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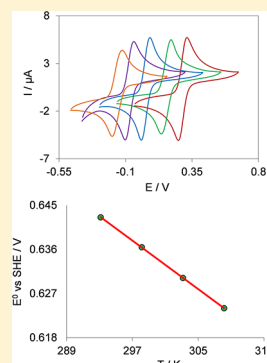
Electrooxidation of Homogentisic Acid in Aqueous and Mixed Solvent Solutions: Experimental and Theoretical Studies

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S Supporting Information

ABSTRACT: Electrochemical behavior of homogentisic acid (HGA) has been studied in both aqueous and mixed solvent solution of water–acetonitrile. Physicochemical parameters of the electrochemical reaction of HGA in these solutions are obtained experimentally by cyclic voltammetry method and are also calculated theoretically using accurate ab initio calculations (G3MP2//B3LYP). Solvation energies are calculated using the available solvation model of CPCM. The pH dependence of the redox activity of HGA in aqueous and the mixture solutions at different temperatures was used for the experimental determination of the standard reduction potential and changes of entropy, enthalpy, and Gibbs free energy for the studied reaction. The experimental standard redox potential of the compound in aqueous solution was obtained to be 0.636 V versus the standard hydrogen electrode. There is a good agreement between the theoretical and experimental values (0.702 and 0.636 V) for the standard electrode potential of HGA. The changes of thermodynamic functions of solvation are also calculated from the differences between the solution-phase experimental values and the gas-phase theoretical values. Finally, using the value of solvation energy of HGA in water and acetonitrile solvents which calculated by the CPCM model of energy, we proposed an equation for calculating the standard redox potential of HGA in mixture solution of water and acetonitrile. A good agreement between the result of electrode potential calculated by the proposed equation and the experimental value confirms the validity of the theoretical models used here and the accuracy of experimental methods.



1. INTRODUCTION

Many compounds containing a quinone–hydroquinone moiety are involved in electron and proton transfer reactions in biological systems.^{1–6} They are also important in biology because of their functions in cellular respiration as well as their antitumor activities.⁷ For rational design of selective bio-reductive agents, it is essential to be able to predict the redox potentials.^{7,8} Among the factors regulating the redox potential of the hydroquinones, the nature of the solvent medium plays an important role.^{9–12} Hydroquinone is much more strongly solvated by water than by benzoquinone. Therefore its reducing power in water is less than it would be in a medium of unit relative permittivity. It is noted that the redox potentials of the biologically important hydroquinone–quinone system should be strongly affected by the surrounding medium, particularly if the corresponding electron transfer is part of an electron transfer chain embedded in mitochondrial inner membrane.^{13,14} Also the thermodynamic parameters such as ΔG , ΔH , and ΔS which do provide a peek at the microscopic processes occurring during the chemical reaction are dependent on the surrounding medium.

Homogentisic acid (HGA, see Scheme 1A for the structure) is a 2-substituted hydroquinone. It is an important intermediate in the metabolism of tyrosine and phenylalanine.^{15,16} In normal persons HGA is oxidized by HGA oxidase to 4-maleylacetoacetic acid and excreted in urine. Therefore the level of HGA in the body of normal persons is extremely small.^{17,18} However, HGA molecules produced by the metabolism of phenylalanine

and tyrosine cannot be further metabolized in alkaptonuric individuals. In fact, it is accumulated to some degree in the tissues and is excreted in the urine. The HGA molecules remaining in the urine are acted upon by the enzyme polyphenol oxidase whose highest activity is in cartilage and skin leading to formation of benzoquinone acetic acid. This acid is the ochre pigment that binds irreversibly to collagen.¹⁸ Oxidation of HGA is much faster under alkaline conditions and is gradually retarded in acidic urine.

