

# New Electrochemical Evaluation of the Antioxidant Capacity of Beverages with Polyoxometalates as Redox Probes

Tadaharu UEDA,<sup>\*†</sup> Takashi OKUMURA,<sup>\*</sup> Yukino TANAKA,<sup>\*</sup> Saki AKASE,<sup>\*</sup> Tomoko SHIMAMURA,<sup>\*\*</sup> and Hiroyuki UKEDA<sup>\*\*</sup>

<sup>\*</sup>Department of Applied Chemistry, Faculty of Science, Kochi University, Kochi 780-8520, Japan

<sup>\*\*</sup>Department of Agriculture, Faculty of Agriculture, Kochi University, Kochi 783-8502, Japan

A new method was developed to evaluate antioxidant activity based on the redox properties of polyoxometalates, which are partially reduced by antioxidants to generate a limiting potential. The polyoxometalates [PMo<sub>12</sub>O<sub>40</sub>]<sup>3-</sup>, [PVW<sub>11</sub>O<sub>40</sub>]<sup>4-</sup> and [SV<sub>2</sub>W<sub>10</sub>O<sub>40</sub>]<sup>4-</sup> formed *in situ* were used as electrochemical probes for the new evaluation method, and their formation conditions were optimized to evaluate the antioxidant activities of gallic acid, ellagic acid, catechin, quercetin, morin, *trans*-ferulic acid, sesamol,  $\alpha$ -tocopherol,  $\delta$ -tocopherol and L-ascorbic acid. The observed difference between initial potential and limiting potential ( $\Delta E$ ) were compared with spectrophotometrically evaluated antioxidant activities. In addition, the antioxidant capacities of five beverages (Japanese green tea, concentrated catechin-containing green tea, grapefruit juice, red wine and Japanese sake) were evaluated.

**Keywords** Antioxidant, activity, capacity, potentiometry, polyoxometalate

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## Introduction

Many people live under oxidative stress, defined as the imbalance between reactive oxygen and nitrogen species, and antioxidant defence. To decrease the risk of developing pathology from oxidative stress, extensive attention has been devoted to the intake of dietary antioxidants, such as polyphenolic compounds, vitamins, and carotenoids from natural products.<sup>1,2</sup> Thus, many researchers and other parties are interested in understanding the antioxidant capacities of foods. Many methods have been developed to evaluate the antioxidant capacities of foods and beverages; these methods are mostly based on spectrophotometric measurements such as UV-Vis spectrophotometry and fluorescence spectrophotometry, and radical-trapping mechanisms of antioxidants have been investigated.<sup>3-10</sup> Most evaluation methods are categorized into three classes on the basis of the reaction of reagents with antioxidants: hydrogen-atom transfer reactions, electron-transfer reactions, and others. In particular, the ORAC and DPPH methods have been widely used to evaluate the antioxidant capacities of foods and beverages, although there is a still-unsolved problem given that the antioxidant capacities obtained from different methods do not match each other.<sup>3,4</sup> However, spectrophotometric measurements require expensive instruments and skills to obtain highly accurate and precise results; in addition, serious errors could arise if a colored and/or muddy sample were to be evaluated, which presents a disadvantage. In contrast, electrochemical measurements do not require expensive instruments, and can be performed on colored and/or muddy

samples. However, there have been few reports on electrochemical evaluation methods for antioxidant activities and capacities. If a selective and sensitive electrode were to be developed to evaluate antioxidant capacity, people could measure antioxidant capacity with simple and easy procedures, similar to using a pH meter. An appropriate probe is needed to enable potentiometric evaluations of the activities of antioxidants, that exhibit different redox potentials.

