# Electrochemical Analysis of Antioxidants using a Bicontinuous Microemulsion

E. Kuraya*, S. Nagatomo**, K. Sakata**, S. Uemura**, M. Kunitake**
*Science and Technology Division, Okinawa National College of Technology.
905 Henoko Okinawa 9052192, Japan
** Graduate School of Science and Technology, Kumamoto University.
2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan

## Introduction

Analysis of bioactive polyphenols in foods is very important to establish evaluation criteria for functional foods. Qualitative analyses and quantitative assays of antioxidant materials in foods are required. Some domestic plants in Okinawa contain ingredients with high physiological activity. Plant-derived polyphenols such as flavonoids, anthocyanins, and catechins are well-known as antioxidants, which inhibit or delay the oxidation of other molecules by interrupting the initiation or propagation of oxidizing chain reactions. The Folin-Ciocalteu method, in which a phenol reagent is reduced by the phenolic hydroxyl group and is colored, is a popular analysis technique [1] in which the antioxidant activity of compounds was evaluated as the reducing power of gallic acid equivalent. However, it is very difficult to evaluate the antioxidant activity of fat-soluble species such as α-tocopherol. In addition, the results can be affected greatly by the solvents used for the extraction process.

Bicontinuous microemulsions (BMEs), in which water and oil phases coexist bicontinuously on a microscopic scale, can dissolve hydrophilic and lipophilic compounds simultaneously. In our previous research, we reported that electrochemical contact with the microsized aqueous and organic solution phases in a BME can be alternately or simultaneously achieved by controlling the hydrophilicity and lipophilicity on the electrode surfaces. [2]. In this paper, we report the electrochemical analysis of several polyphenol analogues in a BME.

## Experimental

BME solutions consisting of phosphate buffer (pH=7.0) including saline, sodium dodecylsulfate (SDS), 2-butanol as a cosurfactant, and toluene were prepared for cyclic voltammetry (CV) analysis. Gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and α-tocopherol (vitamin E) were used as a phenolic standard, lipophilic antioxidant and a standard for antioxidant capacity assay, respectively. These compounds were first adjusted to a concentration of 50 mM, and then dissolved in the BME. As working electrodes, an indium tin oxide (ITO) electrode, and highly oriented pyrolytic graphite (HOPG) disk electrode were used as hydrophilic and lipophilic surfaces, respectively. The electrode potential was recorded against a reference saturated calomel electrode (SCE; +244 mV *vs.* SHE at 25 °C). As a popular hydrophilic antioxidant, ascorbic acid was also monitored in the BME.

## Results and Discussion

Figure 1 shows CVs of 1 mM gallic acid as a phenolic standard measured using an ITO electrode at various scan rates in the BME solution. The oxidative peaks of amphiphilic gallic acid were observed with both the ITO and HOPG electrodes. The peak currents of gallic acid, ascorbic acid and Trolox were proportional to the square root of the scan rate. These results indicate the currents are controlled by diffusion rate. It was also found that peak currents were proportional to concentration.

Figure 2 shows CVs of ascorbic acid, α-tocopherol and their mixed solution measured using an ITO electrode in the BME solution. The current peak of lipophilic α-tocopherol was not observed at the hydrophilic ITO electrode. The peak current of α-tocopherol in the mixed solution was in good agreement with that of α-tocopherol in the solution lacking ascorbic acid. These results suggest that antioxidant activity, even of lipophilic species, can be quantified in BMEs using a simple electrochemical technique.

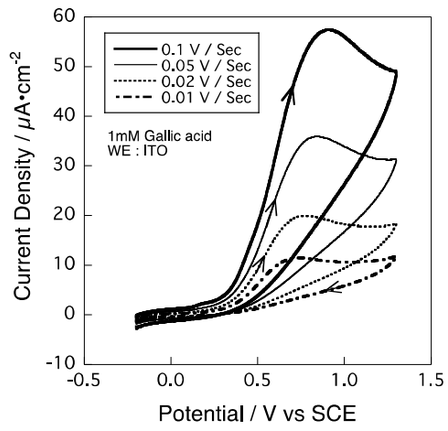


Figure 1. CVs of 1 mM gallic acid measured using an ITO electrode at various scan rates in BME solution.

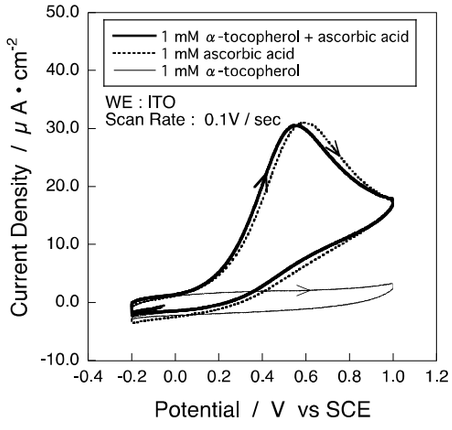


Figure 2. CVs of 1 mM ascorbic acid, 1 mM α-tocopherol and their mixed solution measured using an ITO electrode at 0.1 V/sec in a BME.

## References

[1] V. L. Singleton, Rudolf Orthofer, Rosa M. Lamuela-Raventós, Methods in Enzymology, 299,152-179 (1999).

[2] M. Kunitake, S. Murasaki, S. Yoshitake et al., Chem. Lett. , 34 (10), 1338-1339, (2005)