

Reactive Oxygen in Electrolyzed Anode Water and Anti-Oxidant Activity of Electrolyzed Cathode Water

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ABSTRACT: Hypochlorous acid water prepared from electrolysis of hydrochloride or potassium hypochlorite solution was designated as a specific agricultural chemical in 2014 in Japan. It is considered necessary to investigate the influence of alkali metal ions in the electrolyzed water. Reactive Oxygen in electrolyzed anodic water and anti-oxidant activity of electrolyzed cathode water were investigated. As the cation radius of the alkali ion increased, the amount of active oxygen generated increased. As the cation radius of the alkali ion increased, the anti-oxidant activity of ECW increased. DPPH scavenging activity was stronger than alkali hydroxide of the same pH for all alkali chlorides

KEYWORDS: hypochlorous acid, electrolyzed anodic water, electrolyzed cathode water, alkali chloride

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I. INTRODUCTION

The Hypochlorous acid (HClO) is a powerful oxidizing agent with good bactericidal activity. It reacts with many biological molecules. HClO has advantages over sodium hypochlorite (NaClO) and hydrogen peroxide (H₂O₂) in that within its effective antimicrobial concentration range, it is non-irritating, non-sensitizing, and its cytotoxicity to mammalian cells is low.¹ HClO can be prepared by the following three methods:² (1) hydrolysis of chloride gas, (2) electrolysis of a salt solution, and (3) acidification of hypochlorite. HClO solutions generated through electrolysis of a dilute solution of NaCl are called electrolyzed anodic water (EAW). EAW is used in the medical, dental, food production, and agricultural industries. EAW is advantageous to industry because it allows on-site production of disinfectants, eliminating the need to store or handle chemicals. Electrolyzed water is produced passing a salt solution across a bipolar membrane, resulting in two solutions: an acidic solution that is characterized low pH and a high HClO concentration; the basic solution is composed of a high pH. The bactericidal effects of EAW become maximum between pH4 and 5.³ The bactericidal effects of EAW differ from those of HClO only because substances contained in EAW.⁴ The hydroxyl radical is detected in EAW, and the amount of DMPO-OH spin adduct has not been changed for ca. 10 days.⁵ The anti-oxidant activities of the electrolyzed anodic solution were reported.^{6,7} The radical scavenging activity of beverages or fruits were evaluated by monitoring the 1-diphenyl 1-2-picrylhydrazyl (DPPH) radicals. Hypochlorous acid water prepared from electrolysis of hydrochloride or potassium hypochlorite solution was designated as a specific agricultural chemical in 2014.⁸ Therefore, it is considered necessary to investigate the influence of alkali metal ions in EAW. We reported that the bactericidal effects of the electrolyzed water prepared from various alkali chloride aqueous solutions and found that the bactericidal effects vary depending on alkali metal ion and electrolysis conditions.⁹ Since differences were observed depending on the alkali chloride used for the bactericidal effect test in the samples 24 hours after electrolysis, it was decided to measure the reactive oxygen species in anodic water 24 hours after the electrolysis. The active oxygen scavenging activity of the obtained cathode water was also investigated.

II. MATERIALS AND METHODS

2.1 Reagents and chemicals

Sodium hypochlorite solution (>5.0%, available as chlorine) and potassium hypochlorite solution (5.0–7.0%) were purchased from Kanto Chemical Co. Inc., and used without further purification. Analytical grade sodium chloride (>99.5%), potassium chloride (>99.5%), rubidium chloride (>95.0%), and cesium chloride (>99.0%) were purchased from Wako Pure Chemical Industries, and used without further purification. Sodium hydroxide solution (1 mol/L, for volumetric analysis), 1 mol/L hydrochloric acid (for volumetric analysis), sodium standard solution (Na 1000), potassium standard solution (K 1000), rubidium standard solution (Rb 1000), and cesium standard solution (Cs 1000) were purchased from Wako Pure Chemical Industries, and used without further purification. DMPO (5, 5-dimethyl-1-pyrroline-N-oxide) (>99%) was purchased from LABOTEC DPPH (1,1-Diphenyl-2-picrylhydrazyl) was purchased from TCI, and used without further purification. 4 mol/L Lithium Hydroxide Solution, 1 mol/L Sodium Hydroxide Solution, 1 mol/L Potassium Hydroxide Solution, Rubidium Hydroxide n-Hydrate, Fused Solid, Cesium Hydroxide, 50 % Aqueous Solution were purchased from Wako Pure Chemical Industries, and used without further purification.

