A Simple But Highly Selective Electrochemical Sensor for Dopamine

R. Doyle,* C. B. Breslin, and A. D. Rooney
Department of Chemistry, NUI Maynooth,
Maynooth, Co. Kildare, Ireland

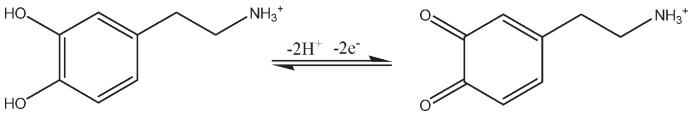
Original scientific paper
Received: July 10, 2008
Accepted: September 3, 2008

A modified platinum electrode was fabricated by the electropolymerization of pyrrole using a sodium *p*-sulphonatocalix[6]arene as the supporting electrolyte. The modified electrode acts as a reasonably sensitive electrochemical sensor for dopamine giving a linear calibration curve in the range 0.075 – 1.00 mM dopamine. The sensor shows no ability to sense the common interferent ascorbic acid, therefore the concentration for dopamine can be directly sensed in a large excess of ascorbic acid with no need to make adjustments for the signal for ascorbic acid. Investigations are included to study the mode of sensing of the modified electrode.

Key words:
Modified electrode, dopamine sensor, sulphonated calixarene

Introduction

Dopamine is a naturally occurring catecholamine that acts as an important neurotransmitter. Dopamine plays an integral role in the function of the central system; it affects brain functions that control movement, emotions, and the ability to feel pleasure and pain. Abnormalities in dopamine concentrations have been linked to a number of conditions, such as Parkinson's disease and Schizophrenia. Also, dopamine plays a central role in the mechanism of action of major substances of abuse – leading to drug addiction. Accordingly, the development of simple materials that are both highly selective and sensitive to dopamine is an important goal for neurochemistry. Currently, the most established method for determining dopamine levels in urinary and plasma samples is high performance liquid chromatography.[1] Chromatography is a reasonably complex, relatively expensive technique. Thus, as dopamine is reversibly oxidised, (eq. 1) its concentration can be determined using electrochemical means and there is much interest in developing a fast, simple to use, electrochemical sensor for dopamine. Moreover, as the electrodes used in the sensing can be miniaturised they can be conveniently placed in the living organism to give data at the sub-second timescale. Many materials have been considered for the modified electrodes, including; nafion-coated electrodes,[2] cysteamine nanosensors,[3] titanate-nanotubes,[4] carbon nanotubes[5] and fullerenes.[6] However, many of these electrode systems exhibit certain drawbacks. Firstly, dopamine exists in the body with other biomolecules, particularly ascorbic and uric acid, which are both oxidised in the same potential region as dopamine. Generally, the modified electrodes listed above function by separating the oxidation waves of ascorbic acid and dopamine. Secondly, dopaminoquinone, the oxidation product of dopamine shown in eq. 1, can undergo a series of reactions to form melanin polymers that will foul the electrode surface. Currently, there is much interest in trying to develop systems that will overcome these problems.



Eq. 1 – *The oxidation of dopamine to dopamineoquinone*

Herein we describe a modified electrode system based on a calix[6]arene anion (Fig. 1) which is doped into a polypyrrole backbone. A calixarene is a macrocycle based on a product of a phenol and an aldehyde. They have hydrophobic cavities that can include molecules or ions, and a number of studies have shown that calixarenes can form inclusion complexes with dopamine and related chemicals.[7–9] In addition, there have been a number of studies

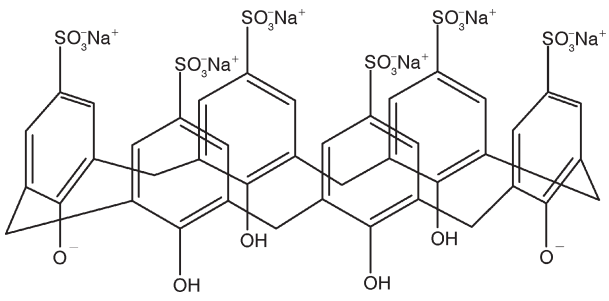


Fig. 1 – *Structure of p-sulphonatocalix[6]arene*

*Corresponding author: Richard Doyle
Email: RICHARD.L.DOYLE@nuim.ie; Tel.: 0035317083770

