 0.1 M NaOH or 0.1 M H_2SO_4.

2.2. Apparatus

Electrochemical experiments were conducted with a CHI 660 electrochemical workstation, or a CHI 1232 bipotentiostat (CH Instruments TX, USA). A glassy carbon electrode (ϕ 1.0 mm) and an interdigitated array (IDA) Pt microelectrode (BAS Japan, Tokyo, Japan) were used for the voltammetric measurements. The IDA Pt microelectrode was formed on a quartz glass substrate. Each segment contained 65 fingers with the length of 2 mm and the width of 3 μm, and the gap between adjacent fingers of two segments was 3 μm. An Ag/AgCl (3.3 M KCl) reference electrode and a platinum wire were used as reference and auxiliary electrodes, respectively.

The micro-FIA system consisted of a LC-100 micro LC pump (BAS Inc, USA), Model 7520 micro injector (Rheodyne, USA) with a sample loop volume of 0.5 μl, and a thin-layer radial flow cell (BAS Japan, Tokyo, Japan). 10 mM KBr in 0.1 M PBS (pH=7) – acetonitrile (MeCN) (v/v=9:1) mixture solution was employed as the mobile phase. The flow rate was maintained at 30 μl/min, and the experiments were carried out at room temperature. To deal with any external sources of noise, such as pump pulsation, an ODS packed capillary column (0.5×10 mm) serving as pulse dampener was placed before the injector. The carbon film ring-disk electrodes (CFRDE) were presented from BAS Japan (Tokyo, Japan). The carbon film was deposited on the plastic films by photolithography and dry etching techniques. As is shown in Fig. 1, the disk electrode (3 mm in diameter) of the CFRDE was separated from the ring electrode (1 mm width) by a gap of 0.5 mm and its thickness of two inches. The CFRDE was fitted into a thin-layer radial flow cell with a 25 μm thick Teflon gasket in amperometric experiments (Fig. 1). The disk electrode was held at +1.3 V *vs*. Ag/AgCl to generate the bromine electrochemically, while the ring electrode, which serves as the collector electrode (amperometric indicator), was held at +0.2 V *vs*. Ag/AgCl. The dual electrode potentials were controlled by a HCES 972 potentiostat coupled with a HCES 366 Twin unit (HUSO Co, Kawasaki, Japan), respectively. The current responses were recorded on a Macintosh computer interfaced with a PowerLab data acquisition module using PowerChrom software (AD Instruments, NSW, Australia).

UV-Vis spectra were obtained with a Shimadzu UV-2550 spectrophotometer (Shimadzu Co., Kyoto, Japan). *In-situ* spectra were measured with a thin-layer quartz glass spectroelectrochemical cell (BAS Japan, Tokyo, Japan) with an optical path length of 1

mm. A 80 mesh platinum gauze electrode was used as working electrode, an Ag/AgCl (3.3 M KCl), and a platinum wire were served as reference and auxiliary electrodes, respectively.

3. Results and Discussion

3.1. Voltammetric behavior of KBr in aqueous solution

Figure 2 displays effect of pH on voltammetric behavior of KBr at a glassy carbon electrode in aqueous solutions. In acidic solution (e.g. pH=1), KBr exhibited a pair of reversible wave corresponding to the redox of Br_3^-/Br^- around + 1.0 V *vs*. Ag/AgCl. When the pH increases, the voltammetric response became irreversible, with cathodic shift of the reduction peak potential and decreasing its peak current. Figure 3(A) shows a plot of the ratio of anodic to cathodic peak current (i_{pc} / i_{pa}) as a function of scan rate. The increase in the ratio of i_{pc} / i_{pa} with an increase of scan rate reflects the EC mechanism, i.e. the following homogeneous chemical reaction involving in the electrochemical oxidation process of KBr at higher pH region. As will be discussed in the next section, the electrogenerate bromine (Br_2) at electrode surface can react with bromide ion to form tribromide ion (Br_3^-) in strong acidic solution.

$$2Br^- - 2e^- \rightarrow Br_2 \qquad (1)$$

$$Br^- + Br_2 \rightleftarrows Br_3^- \qquad (2)$$

The ratio i_{pc} / i_{pa} was decreased with increasing of scan rate in sulfuric solution (pH=1), indicating that the electroactivity of Br_3^- might be higher than that of Br_2. When the pH was raised, bromine was hydrolyzed to hypobromous acid (HBrO) and further dissociated into hypobromite (BrO^-), as were shown in equations (3), (4). The proportions of each product will depend on the pH of solution.

$$Br_2 + H_2O \rightleftarrows HBrO + H^+ + Br^- \qquad (3)$$

$$HBrO \rightleftarrows BrO^- + H^+ \qquad (4)$$

The decreasing of ratio i_{pc} / i_{pa} with increasing of pH value might be due to the lower electroactivity of HBrO and BrO^-.

