NON-COTTRELL BEHAVIOR OF THE DOPAMINE REDOX REACTION OBSERVED ON THE CARBON FIBRE MICROELECTRODE BY THE DOUBLE-STEP VOLTCOULOMETRY

Katarína GMUCOVÁ[a,*], Jozef ORLICKÝ[b] and Juraj PAVLÁSEK[c]

[a] *Institute of Physics, Slovak Academy of Sciences, Dúbravská cesta 9, 845 11 Bratislava, Slovak Republic; e-mail: katarina.gmucova@savba.sk*

[b] *Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Limbová 3, 833 34 Bratislava, Slovak Republic; e-mail: umfgorli@kramare.savba.sk*

[c] *Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Slovak Republic; e-mail: unpfpavl@savba.sk*

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The redox reaction of the neurotransmitter dopamine at the carbon fibre microelectrode was studied by several electrochemical methods. It was found that under conditions usual in a living body, the diffusion current fullfils, within experimental errors, the behavior theoretically predicted by the Cottrell equation. Nevertheless, attention should be paid to the fact that unsupported or weakly supported conditions give rise to a non-Cottrell response of diffusion current. Moreover, similar changes were observed if the dopamine concentration was either lower such as several units of μmol l^{-1}, or about 100 μmol l^{-1} or higher. The non-Cottrell behavior of diffusion current involves the nonlinearity of the dopamine calibration curve obtained by pulse techniques. The present work is aimed at pointing out that such behavior of the measured data could lead to misinterpretation of the obtained dopamine concentration. Similar features could be also achieved for the other catecholamines.

Keywords: Dopamines; Redox chemistry; Coulometry; Kinetics; Carbon fibre microelectrode; Cottrell equation; Supporting electrolyte; Electrochemistry.

Since their introduction to the electrochemical detection of catecholamines[1], carbon fibre microelctrodes have been widely used in *in vivo* experiments. Even though the diameter of such electrode is much smaller than the diffusion length for the time scale of a typical electrochemical experiment, its response was shown to be predicted by the Cottrell equation[2]. In the present paper the real electrochemical response is described as a power function, *i.e.* $\propto t^{\beta}$. That power function expanded to the polynomial terms can be, in conformity with ref.[3], regarded as a Cottrell term, multiplied by a series of polynomial terms. Such terms are usually used to involve similar corrections of the Cottrell equation as was described in ref.[3]

The determination of the respective coefficients might be in our conception indirectly mediated through the determination of the real value of parameter β.

EXPERIMENTAL

In the present study an *in vitro* experiment with dopamine (3-hydroxytyramine hydrochloride p.a., SERVA, Feinbiochemica Heidelberg) was conducted. Sodium chloride (Specpure, Johnson Matthey Chemicals Limited, London) dissolved in deairated (Ar-bubbled) redistilled water, prepared in a silica glass apparatus was used to prepare the supporting electrolyte. In the course of all measurements the electrochemical cell was kept at room temperature. It was equipped with a carbon fibre microelectrode as the working electrode and an Ag|AgCl structure as the reference electrode. Before starting the experiment the electrochemical pretreatment of the carbon fibre microelectrode was performed in a 0.15 mol l^{-1} NaCl solution. First, the cathodic potential of –0.8 V was applied for 40 s (against Pt electrode), followed by a triangular waveform from 0 to +3 V for 10 s and finally an anodic potential of +1.5 V was applied for 10 s.

The electrochemical analyzer used in the present work was developed in our laboratory. The descriptions of the apparatus and the basic principles of its operation were published in ref.[4] The kinetics-sensitive double-step voltcoulometric method (DSVCM) was detailed also in our previous works[5–7]. The transient current flowing in response to a potential step through the electrochemical cell is integrated and processed by the selected time-domain filter, while scanning the applied potential. As an alternative to the scheme of sampling the transient current just before and at the end of the excitation pulse, three values of the transient charge are sampled in the interval between subsequent excitation pulses. Such a filtering scheme is capable of eliminating both the constant and linear components in the transient charge, which results in significant suppression of both the steady-state and capacitive contributions of the transient current with respect to the diffusion current contribution. Moreover, each measurement period is preceded by a single measurement of the steady-state current with the excitation pulse being switched off. In such a way, during the single potential scan both the steady-state voltammetric wave, and the double-step voltcoulometry signal are obtained.

