

Electrochemical Activity Determination of Catechol-O-methyl Transferase by Selective Dopamine Detection

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For the treatment of Parkinson's disease, as one of the most frequent diseases of the central nervous system, several key enzymes for dopamine metabolism [e.g. catechol-O-methyl transferase (COMT)] are drug targets. For an efficient and long-lasting treatment, the activity of this enzyme should be monitored. In this study, an electrochemical approach using differential pulse voltammetry (DPV) is introduced for the activity determination. The applied electrode material, fluorine-doped tin oxide (FTO), is characterized by a clear discrimination between substrate and product of COMT, a high stability of the dopamine signal during consecutive measurements, and a linear dependency on the dopamine concentration in the range

of the maximum reaction rate of COMT. Despite these advantageous results, dopamine detection in the complete activity assay is influenced by each of the added essential assay components, even though none of the added components reveal a current signal at the FTO electrode itself. After adjusting the potential range and the assay composition, these effects can be circumvented. By following the dopamine concentrations during COMT action, it can be shown that the activity of COMT can be detected by using differential pulse voltammetry (DPV) at an FTO electrode and, by analyzing different COMT amounts, quantification can be demonstrated.

1. Introduction

Morbus Parkinson is the second most frequent disease of the central nervous system. This neurodegenerative disease is characterized by a slow, but progressive deterioration of the health status of affected patients caused by the successive dying of dopaminergic cells. Consequently, certain areas of the brain reveal a deficiency of dopamine. This lack leads to movement disorders, which are the most typical symptoms of Parkinson's disease (PD).^[1] Hence, for the treatment of this disease the defect of dopamine can be compensated by the administration of levodopa, the precursor of dopamine, which can pass the blood-brain-barrier. Dopamine acts not only as neurotransmitter, it is also a central component of the catecholamine metabolism. Due to the wide variety of biochemical pathways for synthesis and degradation of catecholamine-derived neurotransmitters the dopamine concentration in the brain is influenced by numerous enzymes. For the treatment of PD, the activity of enzymes, such as dopamine decarboxylase (DDC) or catechol-O-methyl transferase (COMT), can be controlled by inhibitory drugs. While the peripheral decarboxylation of levodopa can be blocked by inhibitors of the DDC (e.g.

carbidopa or benserazide) the O-methylation of dopamine in the brain can be prevented by the administration of inhibitors of the COMT (e.g. entacapone or tolcapone). In this way the bioavailability of dopamine can be improved.^[2] The efficiency of this inhibition however, decreases during a long-lasting treatment due the development of a tolerance^[3] and should be monitored by the determination of the activity of these enzymes. For that, a reliable detection of the substrate and/or the product of the enzyme conversion is needed.

The COMT metabolizes dopamine in the presence of the co-substrate S-adenosyl methionine into methoxytyramine and S-adenosyl homocysteine (Figure 1). Beside dopamine, it can also convert compounds with a similar structure, such as catechol, epicatechin, levodopa, adrenaline, noradrenaline, hydroxyestradiol and others.^[4–6]

In literature numerous procedures for the detection of dopamine and its derivatives are reported.^[7,8] Generally, dopamine and related catecholamines can be determined and discriminated by spectroscopic methods based on UV/Vis absorption,^[9] fluorescence^[10] and Raman scattering^[11] as well as

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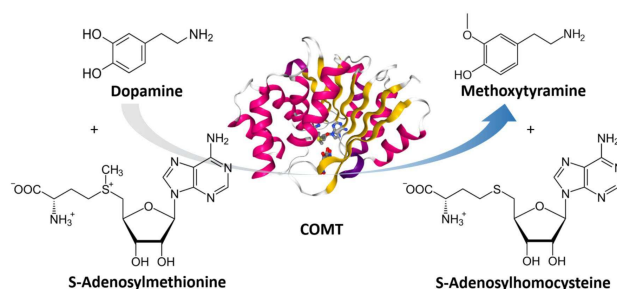


Figure 1. The reaction of catechol-O-methyl transferase (COMT): conversion of dopamine in the presence of S-adenosyl-methionine into methoxytyramine and S-adenosyl-homocysteine.

by FTIR^[12] or mass spectrometry.^[13] Furthermore, histochemical,^[14] electrophoretical,^[15] HPLC-based procedures^[16] or flow injection analysis^[17] can be used for the analysis of these biogenic amines.