showing that calixarenes can be incorporated into a conducting polymer matrix[10–11] and in some cases the calixarene retains its sensing properties.[10] Encouragingly, one sensor based on a non-biocompatible conducting polymer has shown a high level of sensitivity towards dopamine.[12] Our strategy was that the calixarene doped into the biocompatible polypyrrole would recognise dopamine, while the negative charge located on its upper ring would repel the anionic interferents – ascorbic and uric acid. The results outlined in this paper would indicate that this is the case, and a highly selective, reasonably sensitive, electrochemical sensor for dopamine has been developed.

Experimental

Reagents and apparatus

Pyrrole was obtained from Sigma-Aldrich and purified by distillation before use. *p*-sulphonatocalix[6]arene was synthesised according to the procedure outlined by Shinkai and co-workers.[13] The crude product was carefully purified by extraction into singly distilled water. The water was then removed under vacuum and the product was precipitated from ethanol. All other reagents used were of analytical grade and were obtained from Sigma-Aldrich.

All electrochemical experiments were carried out in a single compartment three-electrode cell using a Solartron 1285 potentiostat. A platinum electrode (4 mm diameter) was used as the working electrode. Platinum wire and a saturated calomel electrode (0.241 V vs. SHE) were used as the counter and reference electrodes respectively. All cyclic voltammetry experiments were run using a scan rate of 100 mV s^{-1}. The background electrolyte was 0.1 M Na_2SO_4.

All NMR experiments were carried out in deuterated water on a Bruker Avance 300 MHz spectrometer operating at 300.13 MHz for ^1H.

Preparation of the *p*-sulphonatocalix[6]arene/polypyrrole modified electrode

The *p*-sulphonatocalix[6]arene/polypyrrole film was electrosynthesised from an aqueous solution containing pyrrole (0.2 M) and *p*-sulphonatocalix[6]arene (0.01 M). Prior to each experiment the platinum working electrode was polished using diamond paste and cleaned in an ultrasonicator. The film was then grown potentiostatically at 0.5 V to a charge of 0.03 C.

NMR titration and Job's plot

All NMR experiments were carried out in excess KCl (0.3 M) to ensure that any chemical shift changes observed were not due to ionic strength increases in the solution. For the titration, the concentration of dopamine was kept constant (0.03 M) while the concentration of *p*-sulphonatocalix[6]arene was increased from 0 to 0.15 M. Using Job's method, solutions with different molar ratios of dopamine and *p*-sulphonatocalix[6]arene were prepared while maintaining a constant total dopamine + *p*-sulphonatocalix[6]arene concentration. As the solutions were not buffered, control experiments were carried out to ensure that the changes in the chemical shifts of the signals for dopamine did not alter significantly over the pH range associated with the titration.

Results and discussion

Modification of electrodes

The polymer was grown under a range of conditions and its ability to sense dopamine was investigated (discussed in section "Sensing studies"). From these experiments it was determined that growing the polymer at a potential of 0.5 V to a charge of 0.03 C were the optimum conditions in terms of sensor sensitivity and stability. The sensing studies showed that cyclic voltammograms of dopamine using polymers grown at a potential of 0.5 V gave the most stable signal. The signal for dopamine decayed rapidly with successive cycles when polymers grown at higher potentials were used. We believe the reason for this to be the irreversible oxidation of the calixarene. Fig. 2 shows the current response of a solution of calixarene, in

