Determination of Diffusion Coefficients and Antioxidant Activities of Ascorbic Acid in Guava Juice using Cyclic Voltammetry

K. B. A. Ang, C. M. Lee, H. M. O. Yu, M. Uy, A. N. Soriano, N. P. Dugos*
Chemical Engineering Department, Gokongwei College of Engineering, De La Salle University, Taft Avenue, Manila, Philippines
*e-mail: nathaniel.dugos@dlsu.edu.ph

Abstract. Ascorbic acid is the most abundant antioxidant present in guava (Psidiumguajava L.). There had only been few studies concerning the determination of diffusion coefficient and antioxidant activity of ascorbic acid in guava juice using cyclic voltammetry specifically at varying temperatures. Thus, this study on the effect of temperature on diffusion coefficient and antioxidant activity on ascorbic acid found in guava was done using cyclic voltammetry. The temperatures tested were at 15°C, 25°C, 36°C, and 45°C. Electrodes used in the experiment were glassy carbon as the working electrode, platinum wire as the counter electrode, and Ag/AgCl electrode as the reference electrode. The results showed that the peak currents of ascorbic acid in guava juice at 15°C, 25°C, 36°C, and 45°C were -0.5720, -0.5380, -0.5000, and -0.4760 μA, respectively. The diffusion coefficients of the ascorbic acid were obtained using the Randles-Sevcik equation at all given temperatures and the values were 2.1489 x10^{-5}, 2.1711 x10^{-5}, 2.2070 x10^{-5}, and 2.2250 x10^{-5} cm²/s, respectively. The antioxidant activities, in terms of concentration, at the said temperatures were found to be 0.3374, 0.3212, 0.3015, and 0.2899 mM, respectively. It is concluded that at higher temperature, ascorbic acid in guava juice has a higher diffusion coefficient but lower antioxidant activity. The present results can be used by other researchers doing similar work on fate and transport of the studied system.

1. Introduction
The diffusivity of an antioxidant such as ascorbic acid is important in chemical reactions with free radicals since it influences the reaction rate on the preparation of immobilized cells [1]. Moreover, the diffusivity can tell how quickly the antioxidant moves and how it can freely hinder the oxidation of free radicals under a certain medium [2]. Generally, the diffusivity is dependent on the temperature, pressure and concentration of antioxidant [3]. In the study of Han et al. [4], the diffusivity of ascorbic acid in an embedded lipid cast film was obtained to be 1.87×10^{-5} cm² s⁻¹ with electrodes immersed in 5mM norepinephrine for 2 hours at pH = 6.666 and E = 200 mV. Abdel-Kader [5] also studied on the diffusion coefficient of ascorbic acid from peas at different temperature of water ranging from 50 to 90°C and found out that it increases with temperature. The rise in temperature results in the vibration of electrons which increases the diffusivity [6].

Antioxidant activity on the other hand is the ability of a certain antioxidant to inhibit oxidation of the protected substances or the amount of antioxidant which disrupts or prevents further biological damage through oxidation. The antioxidant activity, which can be expressed in terms of the concentration, is affected by temperature [7] and it decreases with increasing temperature [8-10]. In the study of Okiei et al. [11], cyclic voltammetry and titration method were employed at ambient temperature in the study of antioxidant activity of fruit juices that includes guava and found out that it has 50.3 mg per 100 g of active...
antioxidant. Siow and Hui [12] worked on the effect of drying temperature of 40°C at different duration and showed a significant decrease in the antioxidant activity of guava as time increases. Correa et al. [13] studied on the antioxidant activity of guava in ambient temperature using DPPH (2,2-diphenyl-1-picrylhydrazyl) which resulted to activities ranging from 44.66 to 409.77 mg / 100 g.

There are many methods and techniques that can be applied in determining the antioxidant activity and diffusion coefficient of antioxidant in fruits. Some common methods used are Differential Scanning Calorimetry (DSC) [14], Ferric Reducing Antioxidant Power (FRAP) [15-16], Titrimetric method, High Performance Liquid Chromatography and Spectrophotometric method. However, disadvantages occurred in these methods such as the inability to react fast with some antioxidants in Ferric Reducing Antioxidant Power; organic compounds in complex samples tend to exhibit ultraviolet absorbance due to the matrix effect in Direct Spectrophotometric method; and hard to determine the adequate temperature for the isothermal stage in Differential Scanning Calorimetry. Due to these disadvantages, electrochemical methods were used as a means of determining antioxidants. Advantages of electrochemical methods include low cost, the ability to detect low concentration of sample, and short time for preparation and analysis. The electrochemical method that is often used in the study of antioxidants like ascorbic acid is cyclic voltammetry due to its sensitivity, selectivity, and speed in analyzing samples of low concentration [11].