Electrochemical behavior of this compound has been studied previously. For example, the oxidation of HGA to 1,4-benzoquinone-2-acetic acid by $\text{Na}_2\text{Cr}_2\text{O}_7$ has been reported.¹⁹ More studies have focused on the oxidation of HGA by the dissolved oxygen in aqueous solution.^{20–22} Also the effect of HGA concentration in an alkaptonuria disease has been widely studied.^{23–27} Despite the importance of HGA, it seems that the electrochemical behavior of this compound in aqueous and nonaqueous solutions requires more investigations. Moreover, we have not found any specific study on the role of solvent and temperature on redox potential and other thermodynamic parameters of HGA. To the best of our knowledge, the free energy of solvation for HGA and its oxidized form have not been reported either.

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Scheme 1. Structures of HGA (A), Oxidized Form of HGA (B), and DOPAC (C)

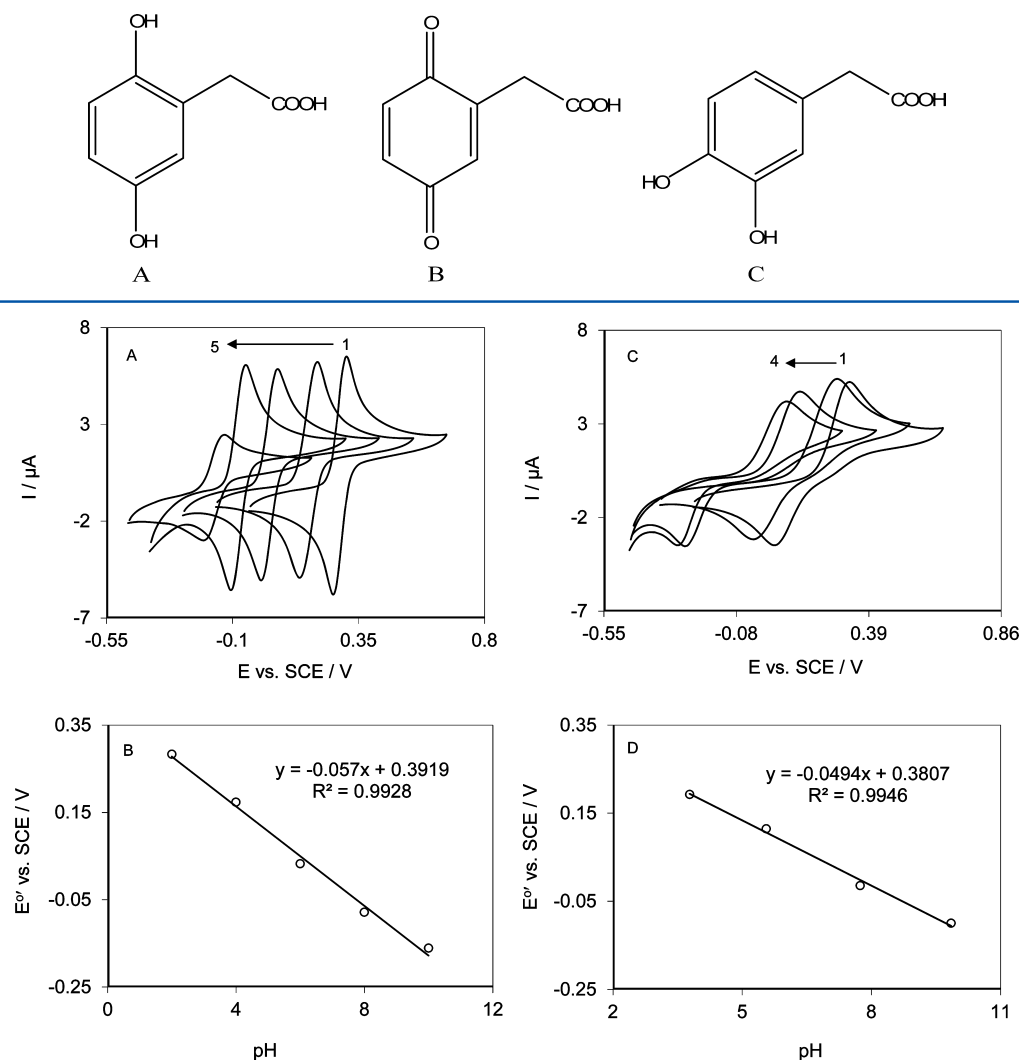


Figure 1. (A) Cyclic voltammograms of a glassy carbon electrode (at 50 mV s^{-1}) in a 0.1 M phosphate buffer solution containing 0.8 mM HGA in different pHs at 25°C . The numbers of 1–5 correspond to pHs 2.0, 4.0, 6.0, 8.0, and 10.0. (B) Plot of the conditional formal potential, $E^{\circ'}$, vs pH in aqueous solution. (C) Cyclic voltammograms of a glassy carbon electrode (at 50 mV s^{-1}) in solution of 50% v/v acetonitrile and water containing 0.1 M phosphate buffer solution with different pHs and 0.8 mM HGA at 25°C . Numbers of 1–4 correspond to pHs 3.78, 5.56, 7.74, and 9.85. (D) Plot of the conditional formal potential, $E^{\circ'}$, vs pH in a mixture solution of acetonitrile and water.

In the present work which is in continuation of our previous studies,^{28–31} we report the effect of temperature on the electrochemical oxidation of HGA in water and binary mixture of water and acetonitrile. The changes of thermodynamic functions of enthalpy, entropy, and Gibbs free energy for the studied electrochemical reaction have been investigated for a range of temperature experimentally and theoretically using standard ab initio calculation. We have also calculated the changes of solvation energy for the electrochemical reaction of HGA using a method which we have proposed recently.