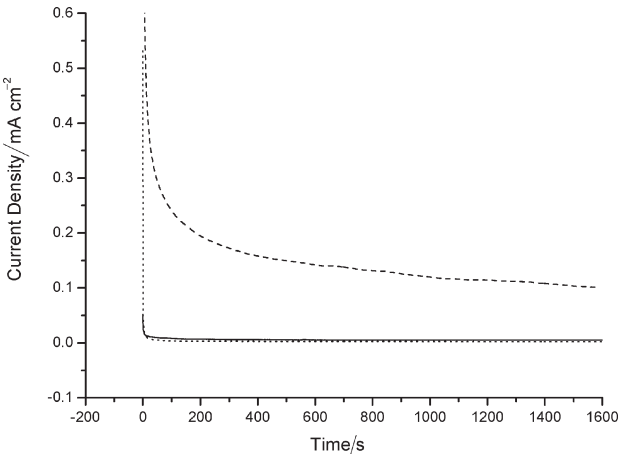
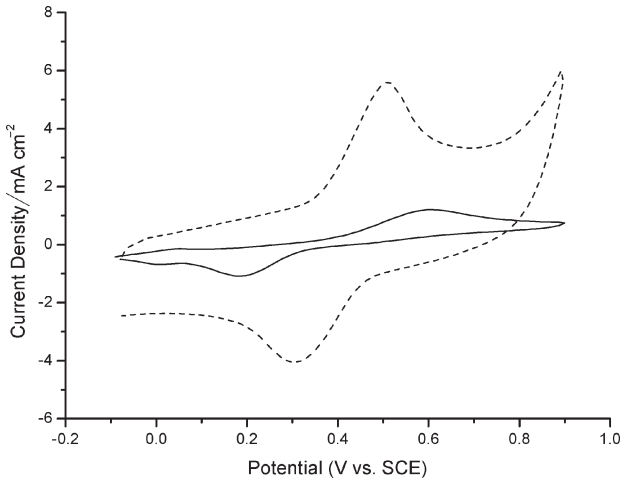


Fig. 2 – *Current response over time for a solution of p-sulphonatocalix[6]arene (0.01 M) in aqueous Na_2SO_4 (0.1 M) at a bare platinum electrode at a constant potential of 0.5 V (——) and 0.8 V (– – –) and for the background electrolyte at a bare platinum electrode at a constant potential of 0.8 V (.....)*

aqueous 0.1 M Na_2SO_4, at a bare platinum electrode held at a constant potential of 0.5 V and 0.8 V. At 0.5 V the current is practically zero whereas at 0.8 V the current is significantly higher. The current response of the background electrolyte at 0.8 V was measured to ensure the current increase was not due to the higher applied voltage. We can conclude that the increase in current is a result of the electroactivity of the calixarene at this potential.

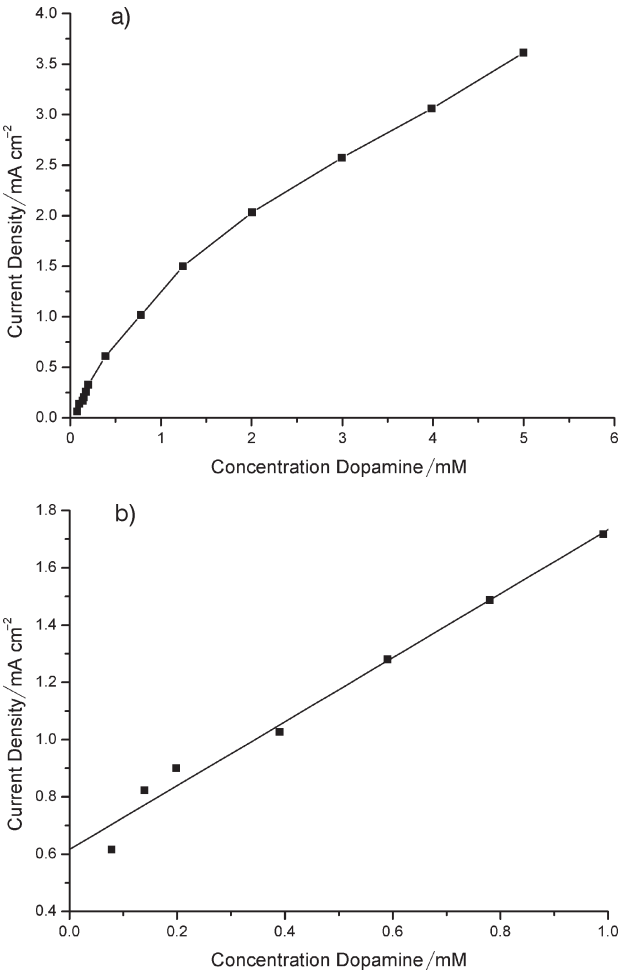
Sensing studies

The ability of the modified electrode to detect the electrochemical process of dopamine was studied using cyclic voltammetry. Analysis of the anodic and cathodic signals shows that at a bare platinum working electrode the process is quasi-reversible (ΔE_p = 426 mV). Fig. 3 shows a comparison of the electrochemical process for dopamine at the unmodified and the modified electrode. Two effects of the polymer can be observed. Firstly, the signal is significantly increased at the modified electrode compared to the unmodified electrode. Secondly, the peak separation is reduced to 200 mV at the modified electrode thereby increasing the reversibility of the electrochemical process. From these observations it can be concluded that the polymer has a kinetic and catalytic effect on the detection of dopamine.