Figure 3(B) shows a plot of cathodic peak potentials as a function of pH. There are two distinct regions. At the pH below 3, the peak potential was independent of pH (the slope dE / dpH≈0). In the acidic region, the redox of bromide underwent the reactions according to equations (1) and (2), which means that no proton is present in the electrode reaction. However, the plot of reduction potential *vs.* pH yields a straight line in the range of pH 3 – 8 with a slope of dE / dpH = −32 mV / pH. In this pH region, bromine was hydrolyzed to HBrO. The potential shift in this pH range suggested that one proton and two electrons may be involved in the electrode reaction as is shown in equation (5).

$$HBrO + H^+ + 2e^- \rightarrow Br^- + H_2O \qquad (5)$$

3.2. Cyclic voltammograms of KBr in presence of ammonium ion

A typical cyclic voltammograms of KBr in presence of ammonium ion with different concentrations at pH 7 is shown in Fig. 4. It was seen that the reduction peak current decreased significantly when NH_4^+ was added into the solution (solid lines in Fig. 4). However remarkable change was not able to be confined in strong acidic media

(pH<2) or alkaline solutions (pH>8.5). Therefore, the possibility of indirect electrochemical detection about ammonium ion involving *in-situ* electrogenerated bromine having the limitation of the pH value of the solution was suggested. The indirect amperometric detection approach with *in-situ* electrochemical generated bromine can be understood by considering the cyclic voltammogram of the bromide in presence of ammonium ion.

3.3. *In-situ* UV-Vis spectra of the electrogenerated bromine

To elucidate the detailed electrochemical oxidation process for bromide, spectral characterization at the electrode surface was carried out by using a thin-layer spectroelectrochemical cell with a light transparent platinum gauze working electrode. Figure 5(A) shows the *in-situ* UV-Vis spectra of the electrogenerated bromine in electrolyte with different pH values. The absorption spectra were measured after electrolysis of 10 mM KBr time for 30 s at +1.0 V vs. Ag/AgCl. Before the measurements were run, the solution was measured at the open circuit potential as reference. The absorption spectra of the electrogenerated bromine were found to be strongly depended on pH of the electrolyte, implying that the complex chemical equilibria of electrogenerated bromine should be taken into account. In strong acidic solution, tribromide ion (Br_3^-) was dominant (equation (2)) which shows the absorption maximum at 266 nm with molar absorptivity value of 40900 $M^{-1}cm^{-1}$ [18]. At pH 7, the absorbance around 260 nm was decreased. According to the equilibrium equation (3), bromine was thought to be disproportionated to HBrO, which shows the absorption maximum at 260 nm but has relatively low molar absorptivity value (95 $M^{-1}cm^{-1}$) [19]. In the basic region, HBrO is dissociated to BrO^- with the absorption maximum at 329 nm [20].

Assuming the average concentration of electrochemically generated bromine is C_{Br2}, the concentrations of each species in the mobile phase can be written as equation (6) based on the law of mass balance.

$$C_{Br2} = [Br_2] + [Br_3^-] + [HBrO] + [BrO^-] \quad (6)$$

In order to determine which of the bromine species is dominant at a specific pH, the equilibriums of equations (2) - (4) must be taken into account. Table 1 summarized the equilibrium constants for equations (2) - (4). K_1 is the formation constant of tribromide in equation (2), K_2 is the hydrolysis constant for the equilibrium of equation (3) and K_3 is the dissociation constant of HBrO in equation (4). By rearranging equation (2) - (4) to solve for all species expect $[Br^-]$, the mole fractions of each species can be expressed as follows.

$$\alpha_{Br_2} = \frac{[Br_2]}{C_{Br2}} = \frac{[Br^-][H^+]^2}{[Br^-][H^+]^2 + K_1[Br^-]^2[H^+]^2 + K_2[H^+] + K_2K_3} \quad (7)$$

$$\alpha_{Br_3^-} = \frac{[Br_3^-]}{C_{Br2}} = \frac{K_1[Br^-]^2[H^+]^2}{[Br^-][H^+]^2 + K_1[Br^-]^2[H^+]^2 + K_2[H^+] + K_2K_3} \quad (8)$$

$$\alpha_{HBrO} = \frac{[HBrO]}{C_{Br2}} = \frac{K_2[H^+]}{[Br^-][H^+]^2 + K_1[Br^-]^2[H^+]^2 + K_2[H^+] + K_2K_3} \quad (9)$$

$$\alpha_{BrO^-} = \frac{[BrO^-]}{C_{Br2}} = \frac{K_2 K_3}{[Br^-][H^+]^2 + K_1[Br^-]^2[H^+]^2 + K_2[H^+] + K_2 K_3} \quad (10)$$