Ascorbic acid which is one of the most common antioxidant that can be found naturally in fruits and vegetables is used in pharmaceuticals as food supplement which can aid in the prevention of diseases by hindering free radicals from forming. Some factors that affect the antioxidant activity of ascorbic acid are temperature, storage time/maturity, type of citrus fruit, process parameters for product formation, containers, handling and storage [17]. The degradation of ascorbic acid occurs when the oxidation of it yields to dehydroascorbic acid (DHAA) which can go back to its original acid form but this reaction does not occur naturally [18]; while study done by Sanusi et al. [19], showed that ascorbic acid in fruit juices decreases with time in storage.

So far, no studies were conducted yet in which the antioxidant activity, as well as, the diffusion coefficients of ascorbic acid in guava juice at different temperatures were determined. Thus, the present work determines the antioxidant activity in terms of concentration and diffusion coefficient of ascorbic acid present in fresh Guava (Psidiumguajava L.) juice with the use of cyclic voltammetry. Specifically it focused on the determination of the effect of temperature in the antioxidant activity and diffusivity of ascorbic acid in guava juice for possible pharmaceutical or industrial food production purposes. In addition, the nature of the reaction of ascorbic acid in guava juice was also verified to determine whether the reaction is reversible, quasi-reversible, or irreversible. Knowledge on the nature of the reaction would indicate whether the free radicals that were hindered by the antioxidants can be formed again [20-21].

The antioxidant activity and diffusion coefficient of ascorbic acid in guava has different applications, therefore knowing and determining these values are important. Knowledge of the antioxidant activity can give the amount of active ascorbic acid in the fruit that would react or are ready to oxidize to prevent free radicals from forming in the body. The variation of temperature in this study can be significant for the storage condition on maintaining the amount of active ascorbic acid in the fruit. Moreover, information on the diffusivity or diffusion coefficient of ascorbic acid is important for the pharmaceutical industry since it can be determined as to how long would ascorbic acid take effect in our body when ingested. Also, the advantage of cyclic voltammetry, is that the nature of the reaction can be determined. Knowing the nature of the reaction can help determine whether formation of free radicals occurs.

2. Methodology

2.1. Flow of the Experiment
The overall flow process of the whole experiment is shown in Figure 1. First, the guava was bought from the market in Malabon. Then the fruit juices were prepared. Next, a calibration curve based from different concentrations of the standards, were made as basis for the analysis of the concentration of fruit extracts
which were obtained experimentally. Lastly, the antioxidant activity and the diffusion coefficient of the antioxidant were determined through the use of cyclic voltammetry. Then the results were analyzed.

![Flow process of the experiment](image)

**Figure 1.** Flow process of the experiment.

### 2.2. Materials

#### 2.2.1. Sample Collection and Preparation

Guava (*Psidium guajava* L.) was bought from the market in Malabon, Philippines. Analytical grade ascorbic acid was bought from Theo-Pam Trading Corporation for the purpose of obtaining the calibration curve.

The guava juice was prepared using a juicer. After the preparation of the juice, 6 grams of the guava juice was then mixed with 30 mL of distilled water. After mixing the mixture thoroughly, the homogenized sample was then filtered through a vacuum filtration set up with the use of glass microfiber filter paper to remove the suspended and settled solids. The filtrate was then transferred to the electrochemical cell and analyzed through cyclic voltammetry.

#### 2.2.2. Electrodes

Glassy carbon was used as the working electrode. It is an electrode that is gas and liquid impermeable. The glassy carbon electrode is known for its properties like high insulation, great conductor and high resistance to chemical attacks [22]. Ag/AgCl solution was used as the reference electrode and platinum wire was used as the auxiliary or counter electrode. The glassy carbon used had a diameter of 1 mm.

#### 2.2.3. Equipment

The potentiostat used was the EG&G Princeton Applied Research Potentiostat Model 273A. The potentiostat supplies the necessary potential to the solution for analysis. The e-corder that was used is an eDAQ brand e-corder. A hot plate with magnetic stirrer was used to keep the temperature constant and the solution homogenized.

### 2.3. Experimental Set-Up

The apparatus was assembled first by putting the working electrode, auxiliary electrode and reference electrode into a beaker with the solution making up the cell. The beaker with solution was placed under a hot plate to maintain constant temperature until the end of the run. The electrodes were connected to the potentiostat through the use of alligator clips. Afterwards, the potentiostat was connected to the E-corder and the E-corder was connected to a computer via a USB. The complete experimental set up is shown below in Figure 2.
2.4. Determination of Calibration Curve

2.4.1. Preparation of Standard
The solutions were prepared using serial dilution starting from 5 mM. Each of the solutions was placed in a 100 mL beaker.