F i g . 3 – *Cyclic voltammograms of dopamine ($5 \cdot 10^{-3}$ M) at the bare platinum electrode (——) and the modified electrode (– – –). The background electrolyte is 0.1 M Na_2SO_4 (pH ~ 6).*

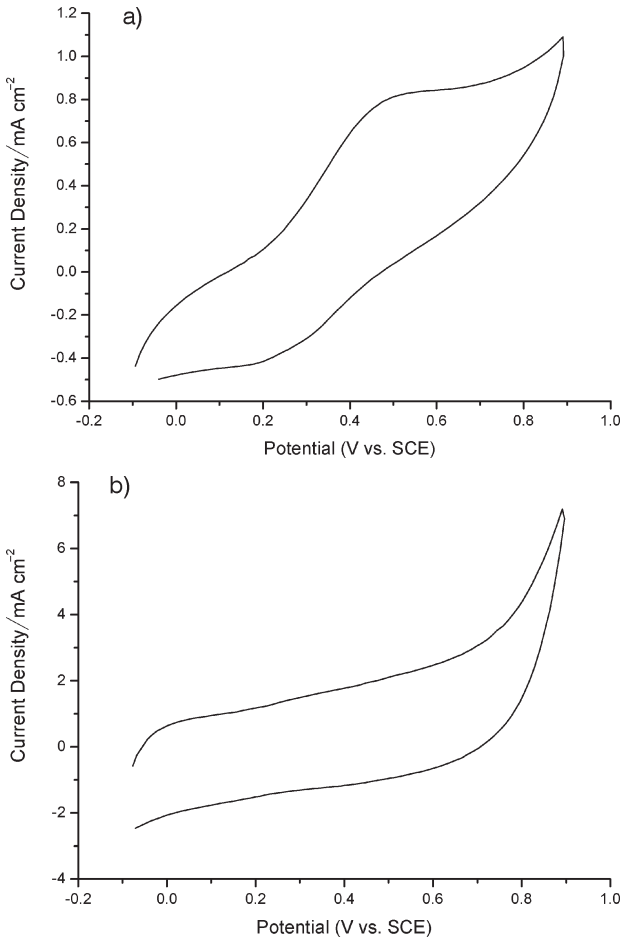
Cyclic voltammetry was carried out on dopamine at the modified electrode as a function of concentration. A plot of the current density of the anodic peak of dopamine against concentration is given in Fig. 4a. The detection limit was found to be $2 \cdot 10^{-5}$ M and the plot displayed a linear relationship within the range $7.5 \cdot 10^{-5}$ to $1.0 \cdot 10^{-3}$ M dopamine (Fig. 4b).



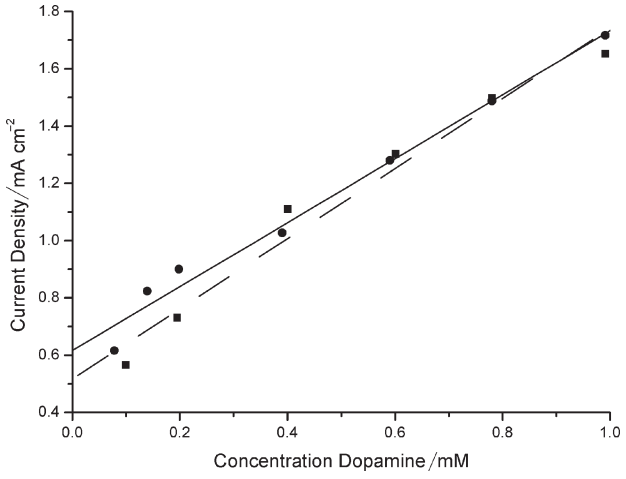
F i g . 4 – *(a) Calibration curve for dopamine in the concentration range $7.5 \cdot 10^{-5}$ M to $5 \cdot 10^{-3}$ M at the modified electrode. (b) Linear region of the calibration curve ($r^2 > 0.99$).*

