Voltammetric Behavior of Bilirubin Based on [bmim][PF₆] As the Supporting Electrolyte in Organic Solvent and Its Analytical Application

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Abstract

The electrochemical oxidation behaviors of bilirubin (BR) at the bare glassy carbon electrode (GCE) were studied in dimethyl formamide (DMF) containing 1 vol.% of 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF₆]) as the supporting electrolyte. Some electrochemical parameters of BR oxidation were investigated by cyclic voltammetry and chronocoulometry. The oxidation products were identified by the spectrophotometric technique. The basic BR showed good linear relationship in the range of 2.6×10^{-5} to 2.5×10^{-4} M, and the neutral BR presented two linear relationships in the range of 5.4×10^{-4} to 1.1×10^{-3} and 1.1×10^{-3} to 1.6×10^{-3} M. The mechanisms for the successive electro-oxidation of BR in different forms were proposed.

Keywords: Bilirubin; Oxidation mechanism; Ionic liquid; Determination; Electrochemical behavior.

1. Introduction

Bilirubin (BR) is a member of the class of bile pigments found in the human body. It is formed daily by the physiological degradation of the porphyrin, such as hemoglobin [1]. BR plays as a protective antioxidant despite its possible toxic action in vivo under certain conditions [2]. Because of the importance of BR level in the physiological system, a great deal of attention has

already been given to the electrochemical study of BR. In previous studies, some authors have described the electrochemical behavior of BR in aqueous solution [3-6]. But further investigations have been limited because of the lack of solubility and stability of BR in aqueous solutions. This has led a number of BR studies being carried out in nonaqueous solvents [7-11]. Van Norman [7,8] has advocated controlled potential coulometry and spectral measurements for assaying BR purity, and

investigated the electrochemical behavior of BR and its metal complexes in dimethyl formamide (DMF). He has observed two oxidation waves ascribed to the oxidation of BR and biliverdin (BV), which located at 0.63 V and 0.8 V (vs. SCE), respectively, whereas no cathodic current could be obtained. Slifstein et al.[9] have applied many electrochemical methods to study the electrochemical redox of BR in dimethylsulfoxide (DMSO). Because of the complexity of the reaction, much is still uncertain concerning the chemistry of BR. For instance, it is difficult to study the successive oxidation process of BR due to its large overpotential, fouling by oxidation products and the different structures of BR.

Recently, a novel nonaqueous media—room temperature ionic liquids (ILs) has received considerable attention. ILs are liquid state molten salts that are generally comprised of an organic cation and an organic or inorganic anion, representing a new class of nonaqueous but polar solvents. They were originally developed by electrochemists for special applications due to their desirable properties such as wide electrochemical windows, good conductivity, extraordinarily high chemical and electrochemical stability, and negligible vapor pressure [12]. These properties make them attractive alternatives to traditional nonaqueous electrolytes. Because the electrolytic conductivity and anionic stability of an organic liquid electrolyte are important electrochemical parameters for the anodic oxidative processes [13, 14], the properties of IL-based electrolytes in organic solvents must be considered. Shiono and Nitta [15] dissolved imidazolium-based ILs in many organic solvents and found these kinds of electrolytes posed good solubility, high reliability and stability. McEwen et al. [16] have also investigated the ionic conductivity, dynamic viscosity, electrochemical window and electrochemical stability for IL-based electrolytes in organic solvents. It was obvious that ILs added to the medium would act as a suitable electrolyte and provide sufficient conductivity for voltammetric experiments. Another major advantage of this IL type electrolyte is the potential for more extensive solubility and dissociated charged species in organic solvents than previously used electrolytes

such as TBAP [17]. More recently, ILs have been widely used as the electrode modified materials [18,19].

In this paper, a hydrophobic IL—1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF₆]) as the supporting electrolyte of DMF enabled conventional electrochemical studies to be employed. The electrochemical behaviors and oxidation products of BR at the bare GCE were studied using a variety of electrochemical techniques, such as cyclic voltammetry (CV), controlled potential coulometry and chronocoulometry, and also the spectrophotometric technique. As we know, this is the first time that the intact Gmelin oxidation series are realized by the electrochemical method.

2. Experimental

2.1. Reagents and materials

BR, obtained from sigma, was of 99% purity. All other chemicals were of reagent grade. DMF was purchased from Chongqing Chemical Reagent Co. (Chongqing, P. R. China). Anhydrous potassium perchlorate (KClO₄) was obtained from Shanghai Chemical Reagent Co. (Shanghai, P. R. China). Tetrabutylammonium perchlorate (TBAP) was from Fluka.