³¹ Also in this study, a general equation for the Gibbs free energy and the electrode potential in the binary mixture of water and acetonitrile has been proposed. The agreement between the theory and experiment is reasonable and confirms the validity of the theoretical models used here and the accuracy of the experimental method.

2. EXPERIMENTAL SECTION

2.1. Equipment and Reagents. Electrochemical measurements were carried out using a computer-controlled potentiostat, Autolab/PGSTAT 101 (Eco-Chimie), with the powerful NOVA software. A three-electrode electrochemical cell was employed for all the electrochemical measurements. The working electrode was a glassy carbon electrode with a diameter of 2 mm , while the counter and reference electrodes were graphite and saturated calomel reference electrodes (SCE), respectively. All the potentials were measured with respect to this reference electrode. Room temperature and elevated temperature experiments were performed in a double-walled glass cell, of 20 cm^3 volume, which was connected to a heated water bath at the appropriate temperature. All pH measurements were performed using a Metrohm 691 pH/mV meter. In order to obtain a clean renewed electrode surface, the glassy carbon electrode was cleaned by successive polishing with $0.05 \text{ }\mu\text{m}$ alumina–water slurry prior to its use in electrochemical experiments. HGA (see Scheme 1A for

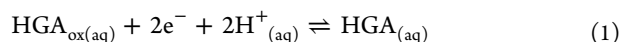
structure) was purchased from Acros Company. Acetonitrile and all other reagents were purchased from Merck and used without further purification. The solutions were prepared with doubly distilled water. Phosphate buffer solutions (0.1 M) were prepared from 0.1 M H_3PO_4 – NaH_2PO_4 , and the pH was adjusted with 0.1 M H_3PO_4 or 2.0 M NaOH.