Cyclic voltammetry studies were then carried out to investigate the ability of the sensor to detect ascorbic acid. Fig. 5 shows a comparison of the electrochemical process for ascorbic acid at the bare platinum and modified electrodes. Strikingly, it can be observed that no signal is observed for ascorbic acid at the modified electrode. This would suggest that our hypothesis, that the negative charge at the upper rim of the calixarene would repel this negatively charged interferent and prevent it being sensed by the modified electrode, is correct.

Further studies were then carried out using cyclic voltammetry to investigate if the presence of ascorbic acid in the solution would alter the modified electrodes ability to sense dopamine. Current densities for the anodic peak of dopamine in the presence of $1 \cdot 10^{-2}$ M ascorbic acid were recorded as a function of dopamine concentration. The plot of the peak current densities for the range $7.5 \cdot 10^{-5}$ to $1 \cdot 10^{-3}$ M dopamine in the presence and absence of the $1 \cdot 10^{-2}$ M ascorbic acid are given in Fig. 6. It can be observed from the plot that the presence of



F i g . 5 – *Cyclic voltammograms of ascorbic acid ($5 \cdot 10^{-3}$ M) at (a) the bare platinum electrode and (b) the modified electrode. The background electrolyte is 0.1 M Na_2SO_4 (pH ~ 6).*



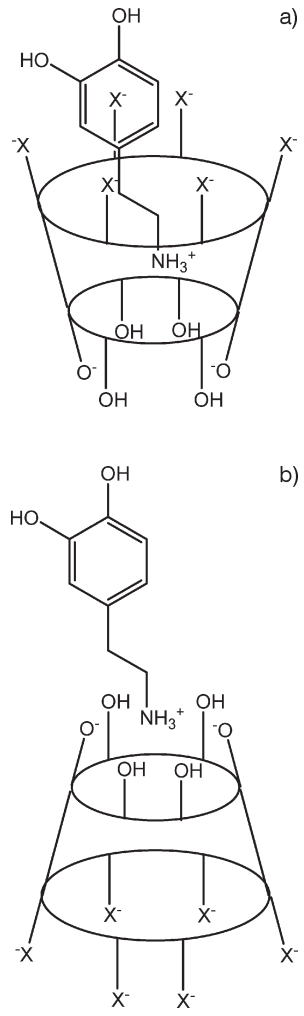
F i g . 6 – *Linear calibration plot for dopamine (——, data points ●) and dopamine + 0.01 M ascorbic acid (– – –, data points ■)*

$1 \cdot 10^{-2}$ M ascorbic acid had essentially no effect on the ability of the sensor to detect dopamine.

Encouragingly, these studies indicate that we have developed a sensor that is highly selective towards dopamine.