2.2 Apparatus

Measurements were made with an ICP-AES (Hitachi High-Tech Science ICP-AES SPS3500, Tokyo Japan), a X-Band ESR (JEOL ESR RE-2X, Tokyo, Japan), a UV-Vis spectrophotometer (JASCO UV/VisV-630, Tokyo, Japan), and Handy pH meter (LACOM Cyber Scan 100, Eutech Instruments, Singapore).

2.3 Measurement of the reactive oxygen species

Flat quartz ESR cells were used in the experiments. The measurement conditions were : field, 336 ± 10 mW width; power, 4 mW; field modulation, 0.200 mT; time constant, 0.1; and amplitude, 500. A manganese signal was used for external standard. 50 μ L of neat DMPO was mixed in 500 μ L of EAW 24 hours after electrolysis. Immediately after mixing, the ESR measurement was done.

2.4 Measurement of the DPPH radical scavenging activity

Flat quartz ESR cells were used in the experiments. The measurement conditions were : field, 336 ± 7.5 mW width; power, 4 mW; field modulation, 0.200 mT; time constant, 0.1; and amplitude, 500. An ethanol solution of a electrolyzed cathode water (ECW) (50~500 μ L) was mixed with 0.02% (0.507 mmol/L) DPPH ethanol solution (1 mL) . Immediately after mixing, the ESR measurement was done.

2.5 Sample preparation

The electrolyzed anodic water (EAW) and electrolyzed cathode water (ECW) were prepared by passing a dilute alkali chloride solution through an electrolytic cell, in which the anode and cathode were separated by a bipolar membrane. A Pt electrode was used for electrolysis. Aqueous HClO solution was prepared by acidification of hypochlorite. The higher the concentration of the electrolyte solution used for the electrolysis, the lower the pH and the higher the hypochlorous concentration. At an electrolyte concentration of 100 mmol/L, the pH and hypochlorous concentration become constant after 30 min of electrolysis. EAW obtained by electrolyzing aqueous solutions of alkali chloride of 100 mmol/L for 60 min and EAW obtained by electrolyzing alkali chloride solutions of 10 mmol/L for 10 min were used. The analytical values and physical data for the samples used in this study are shown in Table 1(EAW) and Table 2 (ECW).

Table 1. Analytical results and physical data for electrolyzed anodic water

Sample (reagent)		Electrolytic condition	pH	ORP ¹⁾ (mV)	HClO + ClO ⁻ (mmol/L)	HClO (mmol/L)	ClO ⁻ (mmol/L)	metal (mmol/L)
Electrolyzed water	LiCl	A	2.4	358.2	0.19	0.17	0.02	6.7
	NaCl		2.4	362.2	0.24	0.21	0.02	6.1
	KCl		2.4	364.1	0.36	0.33	0.03	5.6
	RbCl		2.4	366.9	0.51	0.47	0.04	6.4
	CsCl		2.4	372.7	0.55	0.51	0.04	6.5
Electrolyzed water	LiCl	B	1.6	394.9	12.1	11.5	0.6	11.7
	NaCl		1.6	395.1	13.3	12.3	0.9	9.5
	KCl		1.6	398.1	17.8	16.7	1.1	6.3
	RbCl		1.6	404.6	19.2	17.7	1.5	5.3

	CsCl		1.6	410.2	23.4	21.8	1.6	4.2
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1) Oxidation Reduction Potential

electrolytic condition A: alkali-chloride concentration of 10 mmol/L and electrolytic time of 10 min

electrolytic condition B: alkali-chloride concentration of 100 mmol/L and electrolytic time of 60 min

Table 2. Analytical results and physical data for electrolyzed cathode water and hydroxide solution