Let the working electrode potential be denoted as E, the potential step on the working electrode as ΔE, the standard (formal) potential of the reaction as E^0, the diffusion coefficients of the oxidized and reduced species D_O and D_R, respectively, the concentration of the oxidized species in the solution c_o^*, the Faraday constant F, the gas constant R, the number of electrons entering the reaction n, the area of the working electrode S, the temperature of the analyte T, the sampling time t_1, the parameter introduced to keep the correct dimensions of the transient charge t_0, and the kinetics parameter β. Then, after running a single potential scan, two data sets mathematically described by the following formulas, are obtained

$$\Delta q_D = \frac{2nFSD_O^{1/2}c_o^*}{\pi^{1/2}t_0^{\beta}} \frac{P_A(1-\sigma^2)}{(\sigma + P_A)(1 + P_A\sigma)} [t_1^{\beta} - 2(5t_1)^{\beta} + (9t_1)^{\beta}], \qquad (1)$$

where $\sigma = \exp\left(\frac{nF}{RT}\frac{\Delta E}{2}\right)$, $P_A = \sqrt{\frac{D_O}{D_R}} \exp\left[\frac{nF}{RT}\left(E + \frac{\Delta E}{2} - E^0\right)\right]$,

for the correlated differential charge coming from the diffusion current contribution to the measured signal – according torefs[4,7,8], and

$$I_{\mathrm{lim}} = nF\sqrt{2\pi S}D_{\mathrm{O}}c_{\mathrm{o}}^{*} \qquad (2)$$

for the steady-state voltammetric wave.

Since the choice of slower sampling modes is highly recommended with respect to the expected higher suppression of the parasitic current, the results reported in this paper correspond to the measurements made for sampling time t_1 = 15.96 and/or 10.64 ms. The double step amplitude was set to ΔE = –85 mV. The cyclic voltammetry (CV) experiment was performed using the same experimental equipment by setting ΔE = 0 V; the scan rate was 0.1 V s^{-1}. Because the noise level was originally high, the measured data were mathematically smoothed. The method recommended in ref.[9] was used.

RESULTS AND DISCUSSION

The results obtained for aqueous 80 μmol l^{-1} dopamine with added different amounts of supporting electrolyte (NaCl) are plotted in Fig. 1. As was theoretically predicted for a single electron reaction in ref.[10], if the amount

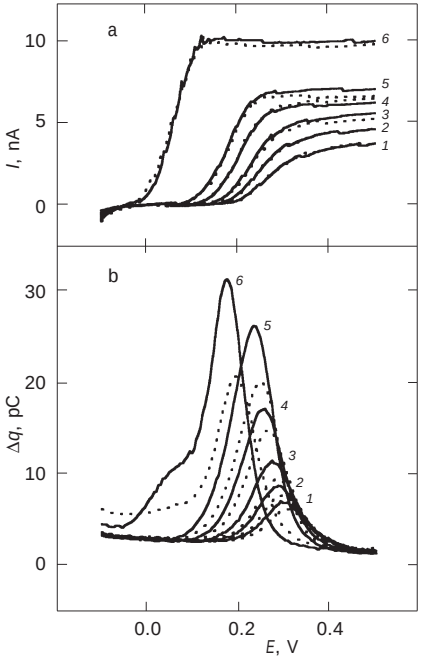

FIG. 1

The steady-state voltammetric wave (a) and voltcoulometric signal (b) obtained with a dopamine solution (80 μmol l^{-1}) under supported (0.9% NaCl (*1*), 0.675% NaCl (*2*), 0.45% NaCl (*3*), 0.225% NaCl (*4*), 0.1125% NaCl (5)) and unsupported (*6*) conditions