[Bmim][PF₆] was prepared as described in the literature [20]. The purity of IL was checked by elemental analysis and ¹HNMR and ¹³CNMR spectroscopy, and the residual water content was analyzed by standard Karl-Fisher titration to be below 0.07% (w/w).

2.2. Apparatus and procedures

All electrochemical measurements were performed with a CHI 660 electrochemical workstation. The voltammetric studies were taken using a pretreated GCE as working electrode with an area of approximately 0.057 cm², a platinum wire as auxiliary electrode, and an aqueous saturated calomel electrode (SCE) as reference electrode. The pretreatment of bare GCE was in the same way as described in our previous paper [21]. The controlled potential coulometric measurements were performed using a large electric glass as the working electrode. For all the electrochemical studies, 1% (v/v) of [bmim][PF₆]

was used as the supporting electrolyte unless specially stated. Potentials were reported vs. SCE. Spectral measurements were taken on a Perking-Elmer Lambda 17 UV-Visible spectrophotometer with wavelength range of 190-900 nm.

BR solutions were prepared daily by weighing or by diluting a more concentrated stock solution. Then the solutions were introduced into the electrochemical cell, and deoxygenated by passage of nitrogen for at least 20 minutes before the measurement. Spectra measurements of the electrolysis solutions were obtained by removing samples with subsequent dilution of 4 times with the blank solution to give appropriate absorbance values.

3. Results and Discussion

Fig. 1 compares the effect of supporting electrolyte on the electrochemical response of BR. Comparing with KClO_4 as the supporting electrolyte, curve c showed that the peak potentials of BR shifted toward the cathodal direction and a new peak at 1.0 V was detected when IL was used as the supporting electrolyte. All the four waves can be well separated from each other. Thus it was promising that 1 vol.% of $[\text{bmim}][\text{PF}_6]$ was chosen as the supporting electrolyte in the experiments.

Fig. 1 CVs of 8.6×10^{-4} M BR in DMF at 0.1 V s^{-1} containing (b) 0.1 M KClO_4 , (c) 1 vol.% of $[\text{bmim}][\text{PF}_6]$, absence of BR containing (a) 1 vol.% of $[\text{bmim}][\text{PF}_6]$ as the supporting electrolyte.

CVs of BR in DMF were shown in Fig.2. No peaks were observed in the blank solvent (curve a). After the addition of BR, the voltammogram showed two anodic waves at approximately 0.3 and 0.63 V, as shown in curve b. With the further addition of BR, the peak at 0.3 V initially increased till reaching a maximum and then gradually decreased, while the peak at 0.63 V always increased and the potential shifted towards the anodic direction. Two new waves at about 0.6 and 1 V would appear when the peak at 0.3 V began to decrease, as shown in curve c. Curve d showed that when the content of BR reached a certain value, the peak at 0.3 V disappeared and the succedent three peaks became discernable: the first peak appeared approximately at 0.6 V, the second at 0.7 V and the third at 1.0 V. No obvious cathodic peak could be observed. For an irreversible system, $|E_p - E_{p/2}| = 47.7/\alpha n$ [22], the electron transfer number (n) of the four oxidation steps can be calculated to be about 2. Generally, α was assumed to be 0.5 in totally irreversible electrode process.

Fig. 2 CVs at 0.1 V s^{-1} in DMF solution containing 1 vol.% of $[\text{bmim}][\text{PF}_6]$ for (a) blank solvent only, (b) $2.2 \times 10^{-4} \text{ M BR}$, (c) $5.4 \times 10^{-4} \text{ M BR}$, (d) $7.0 \times 10^{-4} \text{ M BR}$, (e) $8.4 \times 10^{-4} \text{ M BR}$.

According to Van Norman [8], there was 10^{-5} to 10^{-3} M of basic amine impurities arising from the spontaneous decomposition of DMF. The basic impurities reacted with BR resulting in the formation of a basic BR. Most of the basic amine was removed after the distillation of DMF from

anhydrous CuSO_4 . BR in purified DMF did not exhibit this small wave but the peak at 0.6 V, which suggested that the removal of the basic impurities was adverse for the formation of basic form BR. So the existence of the small prewave at 0.3 V might be ascribed to the oxidation behavior of BR in basic form. The wave at 0.6 V was ascribed to the oxidation of BR in neutral form — another possible form of BR [8].