Sample (reagent)		Preparation condition	pH	ORP (mV)	HClO + ClO ⁻ (mmol/L)	Alkali metal (mmol/L)
Electrolyzed water	LiCl	A	11.6	-356.8	< 0.001	11.3
	NaCl		11.7	-358.2	< 0.001	12.2
	KCl		11.9	-370.6	< 0.001	14.6
	RbCl		12.0	-372.8	< 0.001	18.8
	CsCl		12.1	-376.3	< 0.001	19.9
Electrolyzed water	LiCl	B	13.5	-391.1	0.13	151.0
	NaCl		13.5	-393.1	0.15	177.3
	KCl		14.0	-432.7	0.14	186.7
	RbCl		13.9	-436.0	0.16	161.9
	CsCl		14.0	-432.0	0.16	190.8
Hydroxide solution	LiOH	C	11.7	-383.1	-	11.3
	NaOH		12.1	-371.2	-	12.2
	KOH		12.3	-378.3	-	14.6
	RbOH		12.7	-401.0	-	18.8
	CsOH		12.5	-414.0	-	19.9

Preparation condition A: alkali-chloride concentration of 10 mmol/L and electrolytic time of 10 min

Preparation condition B: alkali-chloride concentration of 100 mmol/L and electrolytic time of 60 min

Preparation condition C: dilution of aqueous solution of alkali hydroxide

III. RESULTS AND DISCUSSIONS

3.1 Reactive Oxygen in Electrolyzed Anode Water

The ESR measurement using the DMPO spin trap method was carried out. The results are shown in Fig. 1. Superoxide was observed in EAW, 100 mmol/L RbCl and CsCl for 60 min. Singlet oxygen is considered to be involved in the hypochlorous acid water. Fig.2 is a plot of the ratio of highest ESR signal intensity of DMPO-OH or DMPO-OOH to ESR signal intensity of Mn²⁺ against the cationic radius of alkali chloride used as electrolytic aid. As the cation radius of the alkali ion increased, the amount of active oxygen generated increased. In all alkali chlorides, the concentration of active oxygen was EAW, 100 mmol/L for 60 min > EAW, 10 mmol/L for 10 min. The paper on the bactericidal effect and the generation of the reactive oxygen species is mostly reported on electrolyzed water produced from aqueous NaCl solutions, and there is little consideration on the difference of alkali chloride. In our study using *E. coli*, *S. aureus*, *P. aeruginosa*, we reported that the bactericidal effect of the electrolyzed water produced from the KCl aqueous solution is better than the electrolyzed water produced from the aqueous NaCl solution in order to exhibit the bactericidal effect with less chlorine amount¹⁰. It can be considered that this result is due to reactive oxygen species generated by electrolysis or decomposition of hypochlorous acid.