Mode of sensing

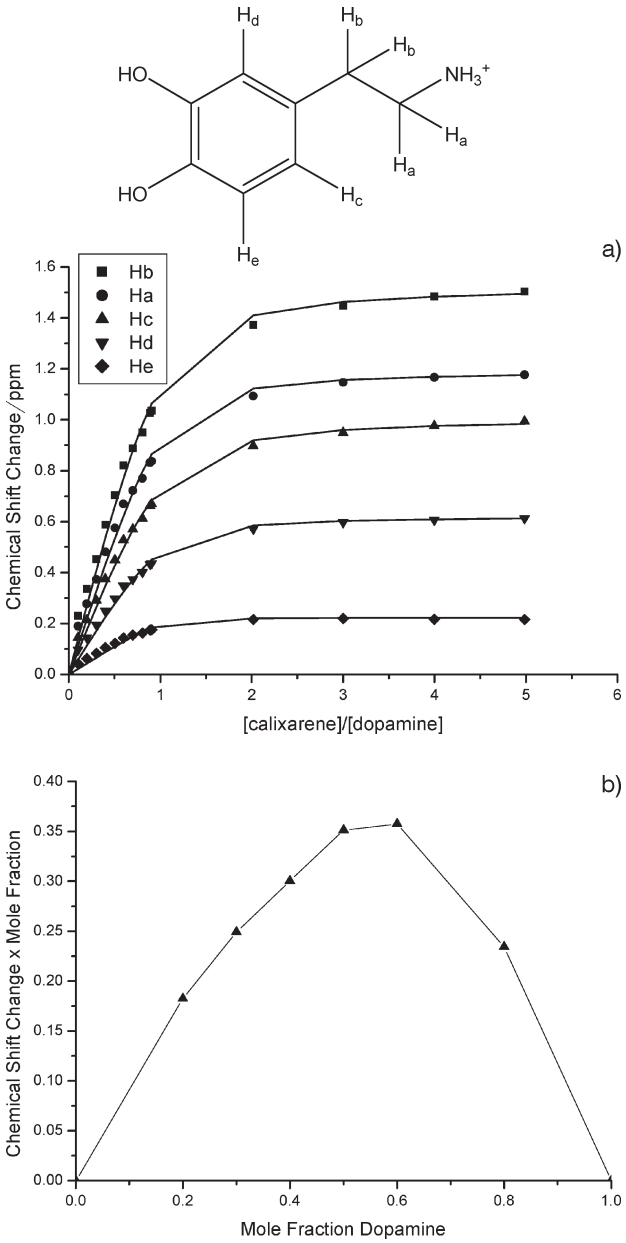
As previously stated, we believe that the inability of the sensor to detect ascorbic acid is due to electrostatic repulsion between the negatively charged calixarene and ascorbic acid. The sensing studies (previous section) have shown that the polymer has a catalytic and kinetic effect on the electrochemical process for dopamine. It is likely that this effect is due to a favourable interaction between dopamine and the calixarene resulting in a pre-concentration of dopamine at the polymer surface. Two possibilities exist for this interaction. Firstly, at the experimental pH (~ 6) dopamine is predominately in its protonated form[14] and the calixarene exists as an octaanion[15](two lower rim hydroxyl protons are dissociated). Therefore, dopamine could be electrostatically attracted to the calixarene, forming an *exo*-complex (Fig. 7a). Secondly, the formation of an inclusion, or *endo*-complex, (Fig. 7b) between dopamine and the calixarene may occur. NMR and cyclic voltammetric studies were used to determine the nature of this interaction.



F i g . 7 – *Dopamine/calixarene Endo-complex (a) and Exo-complex (b)*

NMR studies

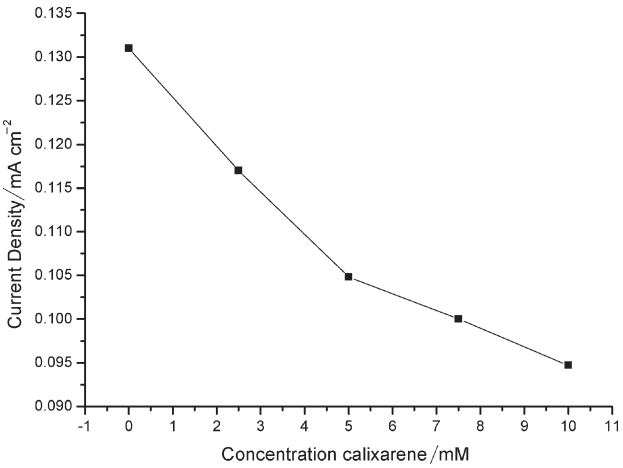
The host-guest properties of the interaction between the calixarene and dopamine were investigated using NMR titration. Fig. 8a shows a plot of the change in chemical shift for the proton signals of dopamine as a function of calixarene/dopamine molar ratio. The large upfield changes in chemical shift are characteristic of inclusion complex formation. It has been reported that various amines can form *exo*-complexes with calixarenes[16,17] and in these cases, the proton signals have shifted in the opposite, downfield, direction. The order of magnitude of these changes Hb>Ha>Hc>Hd>He indicates that dopamine is included through the protonated amine. The stoichiometry of the complex formed was obtained using Job's method. The Job's plot (Fig. 8b) shows a maximum value at 0.6 mole fraction of dopamine. This corresponds to a ratio of 1:1.5 for calixarene:dopamine and suggests that a mixture of 1:1 and 1:2 complexes are formed. The magnitude and direction of the chemical shift change for dopamine upon addition of the calixarene would suggest a partially included species is formed.



