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Short Communication

Electrochemical Characterization of the Hydrophobic Interaction and the Natural Convection within Agarose Gel

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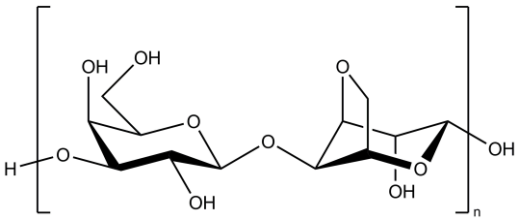
The electrochemical characteristics of redox reaction within agarose as solid electrolyte were investigated. Cyclic voltammetry of ferrocenemethanol within agarose shows the fast charge transfer similar to that in aqueous electrolyte while the difference in chemical interactions between polymeric backbone and redox molecules shifts the formal potential by a few mV. Cyclic voltammetry also demonstrates that diffusion governs the mass transport with the almost same diffusion coefficient to that in aqueous electrolyte in spite of the presence of steric, electrostatic, and chemical interaction. In contrast, the mass-transport behavior in long time region of redox molecules within solid agarose in chronoamperometry shows the reduced natural convection effect than that in liquid electrolyte owing to the presence of polymeric backbone. Analysis by commercial software of COMSOL provides the information about thickness of stagnant layer from natural convection and the effect on chronoamperometry.

Keywords: Ultramicroelectrode, Agarose, Hydrogel, Diffusion

1. INTRODUCTION

Solid electrolyte have great importance due to the versatile and efficient applications in electrochemical storage and energy conversion fields.[1-3] However, electrochemistry of redox molecules in a solid phase has been rarely investigated demonstrating the similar electrochemical behavior to that in solution.[4-7] Recently, our group reported the hydrogel pen for electrochemical reaction called HYPER, which can be applied to localized electrochemistry within nanometer scale.[8] Agarose makes a sharp and solid electrolyte for HYPER and electrochemical current depends on the deformation of agarose tip by contact with macro electrode. The mass transport behavior in agarose was simply assumed as very similar to that in solution except the slightly lowered diffusion coefficient, but the solute-gel interaction, which has been the important subject both in biophysics and applications

of hydrogels, should be interpreted in detail to be utilized as useful electrolyte support in versatile applications.



Scheme 1. Structure of agarose.

Agarose is linear polysaccharide consisting of 1, 3-linked β-D-galactopyranose and 1, 4-linked 3, 4-anhydro-α-L-galactopyranose as a repeating unit as shown in Scheme 1. Cooling of hot agarose solution induce the association of 3D polymer network chains through hydrogen bonding and hydrophobic interaction, that entraps the solution,[9] resulting in agarose gel or solid water having a large amount of water within a small quantity of polysaccharide network structures.[10] Pores in agarose gels shows the wide distribution of pore size ranging from 1 to 900 nm depending on the preparation condition by transmission electron microscopy (TEM),[11] and atomic force microscopy (AFM).[12] In previous study, the diffusion of solutes in agarose gel has been studied by fluorescence correlation spectroscopy (FCS) and was modeled in terms of the steric, electrostatic, and chemical effect.[13] Recently, a local denser layer at gel/water interface of ca. 120 μm was reported implying that diffusion in agarose gel is not homogeneous and would be different from that in liquid aqueous solution.[14]

Ultramicroelectrode (UME), of which the dimension is smaller than 25μm,[15] has led to advances in electroanalysis because its electrochemical behavior deviate from that of macroelectrode owing to unique mass transport when the size of electrode is smaller than the scale of diffusion layer.[16, 17] For rapid heterogeneous electron transfer such as ferrocenemethanol and ferrocyanide, UME shows characteristic cyclic voltammogram, which is sigmoidal in shape and has a diffusion-limited plateau current (i_l) and half-wave potential ($E_{½}$). When the sufficient potential step for reduction or oxidation is applied, current from UME follows Cottrell equation in early transient regimes and then approaches a steady state or quasi-steady sate in long time regime. From both voltammetry and chronoamperometry of UME, one can estimate the concentration of redox molecules, diffusion coefficient, and mechanism of charge transfer.[18]

Herein, we report the electrochemical behavior of redox molecules within agarose gel. Cyclic voltammetry within agarose gel containing ferrocenemethanol with supporting electrolyte were performed to characterize the charge transfer and mass transport behavior. Cyclic voltammetry within agarose gel is nearly identical to that in liquid aqueous solution while slight shift of a half-wave potential was observed, that imply the association of redox molecules with hydrophobic polymer chain. Chronoamperometric result from an UME in agarose gel containing the ferrocyanide solution provides a little difference in long time regime compared with that in liquid aqueous electrolyte, which

is explained by the model related with the stagnant layer caused by natural convection in liquid system and analyzed with the aid of commercially-available, electrochemical finite element analysis.

