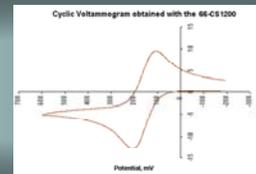


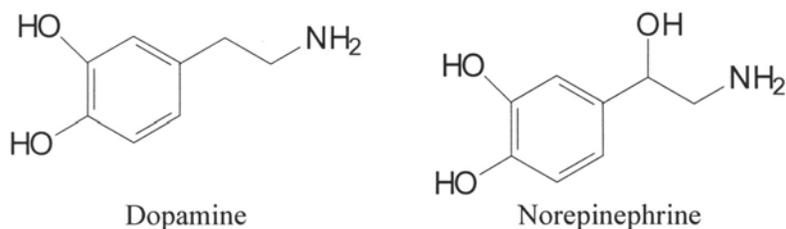
# Experiments in Analytical Electrochemistry



## 4. The Cyclic Voltammetry of Dopamine: an *ec* mechanism

**PURPOSE:** To determine the kinetic rate of a chemical reaction (*c* step) that follows an electron-transfer (*e* step), as illustrated by the oxidation of a neurotransmitter, dopamine.

**BACKGROUND:** Dopamine (DA), 4,5-dihydroxyphenethylamine or 4-(2-aminoethyl)1,2-benzenediol, is a known neurotransmitter that is involved in the chemical transmission of nerve impulses in the mammalian brain. It is a member of the catecholamine family and a precursor to epinephrine (adrenaline) and norepinephrine (noradrenaline) in the biosynthetic pathways.



DA has a molecular formula of  $C_8H_{11}NO_2$  and a formula weight of 153.18 [ref. 1]. It is a water-soluble hormone released by the hypothalamus. Imbalance in dopamine activity can cause brain dysfunction related to two major disorders, Parkinson's disease and schizophrenia [ref. 2,3]. Researchers are also looking at dopamine neurotransmission in drug abuse ranging from stimulants, such as amphetamines and cocaine, to depressants, such as morphine and other opioids, and alcohol [ref. 3].

Several amine neurotransmitters such as DA, noradrenaline (norepinephrine), adrenaline and serotonin are electroactive so that they can be monitored electrochemically. Most undergo a chemical reaction following the initial electron transfer step, an *ec* mechanism, as evaluated by cyclic voltammetry (CV) in this experiment. In biological fluids, prior separation with HPLC is recommended in conjunction with an electrochemical detector (HPLC-ECD). Online illustrative applications can be found at [http://www.esainc.com/applications/esa\\_applications.htm](http://www.esainc.com/applications/esa_applications.htm).

Great strides in learning about the role and fate of DA and other neurotransmitters in brains have come about in recent years due to the ability to monitor these compounds in-vivo. The major breakthrough making this possible came when Adams and co-workers [ref. 4] implanted small carbon electrodes (fibers) in rat brain to detect in-vivo catecholamine neurotransmitters. The development and application of the methodology are discussed in "*Probing Brain Chemistry: Voltammetry Comes of Age*" [ref. 5]. This article is available online: <http://pubs.acs.org/hotartcl/ac/96/jun/jun.html> and is a recommended reading as background to this experiment. Venton and Wightman [ref. 6] in a more recent article propose calling this new subject area "psychoanalytical chemistry" in which sensors, like microelectrodes, can detect neurotransmitter dopamine and determine how its neurochemistry affects and correlates with animal behavior.

**THEORY - What is an “*ec*” mechanism?** Cyclic voltammetry (CV) works well to determine the mechanism and rate of a chemical reaction (*c*) that may follow an electron transfer (*e*) step. The *ec* mechanism is illustrated below for the oxidation of species R to form species Ox, with Ox undergoing a chemical reaction to form product P:



If R/Ox electrode reaction is reversible, that is the heterogeneous electron transfer step is fast, and  $k_f = 0$  so that the follow-up chemical reaction does not occur, the cyclic voltammogram shown in Figure 1, trace A, is observed.