Polyoxometalates (POMs) are a class of inorganic clusters that consist of addenda ions, such as Mo(VI) and W(VI), and hetero ions, such as P(V) and Si(IV). This class of cluster has been extensively investigated for a long time because it includes certain compounds that are very interesting both fundamentally and in terms of potential applications.<sup>11-14</sup> The Keggin-type, [XM<sub>12</sub>O<sub>40</sub>]<sup>n-</sup> (Fig. S1), and the Wells-Dawson-type, [X<sub>2</sub>M<sub>18</sub>O<sub>62</sub>]<sup>n-</sup>, POMs are typical structures. Recently, numerous large POMs have been prepared and characterized. In addition, catalysis studies with POMs have been widespread because POMs exhibit strong acidity, high stability and low corrosiveness.<sup>15-23</sup> POMs exhibit various chemical properties depending on their components and structures. In particular, the electrochemistry of POMs is rich because many POMs, such as Keggin-type POMs, promote multi-step and multi-electron transfer at different redox potentials.<sup>24</sup> In addition, reduced POMs, especially molybdenum-based POMs, produce intense blue colors. In the Folin-Ciocalteu method, [PMoW<sub>11</sub>O<sub>40</sub>]<sup>3-</sup> is reduced by antioxidants to form a blue-colored species, and the antioxidant capacity can be evaluated spectrophotometrically by measuring the absorbance of the reduced species.<sup>25-28</sup> Recently, we investigated the detailed redox mechanisms of vanadium-substituted POMs in CH<sub>3</sub>CN.<sup>29,30</sup> In general, the redox potentials of POMs are more positive than those of many metal complexes, and POMs are very redox-stable. This property implies that

<sup>†</sup> To whom correspondence should be addressed.  
E-mail: chuji@kochi-u.ac.jp

POMs can be used as sensitive probes to evaluate antioxidant activity *via* electrochemical methods because antioxidants, depending on their activity, can reduce some parts of POMs. In addition, the redox potentials of molybdenum-POMs are more positive than those of corresponding tungsten-POMs, and vanadium components incorporated in POMs can be reduced at a more positive potential than molybdenum and tungsten framework parts. In the present study, we developed a new electrochemical method to evaluate the antioxidant capacities of foods and beverages on the basis of the redox properties of POMs. Three POMs,  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$ ,  $[\text{PVW}_{11}\text{O}_{40}]^{4-}$  and  $[\text{SV}_2\text{W}_{10}\text{O}_{40}]^{4-}$ , were selected for study as redox probes due to their redox potentials, stability and easy preparation based on previous work.

## Experimental

Voltammetric measurements were carried out at  $25 \pm 1^\circ\text{C}$  (298.2 K) with a BAS 50W apparatus and a BAS RRDE-3 rotating-disk system. A standard three-electrode electrochemical cell arrangement was employed. A glassy carbon macrodisk with a surface area of  $0.071\text{ cm}^2$ , platinum wire, and a Ag/AgCl electrode were used as the working electrode, counter electrode, and reference electrode, respectively. The open-circuit potential and limiting potential were measured using a Hokuto-Denko HA-501 potentiostat. Before each measurement, the glassy carbon electrode (GCE) was polished with an aqueous  $0.1\ \mu\text{m}$  diamond slurry and washed with distilled water. The solutions used in the electrochemical experiments were purged with argon gas to remove dissolved oxygen, whereas the solution used for measuring the equilibrium potentials was not purged. The limiting potential measurements were conducted at least three times, and the average of the results was calculated. The oxygen radical absorbance capacity (ORAC) values of the antioxidants were measured according to a method of Watanabe *et al.*<sup>31-33</sup> The ORAC value was expressed as the Trolox equivalent ( $\mu\text{mol TE/g}$ ). The Trolox equivalent antioxidant capacity (TEAC) values of the antioxidants were measured according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method reported recently.<sup>34</sup>