3. THEORETICAL SECTION

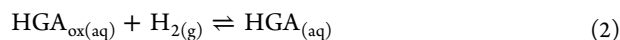
3.1. Computational Details. Gaussian 03³² has been used for all ab initio³³ and DFT calculations.³⁴ The G3MP2//B3LYP level of theory has been employed for the gas-phase calculations. G3MP2//B3LYP, one of the recent methods in the Gaussian-*n* series, provides improved accuracy for relatively large molecules. At the G3MP2//B3LYP method, first the molecular geometries are optimized and the vibrational frequencies are calculated at the B3LYP level of theory using the 6-31G(d) basis set. Then QCISD(T) along with 6-31G(d) basis set and MP2 with the GTMP2Large basis set is applied to derive the electronic energies and Gibbs free energy of the molecules in the gas phase.³⁵ In order to calculate solvation energies, a continuum model of solvation, the conductor-like polarizable continuum model (CPCM), has been used at the B3LYP/6-311G(d,p) level of theory.^{36–38} This level of theory has been recommended for the calculation of solvation energies.³⁸ All geometries of the studied species have been optimized fully in the presence of solvents.

4. RESULTS

4.1. Influence of pH and Temperature on the HGA Voltammetric Response in Aqueous Solution. The effect of pH on the HGA response was investigated by cyclic voltammetry using a 0.1 M phosphate buffer solution containing 0.8 mM HGA at different temperatures (20–35 °C) and at various pH values ranging from 2.0 to 10.0. Figure 1A shows the cyclic voltammograms of the glassy carbon electrode in phosphate buffer solution with different pHs at 25 °C containing 0.8 mM HGA. The voltammetric response of the glassy carbon electrode at the other temperatures is presented in the Supporting Information (Figures S1–S3). The two-electron transfer oxidation mechanism of hydroquinone^{39,40} is generally adopted to explain the electrochemical oxidation of HGA and other substituted hydroquinones in aqueous solution. The oxidation reaction of HGA to its oxidized form, HGA_{ox} , is a two-proton–two-electron reaction (see Scheme 1 for chemical structures):



Considering the half-cell reaction of standard hydrogen electrode (SHE), the overall oxidation is



For this reaction, the conditional formal potential, $E^{\circ'} = E^{\circ} - (2.303mRT/2F) \text{pH}$, is pH dependent (Figure 1B and Figures S1B–S3B of the Supporting Information). In this equation, E° is standard redox potential, T is temperature, and R and F are gas and Faraday constants, respectively.⁴¹ Based on the relation between the formal potential of the redox couple, $E^{\circ'}$, and pH, the standard redox potential of HGA can be obtained from the interception of this equation at pH 0.⁴¹ In this study, the redox potentials of the HGA at different temperatures have been measured versus the saturated calomel electrode (SCE) and then converted to the electrode potentials versus SHE using the

exact potential of SCE at each temperature⁴² following our previous methodology.^{31,43} The standard redox potential of HGA in aqueous solution at 25 °C is found to be 0.636 V versus SHE. The standard oxidation potential of DOPAC, which has the same structure as HGA but different position of hydroxyl groups (see Scheme 1C for the structure), was 0.828 V versus SHE.³⁰ The standard oxidation of HGA is 0.126 V smaller than that of DOPAC. This is in agreement with the difference between oxidation of hydroquinone (para) and catechol (ortho).⁴⁴

We have recently shown that using temperature dependency of redox potential, the thermodynamic parameters of oxidation/reduction of quinone species can be accurately determined.^{31,43} Exactly, the same methodology has been carried out in this work in order to measure the change of enthalpy, entropy, and free energy of reaction 1. Figure 2 shows the resulting variation of redox potentials with temperature in aqueous solution.

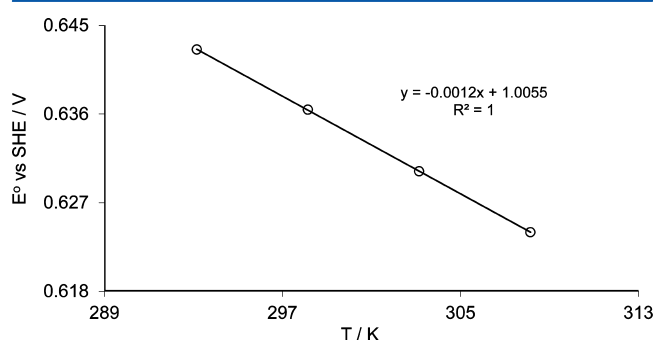


Figure 2. Effect of temperature on the redox potential of HGA in aqueous solution.