As discussed earlier, the anodic peak current,  $I_{pa}$ , and cathodic peak current,  $I_{pc}$ , are equal in magnitude when the transport of species R and Ox in the solution to and from the electrode is controlled only by diffusion. We are assuming that the CV is run at a planar (flat) electrode immersed in a quiet, unstirred solution. The reversible potential,  $E^0$ , is equal to the electrode potential,  $E_{0.85}$ , (the potential found 85% up the CV wave to  $I_{pa}$  (or  $E_{pa}$ )). In this example, the  $E^0 = +0.25 \text{ V}$  so that the oxidative wave is seen in the potential range of the forward scan, going from 0.0 V to 0.5 V. The Ox species is reduced back to R during the reverse scan from 0.5 V back to the initial potential of 0.0 V.

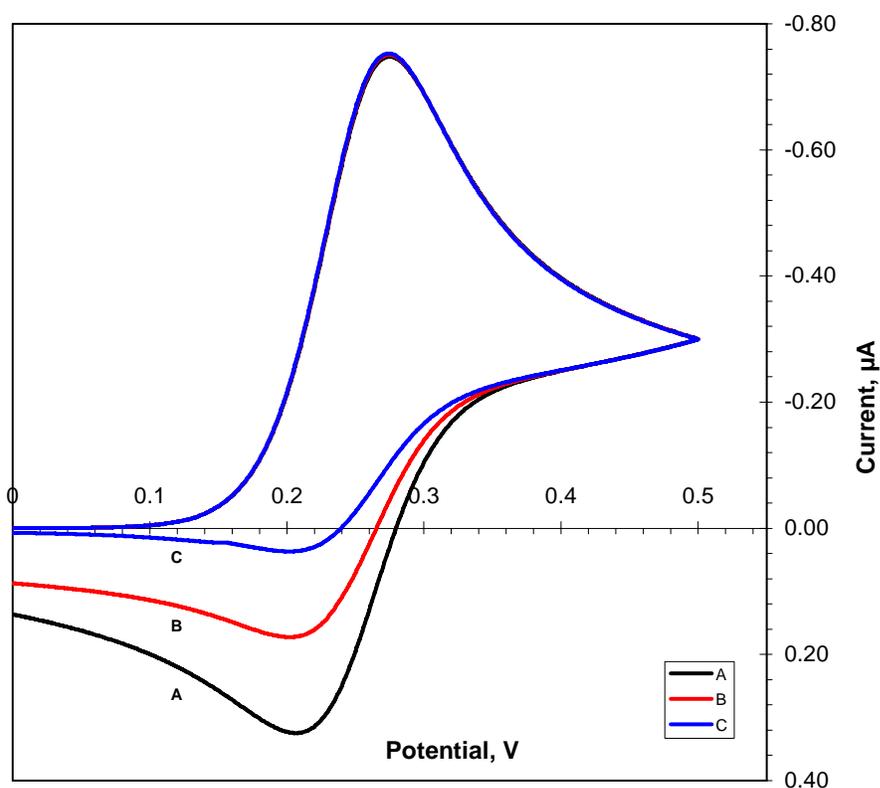


Figure 1. Computer simulated CV waves for an *ec* mechanism.  
 A) Reaction 1 is reversible,  $k_f = 0$ ;  
 B)  $k_f = 0.30$  and  $k_f \gg k_b$ ; C)  $k_f = 1.0$  and  $k_f \gg k_b$ .  
 Simulated for 1 mM of R species, electrode area = 0.010 cm<sup>2</sup>, scan rate 100 mV/s.

When there is a follow-up chemical reaction, as in the case of a *ec* mechanism, and the  $k_f$  of reaction (2) is finite, Ox will be converted to P. This results in less Ox so that the magnitude of  $I_{pc}$  diminishes during the reverse scan (see CV wave B in Figure 1). The time window to capture Ox is determined by the scan rate. The consequence of an *ec* mechanism, where  $k_f \gg k_b$  is illustrated in curve C of Figure 1. The parameters used in computer simulations of the theoretical CV waves, shown in curves A, B and C in Figure 1, are listed under the captions.