The influence of scan rate (ν , from 0.01 to 0.25 V s^{-1}) on the oxidation of BR was investigated (Fig. 3). At low ν ($<0.03 \text{ V s}^{-1}$), the prewave at 0.3 V was discernable (curve a, b). With the increase of ν , the prewave disappeared, while the other three oxidation peaks increased and potentials gradually shifted to more positive potentials. It seemed that low ν was useful for the electrochemical signal of BR in basic form, while fast ν for the signal of BR in neutral form. The shift of potential suggested that there was a kinetic limitation for the reaction between the electrode and BR. The plot of peak current at 0.6 V versus $\nu^{1/2}$ gave linear relationship, demonstrating that the oxidation reaction of BR was diffusion-controlled electrochemical process. Similar results were found for the other two waves.

Fig. 3 CVs for $8.6 \times 10^{-4} \text{ M}$ BR in DMF containing 1 vol.% of [bmim][PF₆] over a range of scan rates (a—h: 0.01, 0.03, 0.05, 0.08, 0.1, 0.15, 0.2, and 0.25 V s^{-1}). Inset: Plots of anodic peak currents at 0.6 V vs. $\nu^{1/2}$.

For a diffusion-controlled irreversible electrochemical process, the diffusion coefficient (D_R) and surface coverage (Γ_o) of BR in neutral or basic form could be determined using chronocoulometry based on the following equation [22]:

$$Q = \frac{2nFAD_R^{1/2}C_0t^{1/2}}{\pi^{1/2}} + Q_{dl} + nFA\Gamma_o$$

Where F is Faraday constant, A the electrode area, C_0 the concentration of BR, n the number of electron, and Q_{dl} is the double-layer charge, which is assumed not changed in the presence and absence of BR in our experiments. Other symbols have their usual significance. Take neutral form BR for example, the potential step was from 0.45 to 0.61 V for the kinetic parameters (Fig. 4). After the subtraction of background charge, the plots of charge (Q) against t were converted into the plot of Q against the square roots of time ($t^{1/2}$). It was obvious that Q had linear relationships with $t^{1/2}$ for the electro-oxidation of BR (date not show). From the slope and intercept, the values of D_R and Γ_o can be obtained. As n involved in the oxidation of BR in basic or neutral form was 2 and $A=0.057 \text{ cm}^2$, $C_0=5.4 \times 10^{-4} \text{ M}$, it was calculated that $D_R = 4.92 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $\Gamma_o = 6.68 \times 10^{-12} \text{ mol cm}^{-2}$ for the BR in basic form and $D_R = 1.03 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $\Gamma_o = 5.47 \times 10^{-11} \text{ mol cm}^{-2}$ for the BR in neutral form. It revealed that the diffusion coefficient and surface coverage amount of BR in neutral form were both larger than BR in basic form.

The calculated values were in accordance with the results of Fig. 2 and Fig.3. If BR was in low concentration, BR existed uniquely in the basic form, which was oxidized at 0.3 V. Once the concentration of BR exceeded the content of basic impurities, basic form BR and neutral form BR coexisted in DMF solution. But because of the larger diffusion coefficient and surface coverage amount, it was more easily for BR in neutral form diffusing and adsorbing onto the electrode surface. So the peak at 0.3 V decreased whereas the wave at 0.6 V increased. When the concentration of neutral BR reached the critical value, the surface of working electrode was basically occupied by neutral BR, and then the wave at 0.3 V disappeared. In the experiment process, it was also

found that the anodic signal of basic form was exhibited in the first circle but not in the following circles when BR in higher concentration was scanned in successive multi-cycles. It was deduced that the applied ν of 0.1 V s^{-1} was so quick that the basic form BR had no time to diffuse from solution to electrode surface in successive circle.

Fig. 4 Chronocoulometry of $5.4 \times 10^{-4} \text{ M}$ BR in DMF containing 1 vol.% of [bmim][PF₆] (curve a). Curve b stands for that in the blank solution. Initial potential 0.4V, final potential 0.61V, and pulse width 0.25 s.

In order to identify the oxidation products, controlled potential coulometry of BR at 0.65 V was performed. The electrolysis was halted at intervals and samples were taken for spectral and electrochemical measurement. The blank solution was pre-electrolyzed at 0.65 V before the addition of the BR. Fig. 5 shows the observed changes in spectrums as a function of electrolysis time. Curve a was the spectrum of BR with a maximum absorbance at about 450 nm, which was in accordance with literatures [7,9]. With the increase of electrolysis time, the peak at 450 nm diminished even disappeared (from curve a to f), and new peaks at 380 and 650 nm appeared (curve e). More passed coulombs resulted in the diminution of peaks at 380 and 650 nm (curve f). Thus curve e showed the produce of BV, while curve f showed the diminution. These results indicated that a potential of 0.65 V was sufficiently positive to oxidize BR to BV but not able to further oxidize BV (no new absorbance peaks appeared). Curve f suggested that the

formed BV was not very stable in DMF, so the characteristic absorbance of BV diminished even disappeared along with the increase of the electrolysis time.