The changes of entropy and enthalpy for the studied reaction were found to be $-231.5 \text{ J mol}^{-1} \text{ K}^{-1}$ and $-191.8 \text{ kJ mol}^{-1}$ as shown with more details in Table 1. As we expected, these values are temperature independent in the studied small range of temperature, whereas the Gibbs free energies are temperature dependent.

4.2. Influence of pH and Temperature on the HGA Voltammetric Response in Mixture Solution of Acetonitrile and Water. As it was described previously, the nature of solvent has a significant effect on the redox potential of

Table 1. Experimental Redox Potentials and Thermodynamic Functions of Reaction 1 for 0.8 mM HGA in Aqueous Solution in the Range of 20–35 °C (293–308 K)

property	temp/K			
	293.15	298.15	303.15	308.15
E°_1 /V	0.642	0.636	0.630	0.624
E_{SCE} /V	0.247	0.244	0.241	0.238
ΔH°_1 /kJ mol ^{−1}	−191.8	−191.8	−191.8	−191.8
ΔS°_1 /J mol ^{−1} K ^{−1}	−231.5	−231.5	−231.5	−231.5
ΔG°_1 /kJ mol ^{−1}	−124.0	−122.8	−121.6	−120.4

^aRelative redox potentials vs SHE; the conversion has been carried out using the exact values of SCE at each temperature.⁴² ^bThe exact potential values of SCE at each temperature.⁴² ^cThe changes of enthalpies and entropies for reaction 1 are temperature independent in the studied range of temperature (linear relation of E vs T , see Figure 2).

hydroquinones. Aprotic solvents are useful because they mimic nonpolar environments of the cell where much of the biological electron transfer processes occur.⁴⁵ Among the aprotic solvents, acetonitrile, which is a polar solvent, has favorable physical properties, and it has been widely used as a solvent both in the study of chemical reactions and for physical measurements involving spectrophotometric and electrochemical techniques.⁴⁶

Therefore, for describing the effect of solvent on E° , ΔG° , ΔH° , and ΔS° of reaction 1, we have also calculated these thermodynamic parameters in 50% v/v acetonitrile and water solution (AWS0%). In this way, the effect of pH and temperature on the redox potential and thermodynamic parameters of HGA has been investigated in this mixture. Figure 1C shows the cyclic voltammograms of the glassy carbon electrode in AWS0% including the phosphate buffer solution with different pHs and 0.8 mM HGA at 25 °C. The voltammetric response of the glassy carbon electrode at different temperatures is presented as Figures S4–S7 of the Supporting Information. It is found that the formal potential of HGA decreases with the increase of pH. This trend is similar to the one in aqueous solution. From the interception of $E^{o'}$ versus pH, the standard potential of HGA in the solution of AWS0% has been calculated as 0.624 V versus SHE at 25 °C. The standard redox potential of HGA is lower in the mixture solution than that in water, suggesting that the lower dielectric constant of the mixed solvents solution is responsible for the decrease of E° . This is in agreement with the fact that HGA has greater solvation energy than its oxidized form. The dielectric constant of a solvent can also be lowered by increasing the temperature.^{42,47} The plot of evaluated standard potential versus temperature is shown in Figure 3.

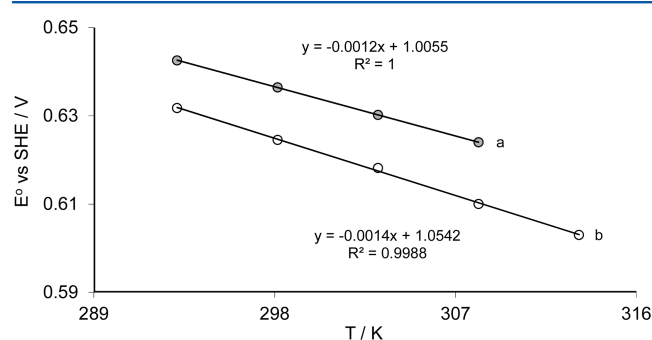


Figure 3. Effect of temperature on the redox potential of HGA in aqueous solution (a) and in the solution of AWS0% (b).