of supporting electrolyte increases, the voltammetric signal falls down. The wave height in the absence of supporting electrolyte for a single electron reaction exceeds that with the electrolyte present by a factor of 2, as was shown in ref.[11] As it is apparent from Eq. (*2*), if the same electrode is immersed into solutions of the same concentrations of the oxidized species and various concentrations of any supporting electrolyte, the diffusion coefficient is the only parameter that affects the steady-state current. If we assume[12] that the diffusion coefficient of dopamine in the physiological solution is $D_O = 2.7 \times 10^{-10}$ m^2 s^{-1}, its dependence on the supporting electrolyte concentration, as obtained from experiment, is depicted in the upper part of Fig. 2. Here, a relatively strong increase in the apparent diffusion coefficient for concentration of supporting electrolyte below 0.1% was observed. This increase in the observed value may be caused by a higher contribution of migration to the transport of dopamine measured in the presence of insufficient amount of supporting electrolyte. A similar influence of the increase in concentration of supporting electrolyte on the height of voltammetric waves of weak acids has been observed in ref.[13] In the case of

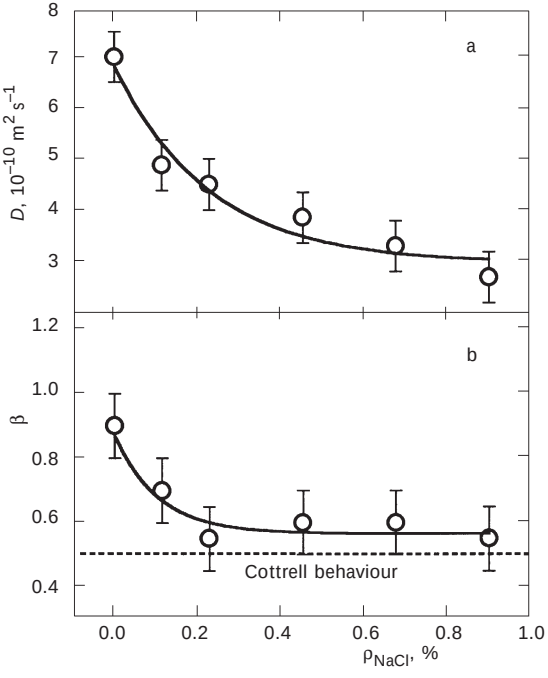

FIG. 2

The dependence of the apparent diffusion coefficient *D* (a) and kinetics parameter β (b) of 80 μmol l^{-1} dopamine on the concentration of the supporting electrolyte

dopamine redox reaction we are dealing with the two-electron reaction[14]. That fact results in the higher ratio of the wave height in the absence of supporting electrolyte to the wave height with the electrolyte present, than in the case of acids – value obtained for DA is 2.8. Simultaneous rough change of the kinetics parameter β for the concentration of supporting electrolyte tending to zero was observed by DSVCM and is shown in the lower part of Fig. 2. The kinetics parameter β was evaluated using Eq. (*1*) from the correlated differential transient charge by the procedure detailed in ref.[6] The measurements were made for both the sampling times t_1 = 15.96 ms (solid line) and t_1 = 10.64 ms (dashed line) – see Fig. 1. For lower concentrations of the supporting electrolyte, the signal becomes more expressive and the maximal value of the correlated differential charge is reached without the supporting electrolyte. Concurrently with decreasing amounts of the supporting electrolyte in the analyte, the apparent diffusion coefficient of dopamine increases and the parameter β recedes from the Cottrell value 0.5 (Fig. 2).

The cyclic voltammograms, obtained with the same analytes, are shown in Fig. 3. As is visible from comparison of Figs 1 and 3, the sensitivity is higher for the DSVCM method. The behavior of the obtained signals (DSVCM and CV) is very similar. The activation potentials of the redox reactions measured by CV are in conformity with the half-wave potentials of steady-state voltammetric waves. The maxima of the DSVCM peaks are shifted toward more positive potentials. This feature of DSVCM signals is