Using the formation and dissociation equilibrium constants listed in Table 1 [21-23], and assuming the concentration of bromide [Br^-] = 10 mM, we can calculate the mole fraction of [Br_2], [Br_3^-], [HBrO] and [BrO^-] at the electrode surface as a function of pH, as is shown in Fig. 6. The pH of the solution has great influence on the bromine species distribution, which, on the other hand, would have great influence on the kinetics of the reactivity with ammonium ion. Figure 5(B) shows *in-situ* UV/Vis spectra after addition of 10 mM NH_4^+ in the solution. Apparently, the chemical reactivity of these species with ammonium ion was different. The absorption bands at 260 nm and 329 nm for HBrO and BrO^- were disappeared after the addition of NH_4^+, indicating the concentration of these species at the electrode surface were almost reduced to zero due to the reaction with ammonium ion. On the other hand, the addition of ammonium ion didn't influence the UV-Vis absorption spectrum of tribromide ion. It thus suggests that ammonium ion do not react with tribromide ion in acidic range (pH=1), but will reaction with HBrO and BrO^- when these species were predominant components in the solution at pH > 5. Because BrO^- is less electroactive, the optimum pH for electrochemical response of ammonium ion can be 7 where an ECC' electrode mechanism was taken into account.

E reaction: $2Br^- \rightarrow Br_2 + 2e^-$ (1)

C reaction: $Br_2 + H_2O \rightleftarrows HBrO + HBr$ (3)

C' reaction: $HBrO + NH_4^+ \rightarrow NH_2Br + H_3O^+$ (11)

Based on the ECC' mechanism, indirect electrochemical detection techniques with *in-situ* electrogenerated hypobromous acid are proposed for the determination of ammonium ion.

3.4. Indirect electrochemical determination of NH_4^+ at an IDA Pt microelectrode

Since the IDA microelectrodes have various advantages concerning their ability to enhance the current response, and offer higher sensitivity than the macro-electrode, they had been used in a variety of electrochemical applications for development of sensors [24, 25]. In this experiment, one segment of the IDA was used to generate bromine (generator electrode) while another was used as the collector electrode at which bromine is reduced. The voltammograms were recorded by scanning the generator electrode from +0.2 to +1.1 V *vs*. Ag/AgCl and measuring the collector electrode current. Because the collector electrode was polarized by a constant potential at of 0.0 V *vs*. Ag/AgCl, the charging current was negligible. Figure 7 shows the voltammograms of 15 mM KBr at an IDA Pt microelectrode in 0.1 M PBS (pH=7) after successive addition of NH_4^+. The current at generator electrode was decreased with increasing of NH_4^+ concentration. The current decrement at collector electrode (Δi at 1.0 V) shows a linear relationship against NH_4^+ concentration in a range of 0 - 150 μM with a correlation coefficient of 0.991 (inset of Fig. 7).

3.5. Indirect amperometric detection of NH_4^+ in a micro-FIA system

The development of micro flow injection analysis (micro-FIA) system is widely adopted in analytical chemistry due characteristics such as high portability, remote

operation, reduced reagent and sample consumption [26]. In contrast to most optical detection methods, the electrochemical detectors are easily miniaturized without loss in sensitivity because the extremely slow flow rate in micro-FIA system leads to an improvement in the electrolysis efficiency [27]. It was confirmed in our previous study that a ring–disk electrode offered the advantages of achieving much higher collection efficiency due to the low flow rate [17]. In this study, the indirect amperometric detection of NH_4^+ was performed at a carbon film ring–disk electrode (CFRDE) in a micro-FIA system. The disk electrode was held at +1.3 V *vs*. Ag/AgCl to generate the bromine, while the ring electrode was served as the collector electrode which was held at +0.2 V *vs*. Ag/AgCl. In the indirect detection mode, NH_4^+ is quantified based on the decreasing of the reduction current at the collector electrode (in the reduction of hypobromous acid). Both the dynamic linear range and the limit of detection were found to be dependent on the concentration of bromide in the mobile phase. Increasing the KBr concentration would lead to a decrease in the S/N ratio of the signal. Hence, a low concentration of KBr is necessary to obtain a relatively lower detection limit; however, this would lead to a small analytical dynamic range. Conversely, a higher KBr concentration would lead to wide analytical dynamic range, but higher detection limit. The optimum concentration of KBr in this study was determined as 10 mM. It was found that the addition of amount of acetonitrile in mobile phase could improve both the stability of the response signal and the detection limit. Fig. 8 illustrates the FIA signals for responding of NH_4^+ with indirect amperometric detection mode using 10 mM KBr and 10%(v/v) acetonirile in 0.1 M PBS (pH=7) as mobile phase.. The detector exhibited a linear response for NH_4^+ in a concentration range of 0-100 μM with a correlation coefficient of 0.994. The detection limit was estimated as 3 μM based on S/N ≥ 3.