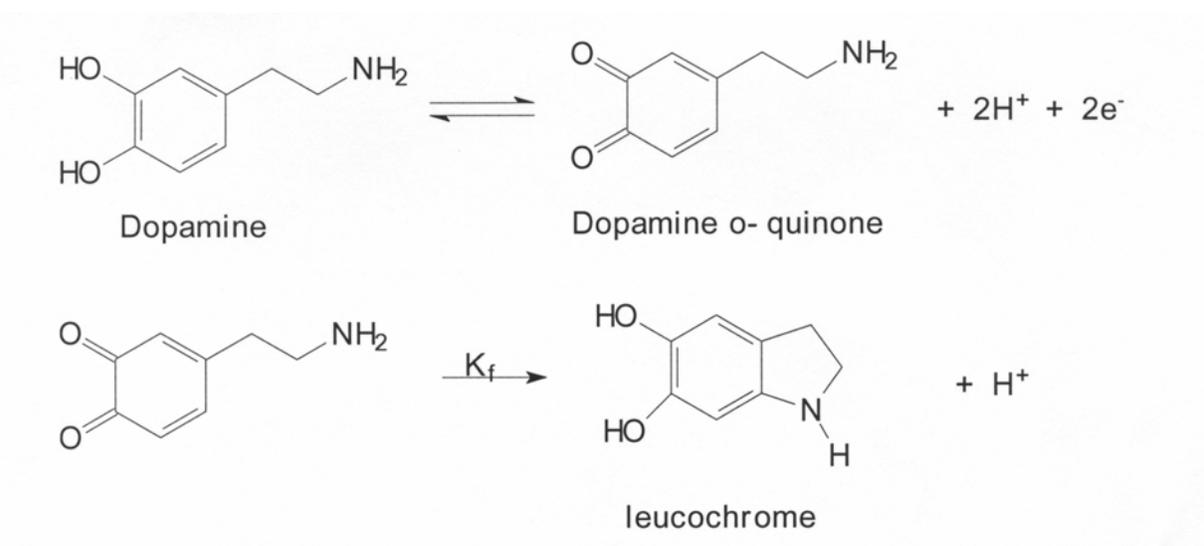
If Ox is long-lived,  $I_{pc}$  appears even at slow scan rates. If Ox is very short-lived, it may not be seen even at very fast scan rates. CV is uniquely able to adjust the time window of observations by the scan rate. Several potentiostats are commercially available that are capable of scanning at very high rates so that  $k_f$  values as high as  $10^5 - 10^6$  l/m/s can be determined.

The ratio of the currents,  $I_{rev}/I_{fwd}$ , can be conveniently measured by the empirical method of Nicholson [ref. 6,7], that requires the evaluation of  $I_{pa}$ ,  $I_{pc}$ , and  $I_\lambda$ , where  $I_\lambda$  is the value of the current at the switching potential,  $E_\lambda$  (where the direction of the CV scan is reversed). These quantities are then used to obtain the current ratio,  $I_{rev}/I_{fwd}$  in equation 3.

$$I_{rev}/I_{fwd} = I_{pc}/I_{pa} + 0.48 I_\lambda / I_{pa} + 0.086 \quad (3)$$

This current ratio is used to calculate the apparent rate constant,  $k_f$ , for the follow-up chemical reaction,  $Ox \rightarrow P$  from the theoretical working curve.

**Mechanism study on Dopamine and Norepinephrine Neurotransmitters:** We can apply CV to determine the *ec* oxidative characteristics of the catecholamines, dopamine and norepinephrine, whose structures are shown below. The catecholamines are oxidized to the corresponding quinone, as illustrated for dopamine. Following the initial oxidation, a pH dependent, 1,4-addition reaction can occur, forming a cyclized product called a leucochrome. At low pH values, the open-chain quinones are protonated to a great extent, and the cyclization reaction is unfavorable. At higher pH values, a sufficient amount of unprotonated quinone is available so that cyclization is observed.



The leucochrome formed in the vicinity of the electrode following the 2-electron oxidation can be observed as a reductive wave at potentials negative of that for the reduction of the non-cyclized quinone. The extent of leucochrome formed is indicated by the ratio of the forward,  $I_{pa}$ , to reverse-scan,  $I_{pc}$ , peak currents for the catecholamine and its corresponding quinone. Further, it is possible to calculate the relative rates at which each cyclization takes place by evaluating the peak currents at different scan rates.