2. EXPERIMENTAL PART

2.1. Materials

Hard agarose, ferrocenemethanol (97%) and Potassium chloride (99.9%) were purchased from Sigma-Aldrich. Gold disk electrode (0.8mm radius), Carbon microfiber electrode (5.5±1μm radius), polishing diamond and polishing alumina were purchased from Bas inc. Polishing micro-cloth pad was purchased from Beuhler. Milli-Q water (18.2MΩ) was used for all aqueous solutions.

2.2. Preparation of agarose gel for electrochemistry

Agarose solution (8.3 wt% agarose in water) was prepared in a container having a sealing cap. The solution was preconditioned in 90°C water bath for an hour and air bubbles were removed by boiling in microwave (700W) for 20s until the solution become viscous and transparent agarose solution. The residual air bubbles were removed slowly in the 90°C bath in sealed container. The prepared hot agarose solution was poured into the plastic mold for square-shaped pad and cooled down slowly (8hrs to room temperature) in a humidity chamber. The solidified agarose pad was seperated from the mold cautiously and stored in distilled water. The agarose gels were soaked in aqueous solution which contains redox molecules and supporting electrolyte for electrochemical reaction. The volume of solution for soaking a piece of agarose pad was 50 times of the agarose volume to minimize dilution of redox molecules and agarose gels were soaked for 8 hours for complete equilibrium before electrochemical measurement.

2.3. Electrochemical measurement

Electrochemical measurements were carried out using a CHI 900b potentiostat and Scanning Electrochemical Microscope system (CHI instruments). The UME was equipped on piezo/stepper system and the position of working electrode was controlled by stepper and piezo controller. the high-sensitivity force sensor is integrated on the system for monitoring the contact of electrode to the agarose gel surface. The whole setup was placed in a home-made faradaic cage (Cu, 1mm square mesh) on optical table. Ag/AgCl reference electrode and Pt (wire, 0.5mm diameter) counter electrode were used.

The hydrogel pad was placed in electrochemical cell, in which reference and counter electrode were connected and the UME approached to the gel surface. Digital microscope (AD7013MTL, Dino-Lite) is used for brief monitoring of the approach of the electrode and for preventing from uncertain events.

2.4 Simulation of Electrochemistry in agarose gel

COMSOL Multiphysics 5.1 with Electrochemistry Module was employed to simulate the mass transfer based on Fick's law of diffusion. COMSOL solves the diffusion equations in Cartesian coordinate system, $\nabla\cdot\left(-D_i\nabla c_i\right)=0$ for stationary problem and $\frac{\partial c_i}{\partial t}+\nabla\cdot\left(-D_i\nabla c_i\right)=0$ for time dependent problem. For a redox couple, Ox and Red species, 2 species are considered, index 1 for reduced state and 2 for oxidized form of redox molecules. The Dirichlet boundary concentration conditions at the semi-infinite surface (stagnant layer). For the chronoamperometry for diffusion-limited diffusion, the sufficient applied potential was condiered ($E_{appl}-E^{0'}>>0$).

3. RESULTS AND DISCUSSION

Figure 1 shows the cyclic voltammograms (CVs) at Au disk electrode (0.8mm radius) in aqueous solution and within agarose gel containing ferrocenemethanol, respectively. Both in aqueous solution and in a agarose gel, characteristic shapes in voltammogram at macroelectrode were observed which is consistent with previous reports.[5] For both cases, the difference between E_{pa} and E_{pc} is ca. 60 mV indicating a reversible, one-electron charge transfer. The identical peak currents in voltammograms also demonstrate the similar diffusion coefficient in agarose gel with aqueous electrolyte based on the Randles-Sevcik equation.