4. Conclusions

The mechanism on the electrochemical oxidation of bromide in the presence of ammonium ion was demonstrated by cyclic voltammetry and *in-situ* UV-Vis spectroscopy. The results indicated that the anodically generated bromine (Br_2) would be hydrolyzed to hypobromous acid (HBrO) in the pH range of 5 - 7 and HBrO was confirmed to be participated in the following homogeneous chemical reaction with the co-existed ammonium ion. The indirect electrochemical method using the reaction of HBrO with ammonium ion was established for determination of ammonium ion at pH 7 with dual-electrode configuration. It was demonstrated that ammonium ion concentrations were proportional to the decreasing of reduction current at collector electrode either in a batch solution with a linear relationship in the range of 5-150 μM (R^2=0.991) or in a micro flow injection analytical (micro-FIA) system with the range of 0 - 100 μM (R^2=0.994). Furthermore, the latter realized the detection limit below 3 μM. The electrochemical indirect detection method shows the advantage of highly selectivity even in presence of a large amount of co-existed cations.

5. Acknowledgements

This work was supported in part by a Grant-in-Aid (No. 18655030) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology in Japan.

References

[1] S. G. Sazhin, E. I. Soborover, and S. V. Tokarev, Russ. J. Nondestructive Testing, 2003, 39, 791.

[2] M. Arauzo, Water Res., **2003**, 37, 1048.

[3] J. Nakajima, Y. Fujimura and Y. Inamori, *Water Sci. Technol*. **1999**, 39, 85.

[4] S.-C. Pai, Y.-J. Tsau and T.-I. Yang, *Anal. Chim. Acta*, **2001**, 434, 209.

[5] W.T. Bolleter, C.J. Bushman, P.W. Tidwell, *Anal. Chem.*,1961, 33, 592.

[6] M.Mori, K.Tanaka, M.I.H. Helaleh, Q.Xu, M.Ikedo, Y. Ogura, S. Sato, W. Wu, K. Hasebe, P.R. Haddad, *J. Chromatogr. A*, 2003,997, 219.

[7] Y. Kitamaki, J. Jin, T. Takeuchi, *J. Chromatography A***, 2003**, 197.

[8] P. Christopher, R. Maria, J. Detlef , K. Jutta, *J. Chromatography A*, **1999**, 850, 239.

[9] S. S. M. Hassan, S. A. Mareib, I. H. Badra, H. A. Aridab, *Anal. Chim. Acta*, **2001**, 427, 21

[10] A.-M. Harbin, C.M.G. van den Berg, *Anal. Chem.*, **1993**, 65, 3411.

[11] X. Ji, R. G. Compton, *Anal. Sci*., **2007**, 23,1317.

[12] G. C. White, *Handbook of Chlorination and Alternative Disinfectants*, 4th edn. Wiley, New York, 1999; 212–287.

[13] J. Tanaka, M. Matsumura, *J. Chem. Technol. Biotechnol.*, **2002**, 77, 649.

[14] X. Hu, N. Takenaka, S. Takasuna, M. Kitano, H. Bandow, Y. Maeda, *Anal. Chem.*, **1993**, 65, 3489.

[15] X. Hu, N. Takenaka, M. Kitano, H. Bandow, Y. Maeda, *Bull. Chem. Soc. Jpn.*, **1996**, 69, 1179.

[16] W. P. King, P. T. Kissinger, *Clin. Chem*, **1980**, 10, 1484.

[17] K. Sato, J. Jin, T. Takeuchi, T. Miwa, Y. Takekoshi, S. Kanno and S. Kawase, *Analyst*, **2000**, 125, 1041.

[18] T.X. Wang, M.D. Kelley, J. N. Cooper, R. C. Beckwith, D. W. Margerum, *Inorg. Chem*, **1994**, 33, 5872.

[19] R. B. Faria, I.R. Epstein, K. Kustin, *J. Phys. Chem*., **1994**, 98, 1363.

[20] R. C. Troy, D. W. Margerum, *Inorg. Chem*, **1991**, 30, 3538.

[21] D.B. Scaife, H.J.V. Tyrrel, *J. Am, Chem. Soc.*,**1958**, 80, 386. (K1)

[22] Z. Toth, I. Fabian, *Inorg. Chem*, **2004**, 43, 2717.(K2)

[23] A. E. Martell, R. M.Smith, *Critical Stability Constants, New York*, Plenum Press, 1976. (R. C. Troy and D. W. Margerum, Inorg. Chem., 1991, 30, 3538) (K3)

[24] M. Morita, O. Niwa, T. Horiuchi, *Electrochim. Acta*, **1997**, 42, 3177.

[25] S. Milardovic, I. Kerekovi´c, R. Derrico, V. Rumenjakb, *Talanta*, **2007**, 71, 213.

[26] J. Ruzicka, E.H. Hansen, *Anal. Chim. Acta*, **1984**, 161, 1
[27] J. Jin, Y. Suzuki, N. Ishikawa, and T. Takeuchi, *Anal. Sci*., **2004**, 20, 205.

Figure Captions

Fig. 1 Schematic diagram of a thin-layer electrochemical flow cell with CFRDE

Fig. 2 Cyclic voltammograms of 10 mM KBr at a glassy carbon electrode in 0.1 M H_2SO_4 (pH=1) and PBSs with the pH values of 5, 7 and 8, respectively. Scan rate: 50 mV/s.