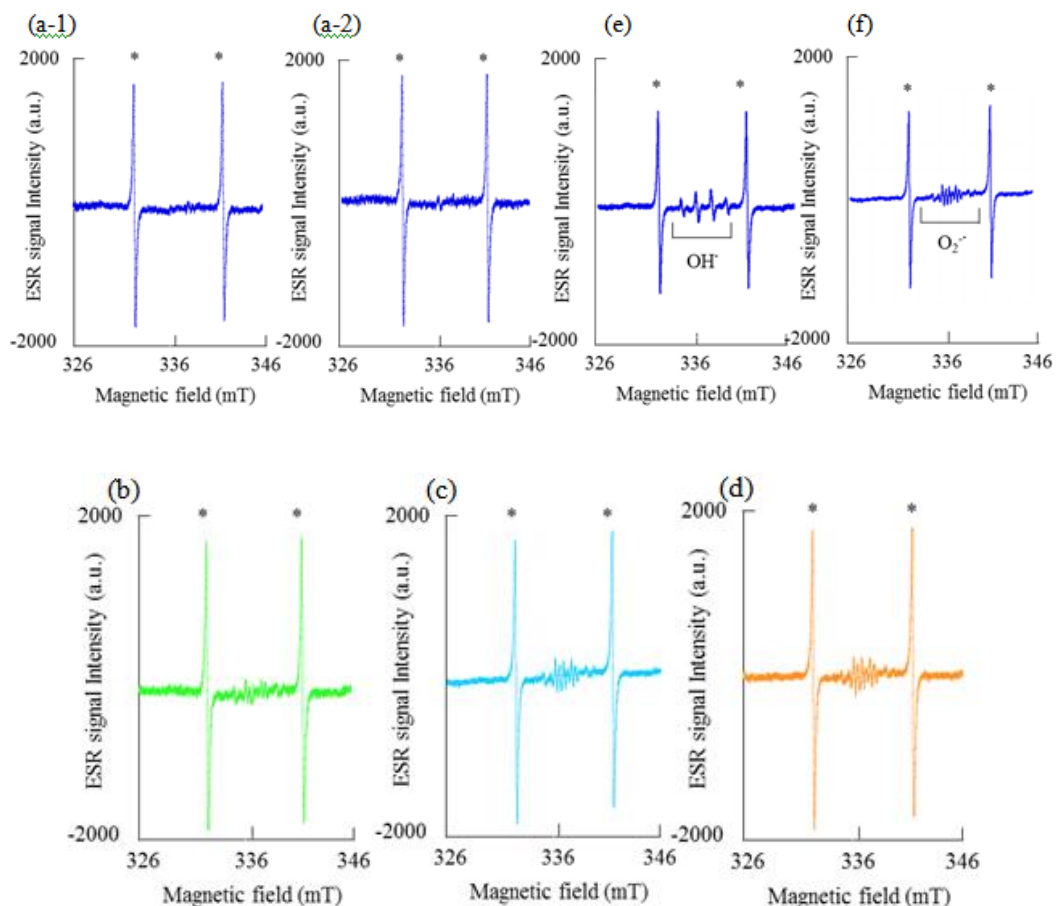


Fig. 1 ESR spectra in the presence of DMPO 24 h after preparation.

- (a-1) Electrolyzed anode water (EAW) prepared from electrolysis of 10 mmol/L NaCl solution for 10 min,
 - (a-2) EAW from electrolysis of 100 mmol/L NaCl solution for 60 min,
 - (b) EAW prepared from electrolysis of 100 mmol/L KCl solution for 60 min,
 - (c) EAW prepared from electrolysis of 10 mmol/L RbCl solution for 60 min,
 - (d) EAW prepared from electrolysis of 100 mmol/L CsCl solution for 60 min,
 - (e) hydroxyl radical (OH[•]), and
 - (f) superoxide (O₂^{•-}).
- *: Mn²⁺.

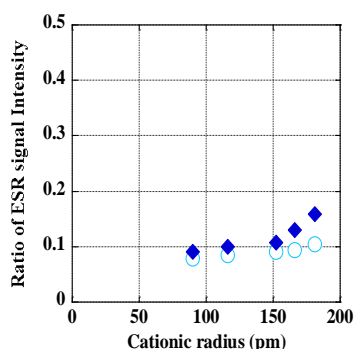


Fig. 2 Ratio of ESR signal intensity of DMPO-OH or DMPO-OOH to the signal of Mn²⁺ as the external standard.

- : EAW prepared from electrolysis of 10 mmol/L alkali chloride solution for 10 min
- ◆: EAW prepared from electrolysis of 100 mmol/L alkali chloride solution for 60 min

3.2 Anti-oxidant Activity of Electrolyzed Cathode Water

The anti-oxidant activities of the electrolyzed anodic solution prepared from NaCl aqueous solution were reported.^{6,7} In all alkali chlorides, the concentration of active oxygen was EAW, 100 mmol/L for 60 min > EAW, 10 mmol/L for 10 min. Therefore, the anti-oxidant activity of ECW, 100mmol/L for 60min was picked up. Fig.3 shows the ESR spectra of 1 mL of DPPH with 0.01 mL of electrolyzed water prepared from 100 mmol/L alkali chloride soln. for 60 min. As electrolyzed water was added, the ESR signal intensity of DPPH decreased and the rate of decrease became larger as the atomic number of alkali metal in the alkali chloride aqueous solution increased. Fig. 4 shows the ratio of ESR signal intensity of DPPH added with hydroxide solution and electrolyzed water to the intensity before addition. As the cation radius of the alkali ion increased, the ESR signal intensity of DPPH decreased. With the same addition volume, ECW has strong DPPH scavenging activity than alkali hydroxide. Regarding Na, it is reported that ECW exhibits superoxide inactivating activity from aqueous NaOH solution at same pH⁽¹⁾. Our results show that DPPH scavenging activity is stronger than alkali hydroxide of the same pH for all alkali chlorides, and it is considered to be a result showing one characteristic of electrolyzed water. It has also been reported that ECW produced from the NaCl aqueous solution has lower superoxide inactivating activity than ECW produced from the KCl aqueous solution⁽¹⁾. Our results are not inconsistent with this fact.