Stock solutions of Mo(VI), W(V) and P(V) were prepared by dissolving  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  in water, respectively. The V(V) stock solution was prepared by mixing  $\text{V}_2\text{O}_5$  in NaOH solution in a teflon beaker. The POMs, specifically  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$  ( $\text{PMo}_{12}$ ),  $[\text{PVW}_{11}\text{O}_{40}]^{4-}$  ( $\text{PVW}_{11}$ ) and  $[\text{SV}_2\text{W}_{10}\text{O}_{40}]^{4-}$  ( $\text{SV}_2\text{W}_{10}$ ), used as redox probes for the electrochemical evaluation of antioxidant activity were prepared as follows.  $\text{PMo}_{12}$  formed after a solution of 10 mM Mo(VI), 1 mM P(V), 0.1 M HCl, and 50% (v/v) EtOH (70% (v/v) EtOH or 50 or 70% (v/v) MeOH for the optimization of measurement conditions) was allowed to stand for 1 day at room temperature to complete the conversion reaction of the  $\alpha$ -form of Keggin-type  $\text{PMo}_{12}$  to the  $\beta$ -form.<sup>35</sup>  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$  formed after solutions of 10 mM W(VI), 1 mM P(V), 1 mM V(V), 0.1 M HCl, and 50% (v/v) EtOH and 10 mM W(VI), 1 mM V(V), 0.1 M  $\text{H}_2\text{SO}_4$ , and 50% (v/v) EtOH, respectively, were heated at  $70^\circ\text{C}$  for 1 day. All of the antioxidants, which were of analytical grade, were purchased from Wako and were used as received. Stock solutions (0.10 M) of antioxidants were prepared to use for potential measurements, and were kept refrigerated after purging oxygen with argon gas so as to avoid their oxidation. Each stock solution was used within 1 month. Only the stock solution of ascorbic acid was prepared fresh for every measurement.

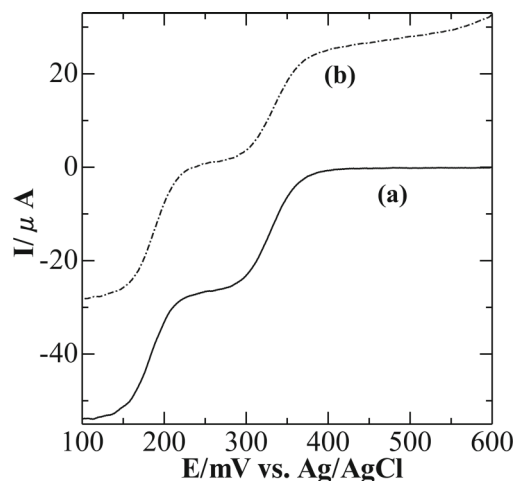
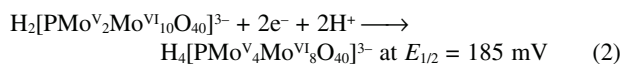
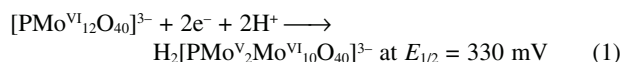


Fig. 1 Hydrodynamic voltammograms of  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$  that form *in situ* in a solution of 10 mM Mo(VI), 1 mM P(V), 0.1 M HCl, and 50% (v/v) EtOH in the absence (a) and in the presence (b) of 10 mM ascorbic acid. The rotation rate of the working electrode was set at 1000 rpm.

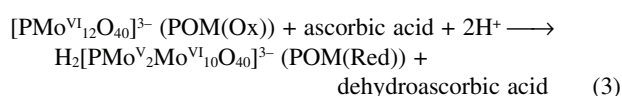
At first, the initial resting equilibrium potentials were measured for the POM solutions (10 ml) prepared as described above (*ca.* 380, 520 and 580 mV for  $\text{PMo}_{12}$ ,  $\text{PVW}_{11}$  and  $\text{S}_2\text{VW}_{10}$ , respectively). Potential was measured every 30 s after the addition of antioxidants or beverages (0.1 ml) to obtain potential shifts ( $\Delta E$ ). During measurements, the temperature of the solutions was kept at  $25 \pm 0.1^\circ\text{C}$ .

## Results and Discussion

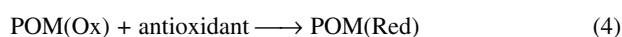
The voltammetric behaviors of the POMs under conditions related to the evaluation of antioxidant activity were investigated in a solution containing 10 mM Mo(VI), 1 mM P(V), 0.1 M HCl, and 50% (v/v) EtOH, in which the formation of  $\text{PMo}_{12}$  occurs. Two two-electron transfer reduction waves were obtained at  $E_{1/2} = 330$  and 185 mV; these waves are attributed to the reduction of Mo(VI) to Mo(V) at two different positions in the  $\text{PMo}_{12}$  molecule (Fig. 1(a)).