Fig. 3 (A) Dependence of i_{pc}/i_{pa} ratio on the potential scan rate; (B) dependence of reduction potential (E_{pc}) on pH.

Fig. 4 Cyclic voltammograms of 10 mM KBr at a glassy carbon electrode in PBS (pH=7) after addition of NH_4^+ with different concentrations. The dashed line represents the background signal. The expanding of the reduction waves are shown in the inset of the figure.

Fig. 5 (A) *In-situ* UV/Vis spectra of electrogenerated bromide ion solution in 0.1 M H_2SO_4 (pH=1) and PBS (pH=7, 12) and (b) after addition of 10 mM NH_4^+. The concentrations of bromide ion were 10 mM in the solutions of pH 1 and pH 7, but was 40 mM in PBS (pH=12) The solutions at open circuit potential were used as references for the measurements.

Fig. 6 Distribution of the bromine species as a function of pH. $[Br^-]$ = 10 mM.

Fig. 7 Liner sweep voltammograms of 15 mM KBr at an IDA Pt microelectrode in 0.1 M PBS (pH=7) in presence of NH_4^+ with various concentration. Inset: Plots of reduction peak current toward various concentrations of ammonium ion.

Fig. 8 FIA responses of various concentrations of NH_4^+. Mobile phase: 10 mM KBr in 0.1 M PBS (pH=7) - MeCN (v/v=9:1) solution, flow rate: 30 μl / min, E_{disk}: 1.3 V *vs*.Ag/AgCl, E_{ring}: 0.2 V *vs*.Ag/AgCl.

Table 1 Formation and dissociation equilibrium constants

Equilibrium	Const.	ref
$K_1=[Br_3^-] / [Br_2][Br^-]$	$16.9\ M^{-1}$	20
$K_2=[HBrO][Br^-][H^+] / [Br_2]$	$7.2\times10^{-9}\ M^2$	21
$K_3=[BrO^-][H^+] / [\ HBrO]$	$2.3\times10^{-9}\ M$	22

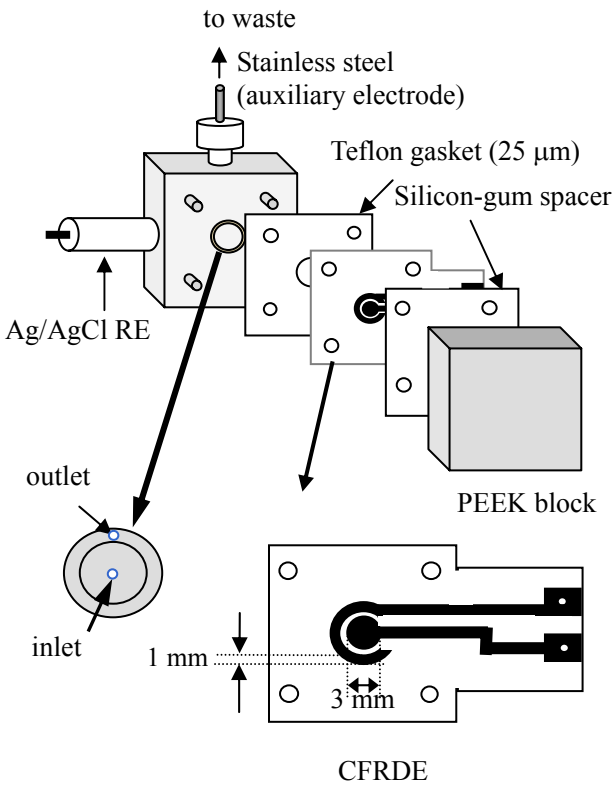
to waste
Stainless steel
(auxiliary electrode)
Teflon gasket (25 μm)
Silicon-gum spacer
Ag/AgCl RE
outlet
PEEK block
inlet
1 mm
3 mm
CFRDE