Fig. 5 Spectra in DMF containing 1 vol.% of [bmim][PF₆] for $2.2 \times 10^{-4} \text{ M}$ BR solution electrolyzed at 0.65 V vs. SCE. (a) Initial solution, (b—f): 12 h passed, 24 h passed, 28 h passed, 32 h passed, 36 h passed.

Fig. 6 CVs in DMF containing 1 vol.% of [bmim][PF₆] at 0.1 V s^{-1} for $8.6 \times 10^{-4} \text{ M}$ BR solution electrolyzed at 0.65 V vs. SCE. (a) Initial solution, (e) 32 h passed, (f) 36 h passed.

Furthermore, three typical samples of curves a, e, f in Fig. 5 have been chosen for the voltammetric measurement. The change in the voltammetric behavior was shown in Fig. 6. The wave at 0.6 V steadily disappeared while the wave

at 0.7 V remained (curve e). This suggested that this peak at 0.6 V might be due to the oxidation of BR to BV and the wave at 0.7 V was ascribed to oxidation of BV to purpurin. Considering the two-electron oxidation process of the wave at 1 V, this wave would attribute to the subsequent oxidation of purpurin. The possible electrochemical oxidation mechanisms for two forms of BR were shown in schemes 1 and 2.

SCHEME 1: Main electrochemical oxidation pathways of BR in neutral form

Fig. 7 shows the relationship of peak current at 0.3 V and concentration of basic BR. The oxidation peak current initially increased linearly with the addition of BR, the linear relationship was observed between 2.6×10^{-5} and 2.5×10^{-4} M (Fig. 7 (inset)). The calibration curve tended to level off till reaching a maximum (also shown in Fig. 7) and then decrease if BR continued to increase (from 2.5×10^{-4} to 5.4×10^{-4} M). When the content of BR exceeded 5.4×10^{-4} , BR only oxidized at 0.6 V (Fig. 8). It presented two linear relationships between the peak current and the concentration of BR from 5.4×10^{-4} to 1.1×10^{-3} and 1.1×10^{-3} to 1.6×10^{-3} M. The correspondingly complicated relationship might imply that the reaction system was not a simple electrode system,

and the ranges of low concentration and high concentration followed different reaction mechanism. The relative standard deviation (RSD) was 2.1% for 8 successive determinations of 8.6×10^{-4} M BR solution with a single electrode.

SCHEME 2: Main electrochemical oxidation pathways of BR in basic form

Fig. 7 CVs at 0.1 V s^{-1} in DMF containing 1 vol.% of [bmim][PF₆] for BR at different concentrations (a—f: 0, 1.0×10^{-4} , 1.5×10^{-4} , 2.2×10^{-4} , 2.9×10^{-4} , 4.5×10^{-4} M). Inset: Linear relationship between the anodic peak currents and the concentrations of BR.

Fig. 8 CVs at 0.1 V s^{-1} in DMF containing 1 vol.% of [bmim][PF₆] for BR at different concentrations (a—f: 0, 5.4×10^{-4} , 7.0×10^{-4} , 8.4×10^{-4} , 1.1×10^{-3} , 1.6×10^{-3} M). Inset: Linear relationship between the anodic peak currents at 0.6 V and the concentrations of BR.

The influence of interferences on the determination of BR was evaluated. The investigation showed that 1000-fold higher level of amyllum did not interfere with the determination, whereas some familiar metal ions such as Cu^{2+} , Fe^{3+} , Ca^{2+} , Zn^{2+} and Mg^{2+} produced serious interference. The possible reason was that BR and metal ions could form the relatively stable BR-metal ion complexes.

4. Conclusions

The electrochemical behaviors of BR at the bare GCE were studied in DMF containing 1 vol.% of [bmim][PF₆] as the nonaqueous supporting electrolyte by a variety of techniques. Some electrochemical parameters of BR oxidation were investigated by CV and chronocoulometry. The experimental results showed that IL had a distinct enhancement effect on the electrochemical responses of BR and could reduce the oxidation overpotential. BR existed in a variety of forms depending upon the solution conditions. If BR was in low concentration, BR existed uniquely in the basic form, which was oxidized at 0.3 V. Otherwise, BR existed in the neutral form, which was oxidized at 0.6 V. The oxidation of BR was marked by four anodic waves in DMF, while all of the peaks were irreversible and two electron

process. The mechanisms for the successive electro-oxidation of BR were proposed.

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