## EXPERIMENT

### Equipment

- See the laboratory instructor about what potentiostat is available and the method of data acquisition (i.e., x-y recorder with analog potentiostat or printed output with a computerized potentiostat).
- Electrochemical cell and electrodes
  - 1.0 or 3.0 mm diameter flat tipped glassy carbon electrode
  - Pt auxiliary electrode
  - Ag/AgCl reference electrode
  - Small volume electrochemical cell
  - Polishing kit
- Timer

### Chemicals

- A. Dopamine [recommend the HCl salt of DA, F. W. 189.64]
- B. Norepinephrine [recommend the HCl salt of NE, F. W. 205.64]
- C.  $\text{Na}_2\text{HPO}_4$  and citric acid for making McIlvaine buffer
- D. Sulfuric acid (1 M)

### Procedure

1. Prepare a solution of dopamine (DA) at a pH near 1.0 by adding approximately 10 mg of solid DA to a 50.00 ml volumetric flask and dissolving it in 1 M sulfuric acid. Record the actual mass of DA. Mix well by shaking the flask. Handle catecholamines with extreme care, as they can have severe physiological effects!
2. Place about 10 ml of this solution into an electrochemical cell and deoxygenate for ~ 10 minutes with nitrogen or argon.
3. Prepare a glassy carbon electrode (1 mm or 3 mm diameter) by polishing it for ~ 1 minute on 0.05  $\mu\text{m}$  alumina. Use a gentle circular motion or trace a figure 8 on a polishing pad that has the alumina on the surface. Clean the electrode carefully with distilled water, sonicate for 10-15 seconds, and then touch the edge with a Kimwipe™ before introducing the electrode into the cell.
4. Please ask laboratory instructor for the directions to set-up and operate the potentiostat.
5. Record cyclic voltammograms for this solution at scan rates of 50 and 100 mV/s between an initial potential of 0.00 V and a positive potential limit of +1.00 V. Make duplicate runs at each scan rate. Measure the pH of the solution before discarding.
6. Prepare a 1 mM solution of dopamine at ~ pH 7.0 by dissolving ~10 mg in a 50.00 ml volumetric flask. Record the actual mass of DA. Pipette 5.0 ml of 0.1 M citric acid, then dilute to mark with 0.2 M disodium phosphate. Together, these ingredients comprise the McIlvaine buffer.
7. Degas the solution and repolish the electrode as previously instructed (see #3 above).
8. Record cyclic voltammograms for dopamine in this pH 7.0 solution at scan rates of 50, 100, 150, 200, 250 and 300 mV/s, adjusting the x-axis (current) sensitivity scale as needed to record the entire I-t curves. Set the scan limits so that you start at -100 mV and scan the potential anodically to the limit of +700 mV, reverse back to -800 mV and end up at -100 mV [sequence of limits: -100, +700, -800 and stop at -100 mV]. Record the pH of the solution before discarding.

- Prepare a 1 mM solution of norepinephrine (NE) near pH 7.0 by dissolving ~11 mg in 50.00 ml of McIlvaine buffer. Record the actual mass of NE.
- Degas the solution and repolish the electrode. Record CV scans of NE at 50 mV/s and 400 mV/s with the same potential limits as in step #7. Run duplicates of the CV scans. Record the pH of this solution before discarding.

**Calculations:**

Table 1

Theoretical Values for the Ratio of Reverse to Forward Peak Currents for Charge Transfer Followed by an Irreversible Chemical Reaction

$k_f t$	$i_{rev} / i_{fwd}$
0.004	1.00
0.023	0.986
0.035	0.967
0.066	0.937
0.105	0.900
0.195	0.828
0.350	0.727
0.525	0.641
0.550	0.628
0.778	0.551
1.050	0.486
1.168	0.466
1.557	0.415