2.4.2. Test at Varying Concentrations and Temperatures
The diluted solutions were all tested. It is very important that the same settings of the voltammetry are made, i.e. the same scan rate, since the basis of the experimentation with the fruit extracts are of the same settings. All of these data were recorded for the next step.

Concentrations of 1, 2, 3, 4, and 5 mM were used for the ascorbic acid solutions. The basis for the concentrations used was from Okiei et al. [11]. The parameters used for the potential range was from 0 to 1000 mV and a scan rate of 50 mV/s.

2.5. Determination of Antioxidant Activity and Diffusion Coefficient

2.5.1. Determination of Antioxidant Activity
The antioxidant activities were analyzed through cyclic voltammetry. The temperature was maintained constant by placing the electrochemical cell over a hot plate. The same setting as the one used for basis of the desired antioxidant is to be used. The obtained peak current was then plugged into the trend line equation from the calibration curve of the standards. Then the antioxidant activities in terms of the concentrations of the antioxidants were found.

2.5.2. Determination of Diffusion Coefficient
Based on the cyclic voltammogram, the nature of the reaction was known. Different formulas were used depending on the nature whether it would be reversible, irreversible or quasi-reversible reaction. To obtain the diffusion coefficient, the Randles-Sevcik equation (Eq. 1) was used. To be able to compare the effects of temperature on the diffusion coefficient and antioxidant activity in terms of concentration. These steps
were used for temperatures at 15, 25, 36, and 45°C. An iced water bath was used to maintain the
temperature to 15°C while a hot plate was used to maintain the temperatures at 25, 36, and 45°C. ANOVA
was used to find out the effect of temperature on antioxidant activity and diffusion coefficient.

\[
    i_p = 0.496(an)^\frac{1}{2}nFAC\left(\frac{FDv}{RT}\right)^\frac{1}{2}
\]  

In Eq. (1), \(i_p\) is the peak current in amperes (A), \(\alpha\) is the transfer coefficient which is often assumed to be
near 0.5 for organic substances, \(n\) is the number of electrons involved in the reaction, \(F\) is the Faraday
constant which is in (C/s), \(D\) is the diffusion coefficient or diffusivity in (cm²/s), \(C\) is the ascorbic acid
concentration in (M), \(T\) is the temperature in Kelvin (K), \(v\) is the scan rate in (V/s), and \(R\) is the universal
gas constant in (J/mol-K) [23].

3. Results and Discussions

3.1. Peak Current of Solutions of Ascorbic Acid at Varying Temperature

The peak current is the maximum amount of current produced by the reaction when subjected to cyclic
voltammetry. This can be used to quantify the amount of ascorbic acid that reacted. The antioxidant
activity of ascorbic acid in guava juice was determined using the calibration curve which can be seen in
Figure 3 where the magnitude of the peak current is directly proportional to the concentration. The
coefficients of determination (\(R^2\)) for the curves obtained were 0.9973, 0.9975, 0.9972, and 0.9965 for
temperatures 15, 25, 36, and 45°C respectively.

![Figure 3. Peak current of ascorbic acid at varying temperature.](image)

3.2. Verifying the Nature of Reaction

Using the eDaq E-Chem software for the e-corder, the graph of peak current (A) vs peak potential (V) was
obtained. In Figure 4a, a peak current of -0.5720 A was obtained based on voltammogram. The ascorbic
acid was detected at the potential of 0.454 V. The shape of the graph shows only one peak which signifies
an irreversible reaction. Likewise, as seen in Figures 4b to 4d, peak current of -0.5380 A, -0.5000 A, -
0.4760 Å were obtained. The ascorbic acid was detected at the potential of 0.454 V, 0.4420 V and 0.4370 V, respectively, for Figures 4b to 4d.