After 10 mM ascorbic acid was added, the voltammograms changed as shown in Fig. 1(b) because ascorbic acid chemically reduced some of the molybdenum from +VI to +V in  $\text{PMo}_{12}$ .



In general, the chemical equation for POMs and antioxidants is as follows:



After this reaction, the open-circuit potential or the limiting

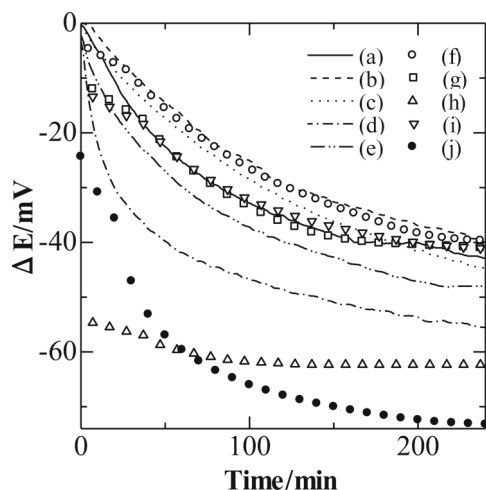


Fig. 2 Time dependence of the potential for  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$  (10 mM Mo(VI), 1 mM P(V), and 0.1 M HCl-50% (v/v) EtOH) formed *in situ* in the presence of 0.1 mM antioxidants: (a) gallic acid; (b) sesamol; (c) catechin; (d) quercetin; (e) morin; (f) *trans*-ferulic acid; (g) ellagic acid; (h)  $\alpha$ -tocopherol; (i)  $\delta$ -tocopherol; (j) L-ascorbic acid.

potential is expressed as

$$E = E^{\circ} + \frac{0.059}{2} \log \frac{[\text{POM}(\text{Ox})]}{[\text{POM}(\text{Red})]} \quad (5)$$

If  $[\text{POM}(\text{Ox})] > [\text{antioxidant}]$ ,  $E$  should be almost proportional to  $-\log[\text{POM}(\text{Red})]$  or  $-\log[\text{antioxidant}]$  for each of antioxidants. However, if an antioxidant is weak in antioxidant activity, it is noted that  $E$  exhibits a mixed potential between the POM and the antioxidant, itself.

The open-circuit potential, or the equilibrium potential, changes simultaneously, implying that the potential would change in proportion to the antioxidant activity, although the slope of  $E$  would be different depending on the strength of the antioxidant activity. Vanadium-substituted Keggin-type POMs,  $[\text{XV}_x\text{M}_{12-x}\text{O}_{40}]^{n-}$  ( $X = \text{Si, Ge, P, As, S}$ ;  $M = \text{Mo, W}$ ), exhibit higher redox potentials than the corresponding parent non-substituted POMs,  $[\text{XM}_{12}\text{O}_{40}]^{n-}$ .<sup>29,30</sup> In addition, heteropolytungstates are generally more rigid than heteropolymolybdates for hydrolysis. These electrochemical and chemical properties imply that these compounds would react smoothly and stably with antioxidants. The cyclic voltammograms of  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$  formed *in situ* are displayed in Fig. S2.  $[\text{SVW}_{11}\text{O}_{40}]^{3-}$ , which occurs in the solution, was not used in the present study because it does not form directly from heating of the  $\text{W}(\text{VI})$ - $\text{V}(\text{V})$ - $\text{H}_2\text{SO}_4$ -EtOH solution.<sup>36</sup> To attain the limiting potential, some conditions for the electrochemical measurements were optimized as described below.