- Use the Nicholson equation to calculate the value of  $i_{rev}/i_{fwd}$  from the CV scans for dopamine at pH 1.0 and at pH 7.0, recorded at the scan rates of 50, 100, 200 and 300 mV/s. Next, do the same calculations for the two CV scan rates with norepinephrine.
- You will need to determine the time,  $t$ , in seconds that it takes to scan the potential from the  $E_{1/2}$  value to the switching potential,  $E_{\lambda}$ , of each cyclic voltammogram. This value of  $t$  will be different for each of the scans (remember that the time it takes is dependent on the distance along the potential axis and the scan rate). The  $E_{1/2}$  value is the potential at  $1/2$  the peak current.
- Table 1 shows the  $i_{rev}/i_{fwd}$  values as a function of the theoretically calculated  $k_f t$  values, as determined by Nicholson. Plot the  $(i_{rev}/i_{fwd})$  vs.  $\log(k_f t)$  to make a working curve. Interpolate the points to obtain a smooth curve.
- Expand the appropriate region of the working curve corresponding to each experimental value of  $(i_{rev}/i_{fwd})$  and measure the value of  $\log(k_f t)$  for each of your current ratios from the working curve. Next, use the experimentally determined value of  $t$  at each of your scan rates to calculate a value of  $k_f$ . Calculate the average value of  $k_f$  for DA and NE.
- The literature values for  $k_f$  are  $0.038 \text{ s}^{-1}$  and  $0.36 \text{ s}^{-1}$  for DA and NE, respectively [ref. 8].
- How close are your values to those in the literature?

Postscript: The *c* step (cyclization) is first-order and irreversible for the oxidized product of both dopamine and norephenephrine. Other examples of this *ec* mechanism are the compounds of *p*-aminophenol and catechol – they undergo a 2-electron electrooxidation. The triol, produced by the *c* step in the case of catechol, is readily oxidized to the quinone form at potentials less positive than the parent.

## REPORT (Data Analysis and Discussion):

Please consult with your supervisor about the content and format of the laboratory report.

Questions for discussion –

1. How close are your  $k_f$  values to those of the literature? If your results deviate by more than 10% from the literature value, can you offer explanation for the deviations?
2. Did your  $E_{pa}$  and  $E_{pc}$  values vary as a function of scan rate? If yes, what reasons can you offer to explain these variations?
3. Show plot of  $I_{pa}$  vs. square root of scan rate for DA in the acidic solution. From the slope of the plot, calculate the value of the diffusion coefficient. [Hint: the calculated value should like between  $0.5$  and  $1.0 \times 10^{-5} \text{ cm}^2/\text{s}$ .]
4. Report the average  $E^\circ$  value for dopamine and norepinephrine.
5. Why is the kinetic rate of the follow-up chemical reaction pH dependent?

## REFERENCES

1. The Merck Index, 11<sup>th</sup> Ed., P. 538, #3415 (1988).
2. <http://en.wikipedia.org/wiki/Dopamine>
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8. M.D. Hawley, S.V. Tatawawadi, S. Pierkarski, R. N. Adams, *Electrochemical Studies of the Oxidation Pathways of Catecholamines*, J. Am. Chem. Soc., 89 (1967) 447-450.

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## General References

1. W. E. Geiger, "Instructional Examples of Electrode Mechanisms in Transition Metal Complexes," in Laboratory Techniques in Electroanalytical Chemistry, 2nd Ed., Editors P. T. Kissinger and W. R. Heineman; Marcel-Dekker, NY 1996, pp. 683-717.
2. The research of Professor Mark Wightman (University of North Carolina, Chapel Hill, NC) has focused on understanding the physiological role of dopamine and related catecholamines in the brain. Wightman has been a leader in the development of electroanalytical methods involving fast scan CV with microelectrodes for detection and quantitation of these compounds. A list of his publications on the subject can be seen by going to the "application" section of [www.cypresssystems.com](http://www.cypresssystems.com) and clicking on Mark Wightman//66-EI400.
3. To computer simulate CV curves for various electrode mechanisms, an online program is available as developed by Professor Vining and linked as ASDL site #005005 titled "Cyclic Voltammetry Simulator." The simulation program can be downloaded from this site: <http://employees.oneonta.edu/viningwj/>

## ACKNOWLEDGMENT:

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## COMMENTARY:

Professor Mark Wightman, UNC, Chapel Hill, NC, has focused on understanding the physiological role of dopamine and related catecholamines in the brain. He has been a leader in the development of Electroanalytical methods involving fast scan CV with microelectrodes to detect and quantify these compounds. Reference #3 gives a website reference to the research of the UNC group.