![Cyclic voltammogram of guava juice at (a) 15°C, (b) 25°C, (c) 36°C, and (d) 45°C.](image)

**Figure 4.** Cyclic voltammogram of guava juice at (a) 15°C, (b) 25°C, (c) 36°C, and (d) 45°C.

According to Pisoschi et al. [24], whose study employed cyclic voltammetry on juices of different fruits excluding guava, they found out that no interferences from other organic compounds were found to influence the analytical signal of the ascorbic acid. A typical cyclic voltammogram exhibits a loop graph due to the redox reaction shown in Figure 8. As shown in Figures 4a to 4d, the shape of the cyclic voltammograms had been consistent showing only a single oxidation peak. The single peak in the voltammograms signified that the ascorbic acid did not undergo reduction reaction. Thus, this indicates that the reaction of the ascorbic acid is irreversible.
3.3. Effect of Temperature on the Antioxidant Activity and Diffusion Coefficient of Guava Juice

Guava juice was obtained from fresh guava fruit using a power juicer. The guava juice was analyzed at the following temperatures: 15°C, 25°C, 36°C, and 45°C. The concentration of ascorbic acid can be associated with the calibration curve using the corresponding peak currents obtained from the cyclic voltammetry.

Results in Table 1 showed that as the temperature increases, the antioxidant activities decreases. The higher the antioxidant activity, the higher the potency of the drug [17]. A higher antioxidant activity indicates that the ascorbic acid is more stable meaning that it degrades slower at a lower temperature [26]. In the food industry, higher antioxidant activity of the ascorbic acid would be more effective in preserving food such as meat [27]. According to Lewis [28], when the temperature rises, the solubility of the oxygen decreases. In relation to our study, the antioxidant activity decreases due to diminishing solubility of oxygen when there is an increase in temperature for the four solutions accordingly. Since there would be less oxygen, there would be less ascorbic acid to react with.

Table 1. Mean antioxidant activity and mean diffusion coefficient of guava juice at different temperatures

| Temperature (°C) | Mean Antioxidant Activity (mM ascorbic acid) | Mean Diffusion Coefficient (cm² s⁻¹) |
|------------------|-------------------------------------------|-----------------------------------|
| 15               | 0.3374                                     | 2.1496 × 10⁻⁵                     |
| 25               | 0.3212                                     | 2.1714 × 10⁻⁵                     |
| 36               | 0.3015                                     | 2.2071 × 10⁻⁵                     |
| 45               | 0.2899                                     | 2.2250 × 10⁻⁵                     |

The diffusion coefficient of the ascorbic acid in guava juice was determined using the Randles-Sevcik equation for irreversible reactions (Eq. 1) and is also shown in Table 1. Based from the redox reaction of ascorbic acid shown in Figure 9, only one electron was released. The surface area of the electrode was then calculated with the diameter of the electrode being 1 mm. The scan rate was based on the parameter used in the experiment. The transfer coefficient (α) was assumed to be 0.47 based on the study of Raoof et al. [29].

In comparison with the literature data, the values of diffusion obtained for 25°C were close to both the studies of Han et al. [4] and Rohani & Taher [30] wherein they obtained 1.87 × 10⁻⁵ cm²/s and 1.028 × 10⁻⁵ cm²/s respectively using cyclic voltammetry. The difference between then present work with that of Han et al. [4] is that the later used a norepinephrine embedded in lipid cast film at glassy carbon electrode and an ascorbic acid and uric acid solution while Rohani & Taher [30] used a Cu (II) zeolite modified electrode. In the pharmaceutical industry, a decrease in diffusion coefficient would mean a decrease in the absorption of ascorbic acid in the body [31].

Increasing the temperature will have a significant increase on the diffusion coefficient. While the antioxidant activity compared with the temperature produced a P-value of 6.1526 × 10⁻⁶ (using ANOVA).
Therefore, increasing the temperature will have a significant change in the measured antioxidant activity. Based from this, keeping the temperature constant for the whole experiment is very important when using cyclic voltammetry as it is sensitive to temperature since a change in temperature would have significant effects on the results.

4. Conclusion

This research studied the effect of temperature on the diffusion coefficient and antioxidant activity in terms of concentration of ascorbic acid in guava juice. Using the eDaq e-cor dner as the measuring device and its complimentary software E-Chem, the cyclic voltammograms of the fruit juice samples were obtained and analyzed.

The average antioxidant activity in terms of concentration obtained at 15°C was found to be 0.3374 mM. At 25°C, the average antioxidant activity was found to be 0.3212 while at 36°C, a value of 0.3015 mM was obtained. Finally, a value of 0.2899 mM was obtained for 45°C. The values are equivalent to 0.2972, 0.2829, 0.2655, and 0.2554 milligram of ascorbic acid per gram of guava juice for temperature 15, 25, 36, and 45°C respectively.

Using the Randles-Sevcik equation for irreversible reactions, the diffusion coefficients for the different temperatures were obtained. The following values were 2.1489 x10⁻⁵, 2.1711 x10⁻⁵, 2.2073 x10⁻⁵, and 2.2250 x10⁻⁵ cm²/s for temperatures 15, 25, 36, and 45°C, respectively. Based on the results, temperature has a significant effect on both the antioxidant activity and diffusion coefficient.