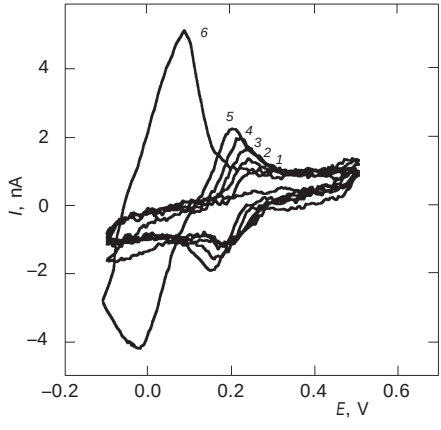

FIG. 3
Cyclic voltammograms (scan rate 0.1 V s^{-1}) of dopamine solution (80 μmol l^{-1}) under supported (0.9% NaCl (*1*), 0.675% NaCl (*2*), 0.45% NaCl (*3*), 0.225% NaCl (*4*), 0.1125% NaCl (*5*)) and unsupported (*6*) conditions

caused by the used sampling scheme comprising charge measurement during three different sampling events.

Another experiment was made to obtain the calibration curve of a supported dopamine solution (0.9% NaCl) within the range from 3 up to 1000 μmol l^{-1}. As is visible from Fig. 4, while the calibration curve obtained from the steady-state measurements (Fig. 4a) is linear within the wide concentration range, the situation is rather different in the case of voltcoulometry (Fig. 4b; the solid line represents the data obtained for the sampling time t_1 = 15.96 ms, the dashed line those obtained for t_1 = 10.64 ms). The nonlinearity of that calibration curve is caused by the change of the kinetics parameter β. Its behavior, computed using the procedure described in ref.[6], is depicted in Fig. 4c. The error bars in both the parts of Figs 2 and 4 were calculated as the experimental error due to a inaccurate subtraction of the background caused by the parasitic capacitive current through the cell.

The measurements conducted on both the supported and unsupported aqueous solutions point to the fact, that the dopamine redox reaction on

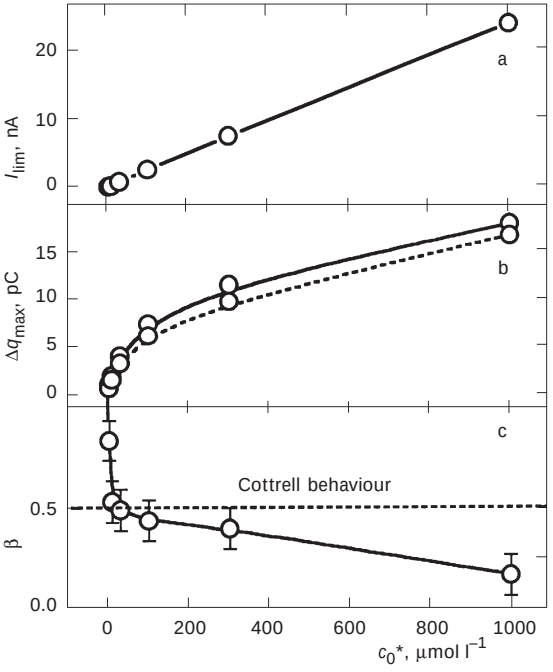

FIG. 4

The calibration curves of dopamine (from 3 up to 1000 μmol l^{-1}) obtained from the steady-state measurements (a) and from the voltcoulometry (b). The calculated kinetics parameter β is in part c

the carbon fibre microelectrode is kinetically controlled. At ionic strengths corresponding to the usual conditions in a living body (supporting electrolyte at concentration similar to the physiological level and/or the concentration of dopamine tens μmol l^{-1}), the kinetics parameter β could be regarded as approximately fulfilling the behavior theoretically predicted by the Cottrell equation. When dealing either with dopamine concentrations less or equal to several μmol l^{-1}, or greater or equal to *ca* 100 μmol l^{-1}, as well as when an *in vitro* experiment on the unsupported analyte is conducted, attention has to be paid to the fact, that a non-Cottrell response could be anticipated. Clearly, similar features of a voltcoulometric response obtained by the kinetics-sensitive electrochemical methods, could be also achieved for other catecholamines. Among the kinetics-sensitive methods we can, to a certain extent, relegate all the techniques based on the pulse approaches.

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