Fig. 1

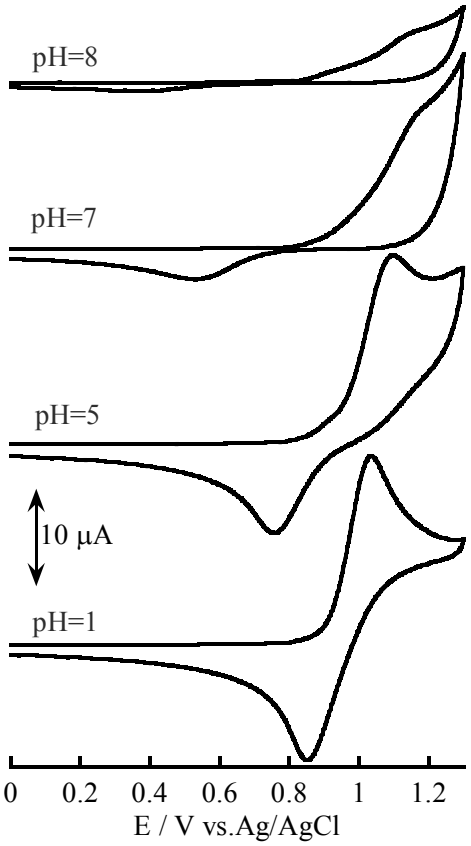

Fig. 2

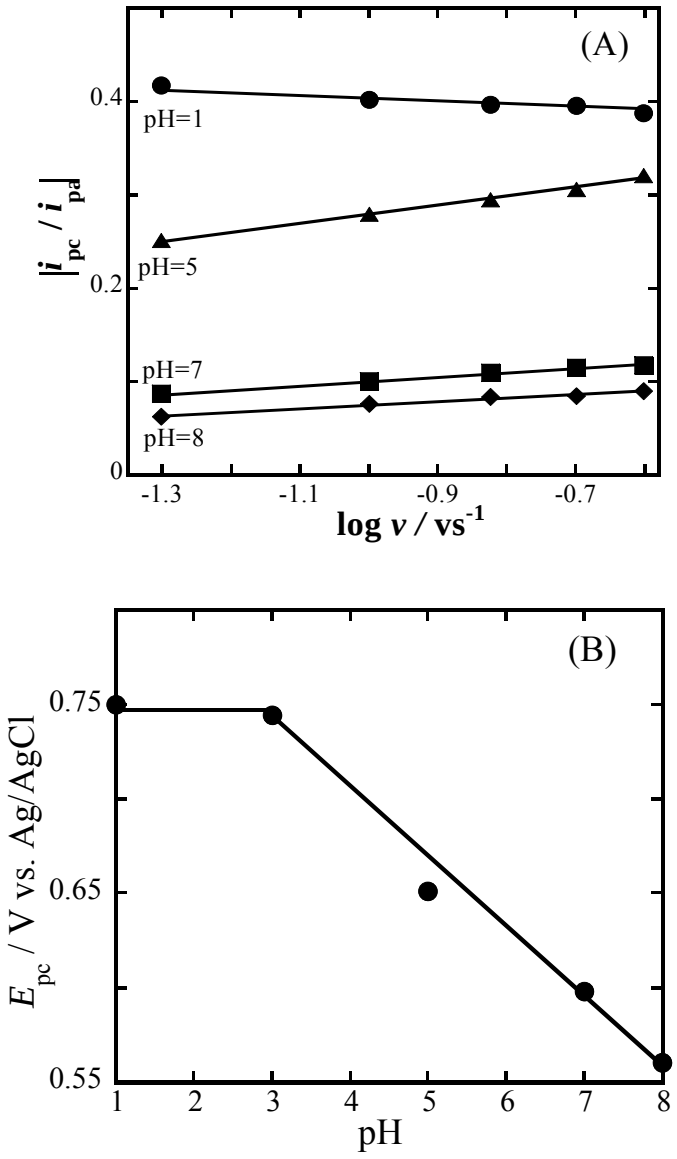
(A)
0.4
0.2
0
pH=1
pH=5
pH=7
pH=8
-1.3
-1.1
-0.9
-0.7
log v / vs-1
|ipc / ipa|
(B)
0.75
0.65
0.55
1
2
3
4
5
6
7
8
pH
Epc / V vs. Ag/AgCl

Fig. 3

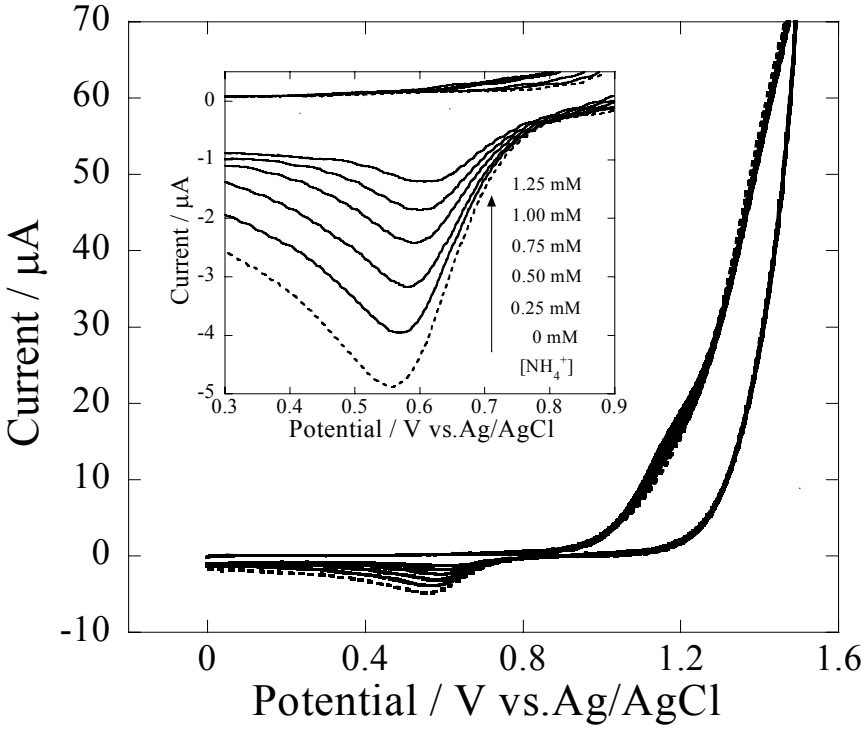
Current / µA
Potential / V vs.Ag/AgCl
70
60
50
40
30
20
10
0
-10
0
0.4
0.8
1.2
1.6
Current / µA
Potential / V vs.Ag/AgCl
0
-1
-2
-3
-4
-5
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.25 mM
1.00 mM
0.75 mM
0.50 mM
0.25 mM
0 mM
[NH4+]