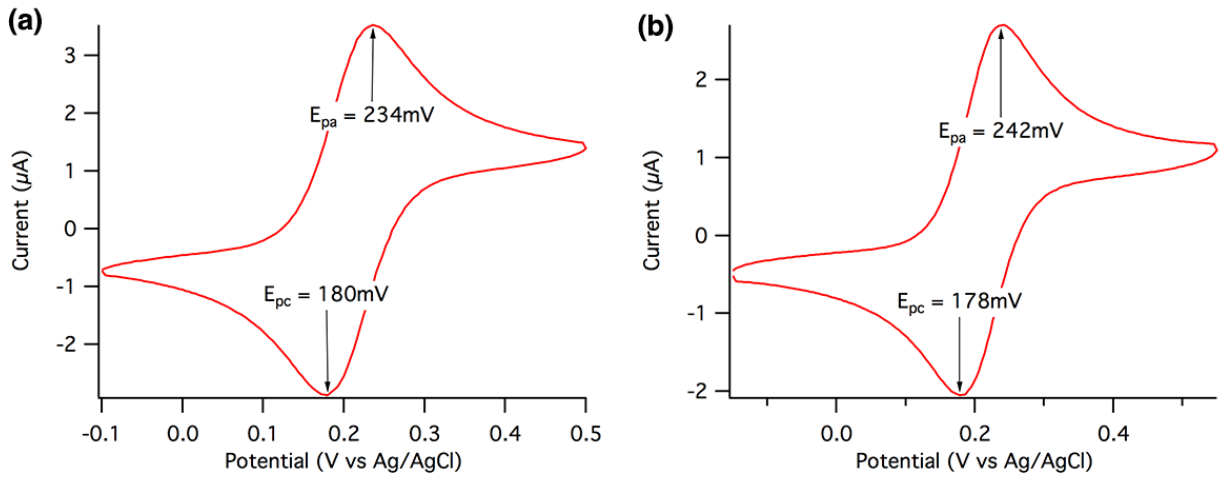


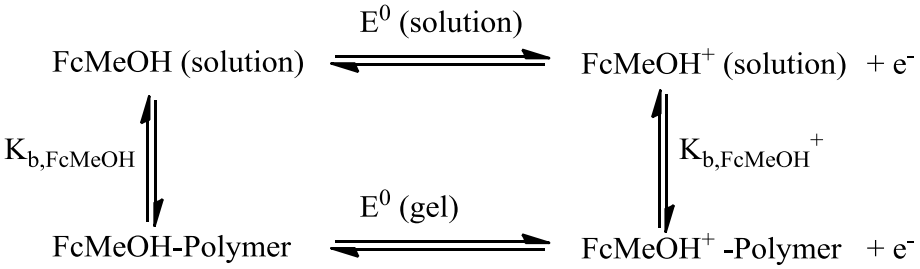
Figure 1. Cyclic voltammograms at Au disk electrode (0.8mm radius) (a) in the aqueous solution and (b) within the agarose gel, both containing the 1mM FcMeOH and 0.1M KCl solution. Scan rate : 50mV/s.

However, CV in agarose gel positively shift by ca. 3mV. Although the shift is small, it is very reproducible. The shift in $E^{0`}$ is characteristics of interaction between redox molecules and host matrices such as agarose polymer.[19-21] The positive shift indicates that the ferrocenemethanol (FcMeOH) slightly binds with polymeric backbone of agarose probably by the hydrophobic interaction

while positively charged $FcMeOH^+$ does not interact with agarose due to the lack of hydrophobicity. The formal potentials of the redox molecules ($E^{0`}$) shown in Scheme 2 is related with the binding constant of the oxidized and reduced species (K_b) as following

$$E^{0`}(gel) - E^{0`}(solution) = \frac{RT}{F}\ln(\frac{K_{b,FcMeOH}}{K_{b,FcMeOH+}}) \qquad \text{(Eq. 1)}$$

where $E^{0`}$ (gel) and $E^{0`}$ (solution) represent the formal potentials of the FcMeOH in agarose gel and solution, and $K_{b,FcMeOH}$ and $K_{b,FcMeOH+}$ stand for the binding constants of the FcMeOH and the $FcMeOH^+$ with agarose, respectively. A ratio of $K_{b,FcMeOH}/ K_{b,FcMeOH+} \approx 1.12$ imply that binding of the FcMeOH with agarose is a little stronger than that of $FcMeOH^+$ due to the hydrophobic interaction. Taking into account that concentration and diffusion constant in agarose are identical to in liquid, however, binding of both FcMeOH and $FcMeOH^+$ is negligible in typical electrochemical experiments. Therefore, it is reasonable that voltammetry of the FcMeOH at Au disk electrode in agarose is nearly identical to that in aqueous electrolyte except slight change in formal potential owing to the different binding constant of redox couples with agarose backbone.