The potentials of  $\text{PMo}_{12}$ ,  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$  were measured as functions of the elapsed time at 25°C (Figs. 2 and S3) in the presence of various antioxidants, including L-ascorbic acid, gallic acid, ellagic acid, catechin, quercetin, morin, *trans*-ferulic acid, sesamol,  $\alpha$ -tocopherol, and  $\delta$ -tocopherol (Fig. S4). Irrespective of the antioxidant added, the potentials of the POMs decreased, although the rate of the potential decrease differed. When  $\text{PMo}_{12}$  was used, the equilibrium potentials became constant 4 h after the antioxidants were added. In the cases of  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$ , the equilibrium potentials were shifted to the negative-potential side until approximately 100 and 50 min, respectively, after the antioxidants were added, indicating that

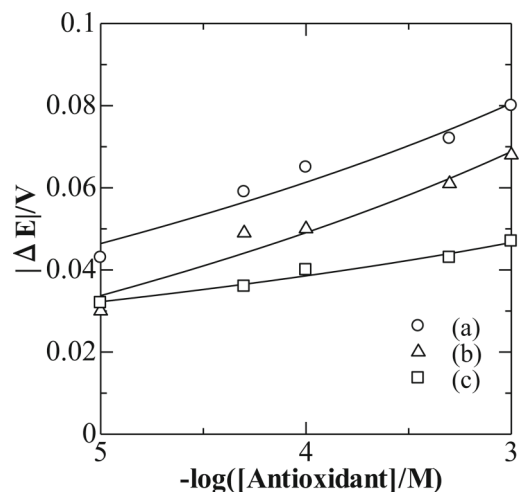


Fig. 3 Limiting potentials of  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$  formed *in situ* in the presence of various concentrations of (a)  $\alpha$ -tocopherol, (b) morin and (c) *trans*-ferulic acid.

the electrochemical evaluation of the antioxidant activity was completed more rapidly than when  $\text{PMo}_{12}$  was used. However, in the cases of  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$ , the equilibrium potential gradually shifted back to the positive-potential side approximately 80 min after  $\alpha$ -tocopherol and L-ascorbic acid were added. The reduced  $\text{SV}_2\text{W}_{10}$  would likely be re-oxidized by air or converted into other species, resulting in a positive equilibrium potential shift, although the details of this mechanism are, unfortunately, not yet clear. The detailed mechanism or phenomenon by which POMs react with antioxidants should be investigated eventually. We have focused on only developing the potentiometric analysis in the present study.

To optimize the concentration of antioxidants evaluated for this system, the limiting potentials were investigated in the presence of designated concentrations (0.01 – 1 mM) of antioxidants (Figs. 3 and S5). The limiting potentials decreased depending on the logarithm of the concentration of antioxidants, as expected in Eq. (5). Since antioxidants possess specific reducing powers, both the value of  $\Delta E$  and the slope of  $\Delta E$  vs.  $-\log[\text{antioxidant}]$  depend on the antioxidant activities. An appropriate antioxidant concentration for investigating limiting potentials under various conditions is 0.1 mM. The effect of the antioxidant concentration on the electrochemical evaluation of the antioxidant activity was also investigated for  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$  using the same procedures used for  $\text{PMo}_{12}$ . Two different slopes were obtained at a boundary of 0.1 mM from plots of  $E$  against the antioxidant concentrations, which were the same results obtained using  $\text{PMo}_{12}$ . On the basis of this result, 0.1 mM was the most appropriate concentration for the system surveyed in the present study because the purpose of this study was to potentiometrically evaluate the antioxidant capacities of beverages containing various antioxidants whose contents and their concentrations are unknown.

$\text{PMo}_{12}$  forms most stably in the presence of water-miscible organic solvents, although it also forms with  $[\text{PMo}_9\text{O}_{34}\text{H}_6]^{3-}$  in aqueous solutions.<sup>37</sup> The solubility of many antioxidants is very low in aqueous solutions, resulting in a negative error in the potentiometric evaluation of antioxidant activity in an aqueous solution. However, the evaluated antioxidant activity would be lower than expected in the presence of high concentrations of organic solvents. Among various water-miscible organic

Table 1 Differences ( $\Delta E/V$ ) between the initial potentials and limiting potentials of polyoxometalates in the presence of 0.1 mM antioxidants