Based on our previous studies,^{31,43} the changes of entropy, enthalpy, and Gibbs free energy of reaction 1 in AWS0% are calculated and listed in Table 2. The thermodynamic behavior of water/acetonitrile mixture differs from that in pure water. There is a notable difference between the value of ΔH° for the aqueous solution and that in AWS0% ($\approx 9.3 \text{ kJ mol}^{-1}$). This is due to the fact that the extent of interaction energy of the species in aqueous and mixture of water/acetonitrile solutions is different. Also, the absolute value of ΔS° for reaction 1 in mixed solvents is higher than that in aqueous solution. This shows water molecules which have stronger interactions play a more important role in the solvation process.⁴⁸

4.3. Theoretical Calculations. The change of standard Gibbs energy of reaction 1 can be computed using the thermodynamic cycle (Figure 4) and equations which were previously introduced in detail.^{31,43}

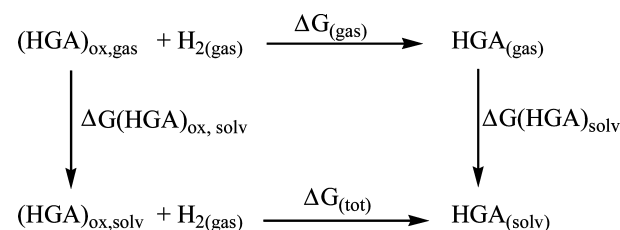


Figure 4. Thermodynamic cycle proposed to convert Gibbs energy of redox reaction in the gas phase to solution.

From this cycle the change of total Gibbs energy is computed by adding the Gibbs free energy of solvation to gas-phase Gibbs energy.⁴³ As detailed in section 3, the gas-phase Gibbs energies are calculated accurately using ab initio calculations at the G3MP2//B3LYP level of theory, and the solvation energies are calculated using the CPCM model of solvation, based on previous investigations.⁴³ Table 3 presents these calculated values of the thermodynamic parameters associated with reaction 1. Using the G3MP2//B3LYP calculated gas-phase energy together with CPCM solvation energies, the redox potential for the two-proton–two-electron reduction, reaction 1, in aqueous solution has been calculated as 0.702 V, which shows a small deviation of 0.066 V in comparison with the experimental value of 0.636 V for the studied reaction. This agreement between the theoretical redox potential and the experimental value shows that the approximations made in calculation of electrode potential are justified.

We have also calculated $\Delta G_{(\text{solv, W})}$, $\Delta H_{(\text{solv, W})}$, and $\Delta S_{(\text{solv, W})}$ for reaction 1 using the differences between the solution-phase

Table 2. Experimental Redox Potentials and Thermodynamic Functions of Reaction 1 for 0.5 mM HGA in 50% v/v Water and Acetonitrile Solution in the Range of 20–40 °C (293–313 K)

property	temp/K				
	293.15	298.15	303.15	308.15	313.15
$E_1^{\circ a}/\text{V}$	0.631	0.624	0.619	0.610	0.601
$E_{\text{SCE}}^b/\text{V}$	0.247	0.244	0.241	0.238	0.234
$\Delta H_1^{\circ}/\text{kJ mol}^{-1}$	−201.1	−201.1	−201.4	−201.0	−200.6
$\Delta S_1^{\circ}/\text{J mol}^{-1} \text{K}^{-1}$	−270.2	−270.2	−270.2	−270.2	−270.2
$\Delta G_1^{\circ}/\text{kJ mol}^{-1}$	−121.9	−120.5	−119.3	−117.7	−116.4

^aRelative redox potentials vs SHE; the conversion has been carried out using the exact values of SCE at each temperature.⁴² ^bThe exact potential values of SCE at each temperature.⁴² ^cThe changes of enthalpies and entropies for reaction 1 are temperature independent in the studied range of temperature (linear relation of E vs T , see Figure 3).