F i g . 8 – *(a) Experimental (data point) and theoretical (——) titration data for the NMR proton signals of dopamine labeled in the structure shown. (b) Job's plot for the complex formed between dopamine and the calixarene.*

Cyclic voltammetry

Cyclic voltammetry experiments were also carried out to investigate the interaction between the calixarene and dopamine. The experiments were carried out on solutions of dopamine ($5 \cdot 10^{-4}$ M) and the concentration of calixarene was altered from 0 to $1 \cdot 10^{-2}$ M. Fig. 9 shows a plot of current density for the anodic signal against calixarene concentration. As can be observed from Fig. 9, the current density for the dopamine signal decreases upon addition of the calixarene. This indicates that the calixarene is slowing the rate at which the dopamine diffuses to the electrode and is consistent with the results obtained from the NMR studies, which show that, in solution, dopamine forms a partial inclusion complex with the calixarene.



F i g . 9 – *Plot of current density for the anodic signal for dopamine as a function of calixarene concentration*

Conclusion

In terms of ease of fabrication and selectivity for dopamine over the common interferent ascorbic acid, the platinum electrode modified with polypyrrole doped with *p*-sulphonatocalix[6]arene is an improvement over most of the electrochemical dopamine sensors produced to date. However, the sensor currently detects dopamine at concentrations of 0.02 mM and therefore studies are continuing to

improve its sensitivity. Studies on the mode of sensing indicate that there is a specific interaction between the dopamine and the calixarene. The most likely interaction is that the dopamine forms a partial *endo*-inclusion complex with the calixarene.

References

1. a) Tsunoda, M., Chromatography **26** (2005) 95.
   b) *Magnusson, O., Nilsson, L. B., Westerlund, D.,* J. Chromatogr. **582** (1992) 1.
2. *Alpat, S. Alpat, S. K., Telefoncu, A.,* Anal. Bioanal. Chem. **383** (2005) 695.
3. *Shervedani, R. K., Bagherzadeh, M., Mozaffari, S. A.,* Sens. Actuators B **115** (2006) 614.
4. *Liu, A., Wei, M., Honma, I., Zhou, H.,* Adv. Funct. Mater. **16** (2006) 371.
5. *Alarcón-Angeles, G., Pérez-López, B., Palomar-Pardave, M., Ramírez-Silva, M. T., Alegrat, S., Merkoçi, A.,* Carbon **46** (2008) 898.
6. *Goyal, R. N., Gupta, V. K., Bachheti, N., Sharma, R. A.,* Electroanalysis **20** (2008) 757.
7. *Oshima, T., Oishi, K., Ohto, K., Inoue, K.,* J. Inclusion Phenom. Macrocyclic Chem. **55** (2006) 79.
8. *Zhang, S., Echegoyen, L.,* Org. Lett. **6** (2004) 791.
9. *Takashi, J.,* J. Inclusion Phenom. Macrocyclic Chem. **45** (2003) 195.
10. *Bidan G., Niel, M. A.,* Synth. Met. **85** (1997) 1387.
11. *Buffenoir, A., Bidan, G.*, Synth. Met. **102** (1999) 1300.
12. *Zhang, Y., Jin, G., Wang, Y., Yang, Z.,* Sensors **3** (2003) 443.
13. *Shinkai, S., Mori, S., Koreishi, H., Tsubaki, T., Manabe, O.,* J. Am. Chem. Soc. **108** (1986) 2409.
14. *Corona-Avendańo, S., Alarcón-Angeles, G., Rosquete-Pina, G. A., Rojas-Hernández, A., Gutierrez, A., Ramírez-Silva, M. T., Romero-Romo, M., Palomar-Pardavé, M.,* J. Phys. Chem. **111** (2007) 1640.
15. *Scharff, J. P., Mahjoubi, M.,* New J. Chem. **15** (1991) 883.
16. *Gutsche, C. D., Iqbal, M., Alam, I.,* J. Am. Chem. Soc. **109** (1987) 4314.
17. *Puchta, R., Clark, T., Bauer, W.,* J. Mol. Model **12** (2006) 739.