Fig. 4

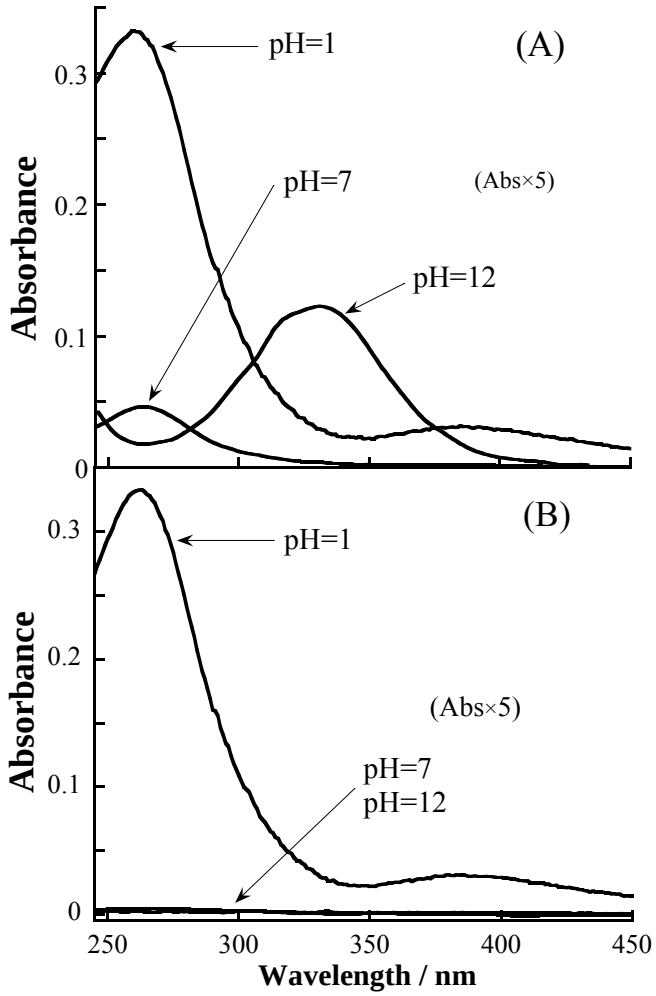
(A)
pH=1
pH=7
(Abs×5)
pH=12
Absorbance
0.3
0.2
0.1
0
(B)
pH=1
(Abs×5)
pH=7
pH=12
Absorbance
0.3
0.2
0.1
0
250
300
350
400
450
Wavelength / nm