Table 3. G3MP2//B3LYP Calculations of Enthalpy, Entropy, and Gibbs Free Energy of Reaction 1 Together with Solvation Energies and Calculated/Experimental Redox Potentials at 298 K

thermodynamic property	G3MP2B3
$\Delta H_{1(\text{gas})}^{\circ}/\text{kJ mol}^{-1}$	−142.4
$\Delta S_{1(\text{gas})}^{\circ}/\text{J mol}^{-1} \text{ K}^{-1}$	−139.5
$\Delta G_{1(\text{gas})}^{\circ}/\text{kJ mol}^{-1}$	−100.7
$\Delta G_{1(\text{solv}, \text{W})}^{\circ a,b}/\text{kJ mol}^{-1}$	−26.9
$\Delta G_{1(\text{solv}, \text{AN})}^{\circ a,b}/\text{kJ mol}^{-1}$	−25.9
$\Delta G_{1(\text{tot})}^{\circ}/\text{kJ mol}^{-1}$	−135.6
$E_{1(\text{calcd})}^{\circ c}/\text{V}$	0.702
$E_{1(\text{expt})}^{\circ c,d}/\text{V}$	0.636

^{a,b}Changes of Gibbs free energies of solvation in water and also in acetonitrile for reaction 1, calculated using CPCM at the B3LYP/6-311+G(d,p) level of theory. ^cStandard redox potentials are relative to SHE. ^dExperimental redox potentials have been measured in this work using cyclic voltammetry in aqueous solution.

experimental values and the gas-phase theoretical values, both of which have been obtained accurately.^{31,43}

$$\Delta H_{(\text{solv}, \text{W})} = \Delta H_{(\text{tot}, \text{expt})} - \Delta H_{(\text{gas}, \text{calcd})} \quad (3)$$

$$\Delta S_{(\text{solv}, \text{W})} = \Delta S_{(\text{tot}, \text{expt})} - \Delta S_{(\text{gas}, \text{calcd})} \quad (4)$$

$$\Delta G_{(\text{solv}, \text{W})} = \Delta H_{(\text{solv}, \text{W})} - T\Delta S_{(\text{solv}, \text{W})} \quad (5)$$

It should be noted that the application of this method allowed us to calculate the components of Gibbs free energy of solvation ($\Delta H_{(\text{solv})}$ and $\Delta S_{(\text{solv})}$). Calculation of the components of Gibbs free energy of solvation is not possible by applying the CPCM model of solvation. The knowledge of these components is important since the Gibbs free energy of solvation at different temperatures can be computed using these components and by the equation of $\Delta G_{(\text{solv})} = \Delta H_{(\text{solv})} - T\Delta S_{(\text{solv})}$. Using the G3MP2//B3LYP results presented in Table 3 for the gaseous thermodynamic quantities, together with experimental value obtained from Figure 2, $\Delta H_{(\text{solv}, \text{W})}$ and $\Delta S_{(\text{solv}, \text{W})}$ are calculated as $-49.5 \text{ kJ mol}^{-1}$ and $-92.0 \text{ J mol}^{-1} \text{ K}^{-1}$ for reaction 1, respectively.