Antioxidant	Condition					
	A		B		C	
	EtOH 50	70	MeOH 50	70	EtOH 50	MeOH 50
Gallic acid	0.041 (0.43)	0.022	0.046	0.056	0.035 (0.13)	0.056 (0.24)
Sesamol	0.037 (0.37)	0.038	0.025	0.068	0.068 (0.37)	0.061 (0.44)
Catechin	0.042 (0.40)	0.015	0.042	0.034	0.020 (0.21)	0.047 (0.57)
<i>trans</i> -Ferulic acid	0.040 (0.26)	0.020	0.051	0.037	0.046 (0.17)	0.009 (0.24)
Quercetin	0.053 (0.45)	0.040	0.061	0.067	0.078 (0.37)	0.090 (0.34)
Morin	0.048 (0.13)	0.018	0.068	0.077	0.075 (0.59)	0.096 (0.13)
Ellagic acid	0.040 (0.24)	0.025	0.041	0.016	0.009 (0.29)	0.021 (0.60)
$\alpha$ -Tocopherol	0.060 (0.24)	0.078	0.060	0.084	0.143 (0.47)	0.186 (1.5)
$\delta$ -Tocopherol	0.042 (0.37)	0.018	0.035	0.030	0.094 (1.3)	0.098 (0.27)
L-Ascorbic acid	0.073 (0.78)	0.071	0.112	0.105	0.181 (0.26)	0.214 (0.13)

The numbers in parenthesis indicate relative standard deviations (%). A: 10 mM Mo(VI), 1 mM P(V), 0.1 M HCl, and 50 or 70% (v/v) EtOH or MeOH. The solution was allowed to stand at room temperature for 1 day.  $\text{PMo}_{12}$  occurs as the main species in this solution. B: 10 mM W(VI), 1 mM P(V), 1 mM V(V), 0.1 M HCl, and 50% (v/v) EtOH. The solution was heated at 70°C for 1 day and then cooled to room temperature to use for evaluation.  $\text{PVW}_{11}$  occurs as the main species in this solution. C: 10 mM W(VI), 1 mM V(V), 0.1 M  $\text{H}_2\text{SO}_4$ , and 50% (v/v) EtOH. The solution was heated at 70°C for 1 day and then cooled to room temperature to use for evaluation.  $\text{SV}_2\text{W}_{10}$  occurs as the main species in this solution.

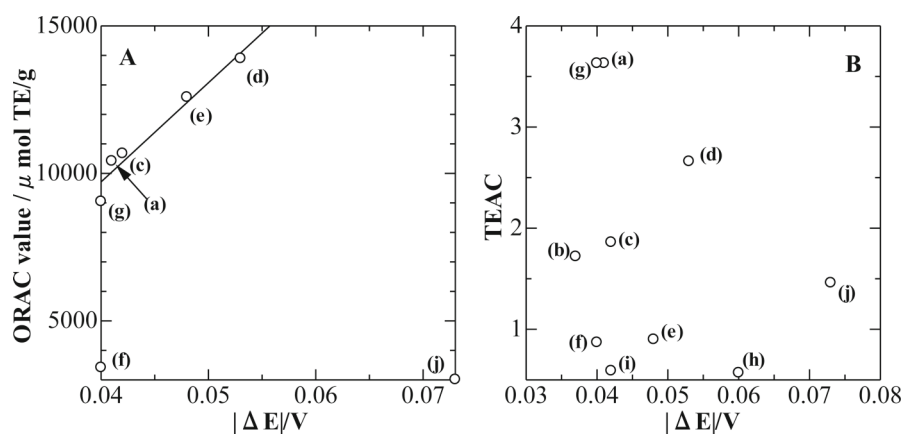


Fig. 4 Comparison of the differences between the initial potential and the limiting potential of  $[\text{PMo}_{12}\text{O}_{40}]^{3-}$  formed *in situ* with the ORAC value (A) and the TEAC value (B) obtained *via* the DPPH method. Antioxidants: (a) gallic acid; (b) sesamol; (c) catechin; (d) quercetin; (e) morin; (f) *trans*-ferulic acid; (g) ellagic acid; (h)  $\alpha$ -tocopherol; (i)  $\delta$ -tocopherol; (j) L-ascorbic acid.