Fig. 5

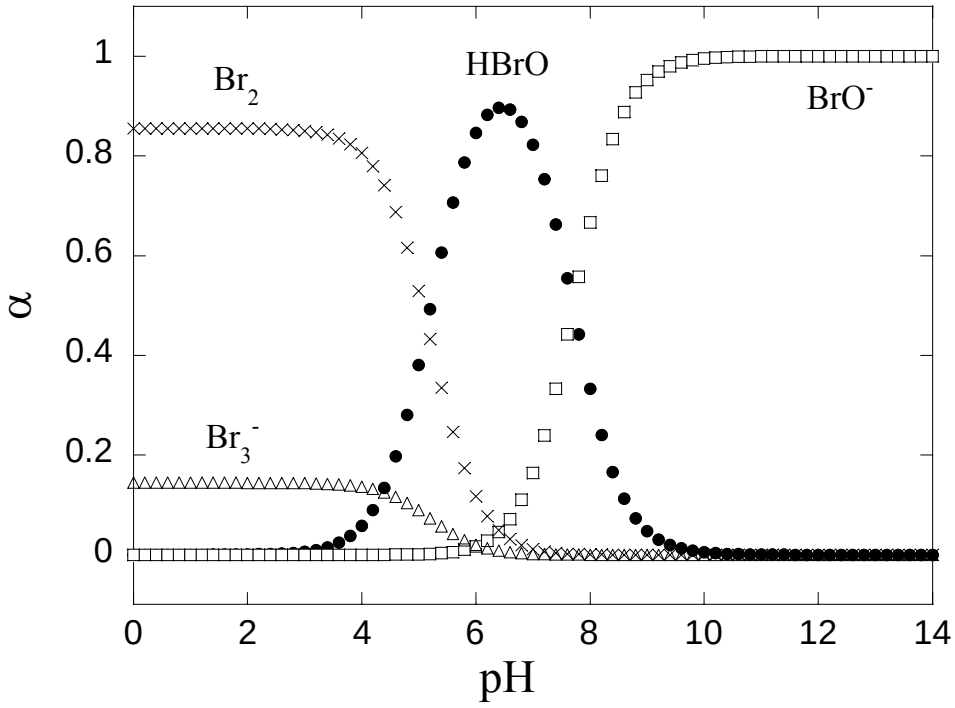

Fig. 6

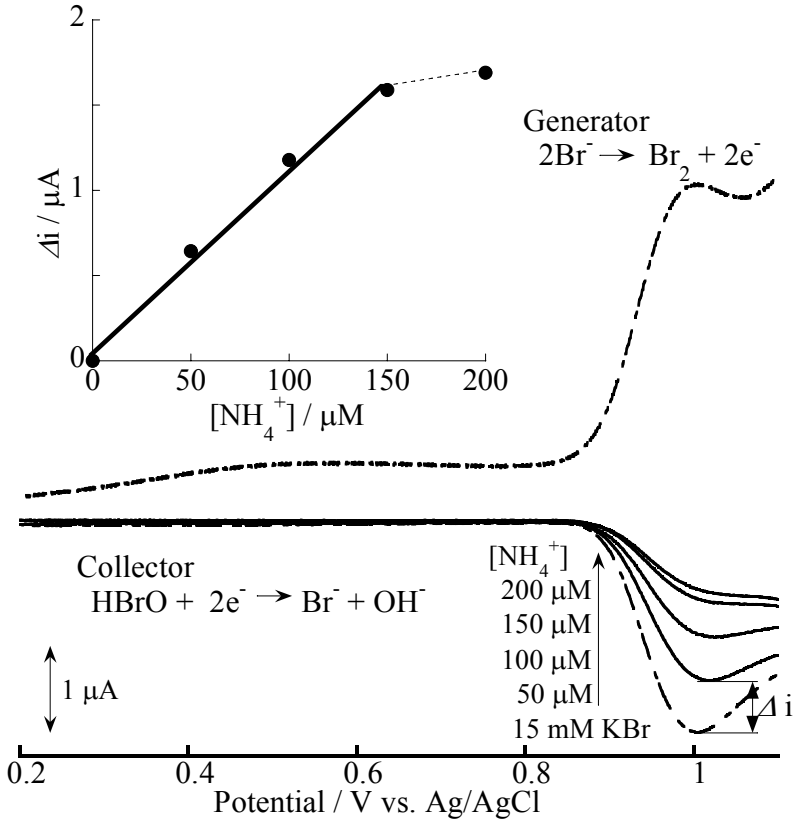
2
1
0
Δi / μA
0
50
100
150
200
[NH4+] / μM
Generator
2Br- → Br2 + 2e-
Collector
HBrO + 2e- → Br- + OH-
1 μA
[NH4+]
200 μM
150 μM
100 μM
50 μM
15 mM KBr
Δ i
0.2
0.4
0.6
0.8
1
Potential / V vs. Ag/AgCl

Fig. 7

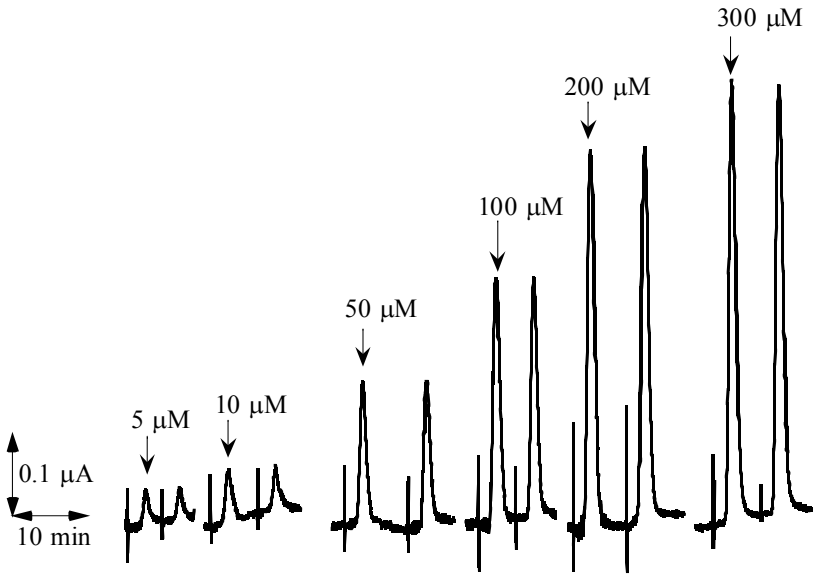

Fig. 8