Scheme 2. Scheme of squares for charge transfer in the presence of chemical interaction with polymer

The diffusion behavior of ferrocyanide in agarose gel was investigated by chronoamperometric responses from UME within agarose gel to estimate local diffusion properties in micrometer scale by changing experimental time scale. Both diffusion coefficient and concentration can be obtained by regression from chronoamperometry,[18] because chronoamperometric current at disk UME is well known,[22] which provides linear relationship between $t^{-1/2}$ and current. In short time regime (large $t^{-1/2}$), the Cottrell current ($\propto 1/t^{-1/2}$) flows due to the thin diffusion layer near UME. In the longer time regime (small $t^{-1/2}$), the current is limited by radial diffusion leading to the steady state, which is the y-intercept value.

$$\text{slope} = nFADC / (\pi D)^{1/2} \qquad \text{(Eq. 2)}$$

$$\text{y-intercept} = 4nFDCr \qquad \text{(Eq. 3)}$$

In aqueous solution, linear relationship of chronoamperometric current at UME was observed in Figure 2a. At long time regime, current is higher than fitted linear line probably due to natural

convection.[23-25] In contrast, chronoamperometric current at UME in agarose gel (Figure 2b) shows the linear relationship over almost entire time scale. This demonstrate that agarose gel reduces the effect of natural convention. Thus, the mass transport within agarose gel is governed only by diffusion. From macroscopic point of view, the unstirred electrolyte in our experiment is immobile. The density driven convection[23] might be negligible owing to low concentration of ferrocyanide. Microscopic motion, however, exists which is dependent on various experimental parameters including vibrations, thermal fluctuation, movement of air, and so on. Natural convection, caused by inevitable microscopic motion, in unstirred electrolyte makes the mass transport more complex. Theoretical model assumed that a stagnant layer of thickness (δ) exists where solute concentration is maintained to bulk concentration beyond x > δ (Figure 3a,b). For planar diffusion, current at the natural convection at long time can be written as following

$$I = nFADC_{bulk} / \delta \qquad \text{(Eq. 4)}$$

where n is the number of electron in redox reaction, F is Faraday constant, A is the electrode surface area, D is the diffusion coefficient, C_{bulk} is the initial concentration of reactant. Based on this model, δ = 230 μm was reported by analysis of chronoamperometry.[24] Briefly, slope in current *vs* $t^{-1/2}$ plot ($nFADC_{bulk} / (\pi D)^{1/2}$) was multiplied by $(\pi D)^{1/2}$ with known D value to give the precise result for $nFADC_{bulk}$ followed by division by the steady state current at long experimental times. When this procedure was applied to our experiment to determine the thickness of stagnant layer, δ is just ca. 20 μm which is too small compared with previous one even though natural convection is random.[24]

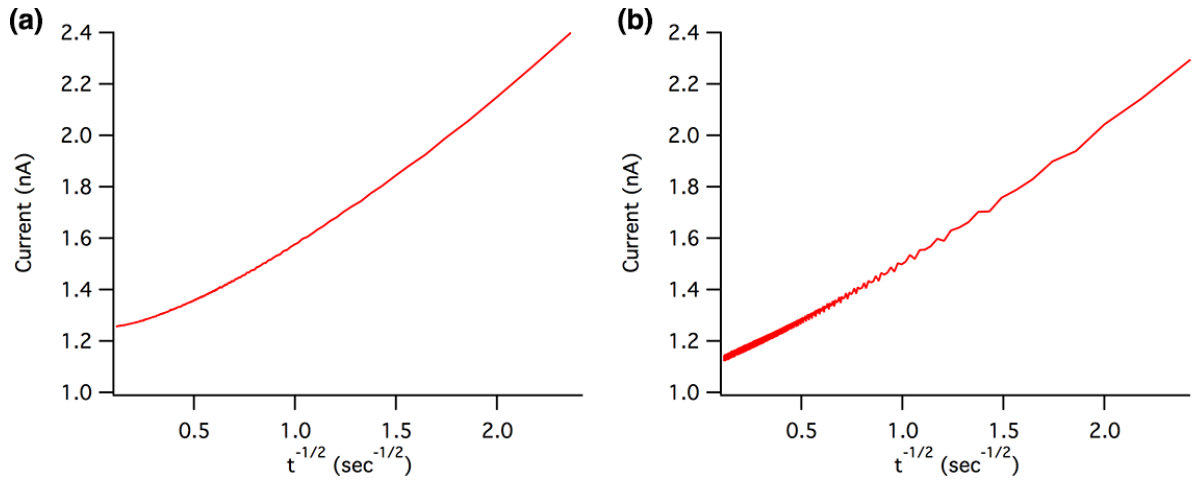


Figure 2. Current *vs* $t^{-1/2}$ plot of chronoamperograms at Carbon microelectrode (5.5μm radius) (a) in the aqueous solution and (b) within the agarose gel. Both systems contain 1mM FcMeOH and 0.1M KCl.