solvents, methanol and ethanol were selected to determine the most appropriate solvent and its most appropriate concentration for the electrochemical evaluation of antioxidant activity because these solvents are cost-effective. The differences ( $\Delta E$ ) between the initial potential and limiting potential for ten antioxidants were measured using  $\text{PMo}_{12}$ , which forms in the presence of 50% (v/v) and 70% (v/v) methanol and ethanol. The results are displayed in Table 1.

The antioxidant activity ( $\Delta E$ ) determined using potentiometric measurement was compared with that measured using well-known techniques, such as the ORAC and DPPH methods (Fig. 4).<sup>31-34</sup> The  $\Delta E$  value was observed to be linearly related to the ORAC value for all of the POMs, except for *trans*-ferulic acid and L-ascorbic acid, for which the reason is not clear at the moment, although the antioxidant activities of sesamol,  $\alpha$ -tocopherol and  $\delta$ -tocopherol could not be measured using the ORAC method.<sup>31-34</sup> This result indicates that the electrochemical

method developed in the present study could also be used to evaluate antioxidant activity. However, the values obtained using the other methods are not linearly related to the  $\Delta E$  values, although, to some extent, a correlation appears to exist for some antioxidants. The observed antioxidant activity has already been argued to strongly depend on the procedure, making a unified correlation difficult to obtain.

The greatest correlativity was obtained between the  $\Delta E$  and ORAC values for most of the antioxidants under the 50% (v/v) ethanol condition. Similarly,  $\Delta E$  was measured using  $\text{PVW}_{11}$  and  $\text{SV}_2\text{W}_{10}$ , which form in 50% (v/v) ethanol; the results are displayed in Table 1. Moreover, these results were also compared with the values obtained by ORAC (Fig. S6). Because a linear relationship was obtained for most of the antioxidants, 50% (v/v) ethanol was determined to be the most appropriate concentration for the potentiometric evaluation of antioxidant activity with all of the POMs used in the present study as

Table 2 Differences ( $\Delta E/V$ ) between the initial potentials and limiting potentials of polyoxometalates in beverages

Sample	Conditon			ORAC	DPPH
	A	B	C		
Red wine	0.054 (1.2) <sup>a</sup>	0.105 (0.75) <sup>a</sup>	0.073 (0.78) <sup>a</sup>		
Green tea (conc. catechin)	0.085 (0.70) <sup>a</sup>	0.129 (1.6) <sup>a</sup>	0.137 (0.93) <sup>a</sup>		
Green tea	0.065 (0.41) <sup>a</sup>	0.095 (0.98) <sup>a</sup>	0.128 (1.0) <sup>a</sup>		
Grapefruit juice	0.080 (1.0) <sup>a</sup>	0.103 (0.59) <sup>a</sup>	0.113 (0.24) <sup>a</sup>		
Sake(100) <sup>b</sup>	0.036 (2.7) <sup>a</sup>	0.024 (5.0) <sup>a</sup>	0.009 (10.3) <sup>a</sup>	19900	46.3 <sup>c</sup>
Sake(50) <sup>b</sup>	0.025 (5.7) <sup>a</sup>	0.023 (2.2) <sup>a</sup>	0.005 (10.1) <sup>a</sup>	6360	41.8 <sup>c</sup>
Sake(30) <sup>b</sup>	0.019 (10.5) <sup>a</sup>	0.025 (3.3) <sup>a</sup>	0.011 (10.3) <sup>a</sup>	3800	37.7 <sup>c</sup>