Since the solvation energy of organic molecules plays a critical role in their reactivity, calculations of solvation energies of HGA in mixture of acetonitrile and water have been also of interest.⁴⁹ In other words, for calculating the standard potential of HGA in mixture solution, it is necessary to introduce a general equation for the Gibbs free energy of solvation in mixed solvents. The solvation Gibbs energy of a substance in binary solution with ideal behavior is calculated by the following equation:

$$\Delta G(\text{solv}, \text{mix}) = X\Delta G(\text{solv}, \text{AN}) + (1 - X)\Delta G(\text{solv}, \text{W}) \quad (6)$$

where X is the mole fraction of acetonitrile. $\Delta G(\text{solv}, \text{AN})$ and $\Delta G(\text{solv}, \text{W})$ are calculated using CPCM model of solvation and are presented in Table 3. Therefore, in AW50% ($X = 0.257$), the value of $\Delta G(\text{solv}, \text{mix})$ is calculated as $-26.15 \text{ kJ mol}^{-1}$. The electrode potential of a solute at a defined temperature is estimated by the following expression:⁴¹

$$\begin{aligned} E^{\circ} &= -\Delta G_{\text{tot}}/nF \\ &= -\{\Delta G(\text{gas}) + X[\Delta G(\text{solv}, \text{AN})] + (1 - X) \\ &\quad [\Delta G(\text{solv}, \text{W})]\}/nF \end{aligned} \quad (7)$$

The theoretical redox potential of HGA at the studied mixture of solvents have been calculated as 0.698 V versus SHE. As it is shown in Figure 3, the experimental value of the potential electrode of HGA in the studied mixture of solvents has been determined as 0.624 V. A small deviation between the theoretical and experimental values indicates that the proposed equation (eq 7) can be used for the calculation of the standard potential of hydroquinones in mixture of water/acetonitrile.

5. DISCUSSION

As mentioned in the Experimental Section, the effects of pH and temperature on the HGA response were investigated in aqueous solution by cyclic voltammetry at different temperatures (20–35 °C) and at various pH values (2.0–10.0). It is shown that the formal potential of HGA is linearly dependent on the solution pH showing that the oxidation reaction is a two-electron–two-proton process. The redox potential has been also found to be linearly dependent on the solution temperature in the studied range of temperature, from which the thermodynamic parameters of the redox reaction have been calculated. The raise of temperature leads to decrease of the redox potential and equilibrium constant. As we expected, the change of entropy for the reduction process was a negative value, and considering the change of enthalpy which was also negative (formation of chemical bonds), the raise of temperature increases the change of Gibbs free energy, based on the equation $\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$. The standard redox potential of HGA in aqueous solution is calculated as 0.636 V versus SHE at 25 °C. Comparing the standard oxidation potential of HGA with a structurally similar compound, DOPAC, with different positions of hydroxyl groups reveals that the reduction of hydroxyl benzoquinones with ortho hydroxyl groups is easier than that with para hydroxyl groups. A simple method (eq 6) has been used to calculate the Gibbs free energy of solvation of the studied species in mixed solvent solution of water–acetonitrile. The increase of solvent with a lower dielectric constant (acetonitrile, in this case) caused the solvation energies and therefore redox potential decrease. The employed method might be extended to other binary mixed solvent solutions as well as to other quinone/hydroquinone systems. The effects of pH and temperature on electrochemical behavior of HGA are in agreement with other quinone compounds we studied previously.^{29,43}

6. CONCLUSIONS

In this work, electrochemical behavior of HGA in aqueous and mixture of water and acetonitrile solutions has been studied both experimentally and theoretically. HGA is oxidized to the HGA_{ox} by a one-step, two-electron–two-proton redox reaction. The standard redox potential of HGA for this reaction is calculated as 0.636 V. High-level ab initio molecular orbital calculations were also used to calculate the standard redox potential of HGA. The theoretical redox potential was in agreement with experiment (within 0.066 V), mutually verifying the experimental and theoretical studies. The changes of solvation enthalpies, entropies, and Gibbs free energies for the studied reaction have been estimated. Also we proposed an

equation for the calculation of redox potential of HGA in mixture of water and acetonitrile. A small deviation between the theoretical and experimental values proves the validity of the proposed equation.

■ ASSOCIATED CONTENT

■ Supporting Information

B3-LYP/6-31G(d) optimized geometries in the form of Gaussian archive entries and corresponding total energies as well as experimental cyclic voltammetry results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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