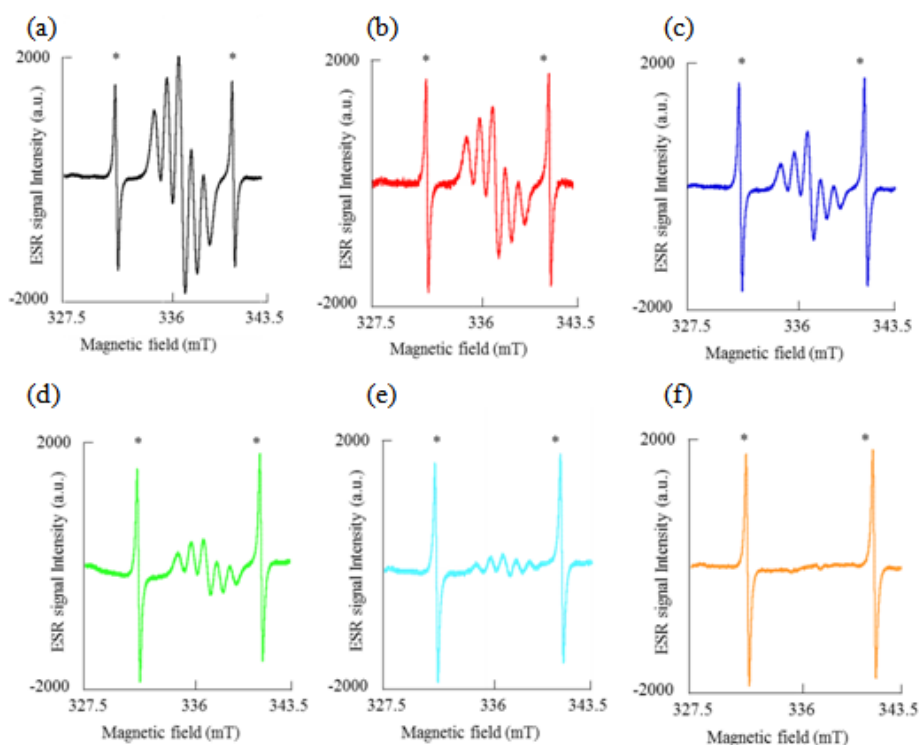


Fig.3 ESR spectra of DPPH (1 mL) with electrolyzed water (0.01 mL) prepared from electrolysis of 100 mmol/L alkali chloride solution for 60 min.

Alkali chloride: (a) without electrolyzed water, (b) LiCl, (c) NaCl, (d) KCl, (e) RbCl, and (f) CsCl.

*: Mn²⁺

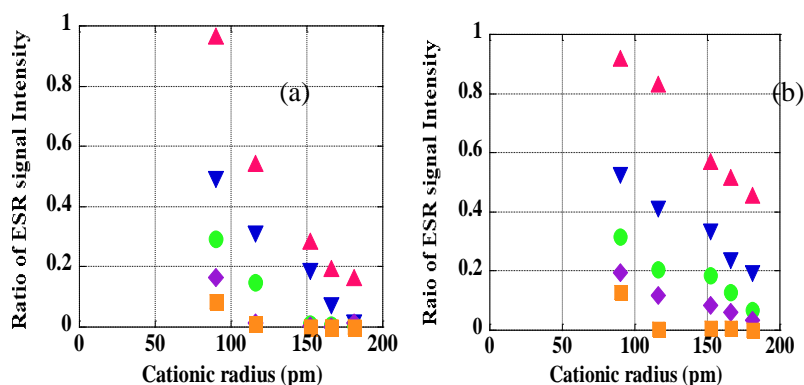


Fig. 4 Ratio of ESR signal Intensity of DPPH added with hydroxide solution and electrolyzed water to the intensity before addition.

- (a) Electrolyzed water prepared from electrolysis of 100 mmol/L alkali chloride solution for 60 min, and
(b) alkali hydroxide solution.

Added volume: ▲ 5 μ L, ▼ 10 μ L, ● 15 μ L, ◆ 20 μ L, ■ 30 μ L,

IV. CONCLUSION

Reactive Oxygen in EAW and anti-oxidant activity of ECW were investigated. As the cation radius of the alkali ion increased, the amount of active oxygen generated increased. As the cation radius of the alkali ion increased, the anti-oxidant activity of ECW increased. DPPH scavenging activity was stronger than alkali hydroxide of the same pH for all alkali chlorides.

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