a. The numbers in parenthesis indicate relative standard deviations (%). b. Ratio (%) indicating the quantity of polished rice gained from a given quantity of brown rice. c. Inhibitory ratio (%) is displayed because  $IC_{50}$  was not obtained. A: 10 mM Mo(VI), 1 mM P(V), 0.1 M HCl, and 50% (v/v) EtOH. The solution was allowed to stand at room temperature for 1 day.  $PMo_{12}$  occurs as the main species in this solution. B: 10 mM W(VI), 1 mM P(V), 1 mM V(V), 0.1 M HCl, and 50% (v/v) EtOH. The solution was heated at 70°C for 1 day and then cooled to room temperature to use for evaluation.  $PVW_{11}$  occurs as the main species in this solution. C: 10 mM W(VI), 1 mM V(V), 0.1 M  $H_2SO_4$ , and 50% (v/v) EtOH. The solution was heated at 70°C for 1 day and then cooled to room temperature to use for evaluation.  $SV_2W_{10}$  occurs as the main species in this solution.

electrochemical probes.

Finally, the antioxidant activities were analyzed by measuring the limiting potentials of the POMs under the optimized conditions; the results are displayed in Table 1. The antioxidant activities could be evaluated with high precision (RSD <1.5%). Among the various antioxidants, the antioxidant activity of L-ascorbic acid was highest, whereas that of ellagic acid was lowest, irrespective of the POM used; however, different results were obtained with different POMs, except in the cases of L-ascorbic acid and ellagic acid. Specific interactions may be essential for different results, although further investigations should be conducted to elucidate the details of the interaction.

Since  $\Delta E$  depends on the reducing power, or the reducing power capacity of the antioxidant, as shown in Fig. 3, the antioxidant capacities of foods and beverages, which contain a variety of antioxidants, could be evaluated based on their  $\Delta E$  values. The antioxidant capacities of five types of beverages—Japanese green tea, concentrated catechin-containing green tea, red wine, and Japanese sake—were potentiometrically evaluated under the optimized conditions; the obtained  $\Delta E$ s are displayed in Table 2. In particular, the antioxidant activities of three types of Japanese sake, brewed using rice polished at different ratios, were evaluated to compare with those obtained *via* the ORAC and DPPH methods. When any POM was used as a probe, concentrated catechin-containing green tea exhibited a high antioxidant capacity, whereas Japanese sake exhibited a low capacity. In addition, Japanese sake brewed using well-polished rice exhibited the lowest capacity, which is in line with the results obtained from the ORAC and DPPH methods. However, the antioxidant capacities of the other beverages and of Japanese sake depended on the POM used as the probe. Notably, the equilibrium potential for evaluating the antioxidant capacities in the beverages could be measured, even when the beverages were colored and muddy, although the POM-dependent results should be investigated in greater detail.

## Conclusions

A new method for evaluating the antioxidant activity has been developed by measuring the limiting potentials of POMs, which are partially reduced by antioxidants. Given the redox potentials of POMs,  $PMo_{12}$ ,  $PVW_{11}$  and  $SV_2W_{10}$  were selected as probes to investigate optimized conditions for measuring the limiting

potential ( $\Delta E$ ). The  $\Delta E$ s obtained under the optimized conditions were compared with the values of the antioxidant activity measured by various well-known techniques, resulting in a linear relationship with the ORAC value for many antioxidants. In addition, the antioxidant capacities of several types of beverages were evaluated by the developed potentiometric evaluation method. Finally, the method developed in the present study, along with spectrophotometric evaluation methods, were determined to be valid for evaluating the antioxidant activities of antioxidants and the antioxidant capacities of beverages. In particular, the new method enables evaluations of the antioxidant capacities of beverages without any complicated treatments, which were found to be very weak for spectroscopic measurements. In addition, the potentiometric evaluation method demonstrates bigger advantages than spectrophotometric evaluation methods as follows: 1) the apparatuses for potentiometric evaluation methods could be much cheaper than those for spectrophotometric evaluation methods. 2) Procedures are very simple rather than spectroscopic measurements, which require complicated sample preparation to obtain  $IC_{50}$  values. 3) The turbidity and coloration of samples are not relevant when considering the results of potentiometric measurements, unlike spectrophotometric measurements.

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## Supporting Information

Cyclic voltammograms and potential change in the case of  $PVW_{11}$  and  $SV_2W_{10}$ . This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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