References

[1] Grunwald P 1989 Biochem. Educ. 17 99–102
[2] Laguerre M, Sorensen A, Bayrasy C, Lecomte J, Jacobsen C, Decker E, and Villeneuve P 2013 In Role of Hydrophobicity on Antioxidant Activity in Lipid Dispersions (pp. 261-296). AOCS Press.
[3] Krynicki K, Green C, and Sawyer D 1978 Pressure and temperature dependence of self-diffusion in water. Faraday Discussions of The Chemical Society.
[4] Han X, Tang J, Wang J, and Wang E 2001 Electrochimica Acta 46 3367–3371
[5] Abdel-Kader Z 2006 A study of the apparent diffusion coefficients for ascorbic acid losses from peas during blanching in water. Nahrung-food
[6] Zhang C, Wu H, and Weng X 2004 Food Chem. 84, 219–222
[7] Prakash A, Rigelhof F, and Miller E 2001 Medallion laboratories analytical progress, 19 1–4
[8] Rěbělová Z 2012 Czech J. Food Sci. 30 171–177
[9] Moschette D, Hinman W, and Halliday E 1947 Ind. & Eng. Chem. 39 994–999
[10] Pokorny J 1986 Czech J. Food Sci. 4 299–307
[11] Okiei W, Ogunlesi M, Azeez L, Obakachi V, and Osunsanmi M 2009 Int. J. Electrochem. Sci. 4 276–287
[12] Siow L and Hui Y 2013 Int. Food Res. J. 20 639–644
[13] Correa L, Santos C, Vianello F, and Lima G 2011 Plant Genetic Resources: Characterization and Utilization 9 384–391
[14] Laye P, Rose D, and Taylor N 1992 Thermochimica Acta 206 95–105
[15] Firuzi O, Lacanna A, Petrucci R, Marrosu G, and Saso L 2005 Biochimica Et Biophysica Acta 1721 174–184
[16] Othman A, Ismail A, Ghani N, and Adenan I 2007 Food Chem. 100 1523–1530
[17] Nagy S and Smoot J 1977 J. Agric. Food Chem. 25 135–138
[18] Cloe A 2013 Do Vitamin C Supplements Go Bad or Lose Potency? | LIVESTRONG.COM. Retrieved May 6, 2015, from http://www.livestrong.com/article/463760-do-vitamin-c-pills-go-bad-or-lose-potency/
[19] Sanusi R, Nwozoh S, and Ogunro Y 2008 Pakistan J. Nutrition 7 730–732.
[20] Adams R 1976 Anal. Chem. 48 1126A–1138A
[21] Wen X, Liu Z, Han Z, and Rieker A 1997 J. Chemical Res. 3 108–109
[22] Zittel H and Miller F 1965 Anal. Chem. 37 200–203
[23] Zanello P 2003 Inorganic electrochemistry: Theory, practice and applications. Cambridge: Royal Society of Chemistry
[24] Pisoschi A, Danet A, and Kalinowski S 2008 J. Automated Methods and Manage. Chem. 937651
[25] Unwin P 2003 Instrumentation and electroanalytical chemistry. Weinheim: Wiley-VCH.
[26] Celestino M, Magalhaes U, Fraga A, Do Carmo F, Lione V, Castro H, Cabral L 2012 Brazilian J. Pharma. Sci. 48 406–415
[27] Verma A, Rajkumar V, Banerjee R, Biswas S, and Das A 2013 Asian-Australas J. Animal Sci. 26 886–895
[28] Lewis M 2006 Chapter 6.2: Dissolve Oxygen. In National Field Manual (2nd ed.). Retrieved from http://water.usgs.gov/owq/FieldManual/Chapter6/6.2_contents.html
[29] Raoof J, Ojani R, and Chekin F 2007 Electroanalysis 19 1883–1889
[30] Rohani T and Taher M 2009 Talanta 78 743–747
[31] howMed 2011 Factors affecting Absorption of Drugs. Retrieved from http://howmed.net/pharmacology/factors-affecting-absorption-of-drugs/
[32] Romero A, Hernández E, Cerón F, and Chávez A 2013 The Exogenous Antioxidants. In J. A. Morales-Gonzales (Ed.), Oxidative Stress and Chronic Degenerative Diseases – A Role for Antioxidants.

Acknowledgments
This work was supported by the Chemical Engineering Department, De La Salle University and the AUN/SEED-Net.