Mass transport in ultramicroelectrode, however, differ from planar diffusion. In previous report, diffusion over the central area of the electrode along the vertical axis is enhanced by natural convection while the radial diffusion transport reactants and products as much as typical

ultramicroelectrode without natural convection.[25] Because the radial diffusion in ultramicroelectrode is not considered in the steady state current from planar model, the steady state current was overestimated resulted in underestimated thickness of stagnant layer(δ). To consider the attribution of radial diffusion and natural convection, we performed the finite element analysis of chronoamperometry using commercially-available COMSOL. As shown in Figure 3c, current was analyzed by assuming that stagnant layer with various thickness by natural convection exist over the electrode surface. The radial diffusion at UME still contribute to the steady state current in the presence of stagnant layer. From the simulation, δ is ca. 200 μm and the concentration profile shows the origin of deviation from linear relationship in in current vs $t^{-1/2}$ plot. Thus, natural convection strongly affects the steady-state current of ultramicroelectrode in the aqueous electrolyte while it is reduced in agarose gel. Probably, polymeric backbone in gel phase hinder the microscopic convection from vibration, temperature, air flow and so on. Because the steady-state current is one of the key advantages of ultramicroelectrode applied to scanning electrode microscopy and others, the well-predicted steady-state current of UME within agarose gel provides the enhancement in various electrochemical technique including sensors and SECM.

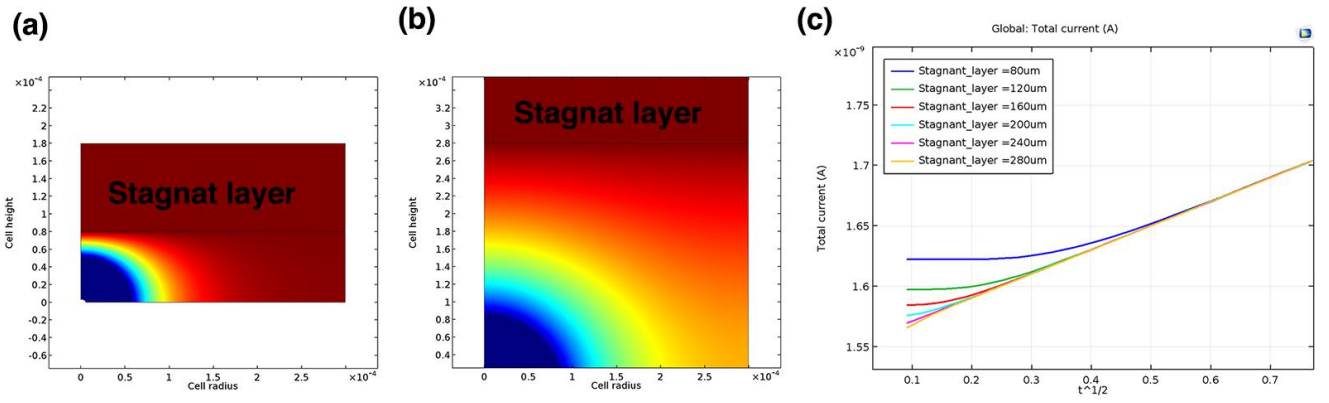


Figure 3. (a, b) Simulated concentration profiles of redox molecules in the electrochemical cell assuming (a) short distance (80μm) and (b) long distance (280μm) to the stagnant layer at the long-time region of chronoamperometry (t = 120s) (c) Simulated current vs $t^{-1/2}$ plot as the various distance to the stagnant layer.

4. CONCLUSIONS

In conclusion, the electrochemical characteristics of redox reaction within solid agarose as an electrolyte were investigated. Cyclic voltammetry of ferrocenemethanol within agarose shows the fast charge transfer, the diffusion coefficient similar to that in aqueous electrolyte, and a small positive shift of the formal potential owing to chemical interactions between polymeric backbone and redox molecules. In contrast, a long-term mass-transport of redox molecules with solid agarose in chronoamperometry shows the reduced natural convection in liquid electrolyte owing to the presence of polymeric backbone. Overall, the electrochemistry within agarose gel keeps the typical properties of

diffusion coefficient, the reversible charge transfer on electrode reaction, and the reduced natural convection providing the ideal model system for diffusion controlled experiments.

ACKNOWLEDGEMENT
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