Because of their redox activity catecholamines can also be detected by electrochemical sensing procedures. Beside cyclic voltammetry,^[18] square-wave voltammetry^[19] and amperometry^[20], especially differential pulse voltammetry^[21,22] is applied for a sensitive detection of dopamine. In order to provide a better selectivity and improve the lower detection limit towards the range of physiological concentrations, applications of enzymatic or electrochemical recycling systems have been successfully demonstrated.^[23,24]

The most often applied electrode materials for the electrochemical detection of dopamine are gold, several semiconductors (indium tin oxide) and carbon. Among the different materials, electrodes based on carbon reveal often a good sensitivity for the electrochemical sensing of this neurotransmitter. Hence, numerous sensor applications for dopamine apply glassy carbon, screen-printed carbon or carbon paste electrodes.^[25,26] A further increase of sensitivity of this kind of electrodes can be achieved by decoration of the electrode surface with nanomaterials such as carbon nanotubes,^[27,28] nanocrystals,^[29] graphene^[30] or metal nanoparticles.^[31]

However, these carbon derived electrode materials show also some drawbacks for the detection of catecholamines. One problem is the passivation of the electrode surface. After the electrochemical oxidation of dopamine a sequence of chemical and electrochemical secondary reactions of the oxidized dopamine leads to the formation of a melanin-like polymer at the electrode surface.^[32,33] This reduces not only the signal intensity for a certain analyte concentration, it also decreases the selectivity and specificity of the sensor construction.

Another critical problem is the influence of the dopamine signal by oxidation currents from interfering substances, which are frequently present in physiological samples. This can be solved by the implementation of selective layers on the electrode surface. For that, electrodes have been modified with Nafion[®],^[34,35] self-assembled monolayers at gold electrodes,^[36,37] polymer-films,^[38] surfactants,^[39] molecular imprinted polymers,^[40] amino acids and DNA.^[41] Also, the covalent modification of CNTs at the electrode surface by different functional groups or the noncovalent adsorption of aromatic compounds can improve the selectivity.^[42,43] SAM layers can also be advantageous for improved signal stability.^[44]

Additionally, the measuring signal can not only be disturbed by interferents as e.g. ascorbate or urate, but is also often superimposed by oxidation signals from dopamine precursors or degradation products. Frequently these related catecholamines have a similar redox behavior to dopamine. Whereas the aspect of redox interferents is devoted much attention in the literature, there are only a few studies investigating the electrochemical behavior of substrates and products from enzymes involved in the catecholamine metabolism. As above mentioned, the COMT is one key enzyme for control of the dopamine level in the brain. For an efficient electrochemical activity assay of this enzyme the applied sensor electrode has

to be able to distinguish clearly between the substrate and the product of the COMT catalyzed conversion reaction. Recently, for activity measurement of the COMT substrate and product of the enzyme conversion are separated via high performance liquid chromatography followed by an electrochemical detection of the single compounds.^[45,46] Another effective separation strategy exploits capillary electrophoresis for the detection of dopamine conversion.^[47]

Fluorine doped tin oxide (FTO) is one electrode material which can be found in actual sensor developments because of its good conductivity and transparency, thus appearing as a valuable alternative to indium tin oxide (ITO) electrodes.^[48,49] First studies on the electrochemical behavior of adrenaline, dopamine and other catecholamines at electrodes using FTO as sensing material have been reported recently.^[50] Most FTO based sensor constructions for the dopamine detection apply different modifications, reduced graphene oxide for instance, in order to separate the dopamine signal from interfering substances.^[51] Recently we could show that the application of FTO as electrode not only allows the suppression of the oxidation of ascorbic and uric acid, but provides a good discrimination between different dopamine derivatives.^[52] With this background the current study investigates the suitability of a FTO electrode for the electrochemical activity determination of the COMT enzyme. Here, the influence of different substances in the assay solution on the selective dopamine detection has been analyzed in detail and strategies to overcome disturbing effects on the voltammetric dopamine detection have been developed. Consequently, an electrochemical assay has been established allowing the quantification of different COMT enzyme activities.

Experimental Section

Materials

Acetone, isopropanol, ethanol, sodium chloride, sodium phosphate (NaH₂PO₄) were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Sodium hydroxide, potassium chloride and sodium chloride were provided by Merck KGaA (Darmstadt, Germany). HIS-Select-HF Nickel Affinity Gel (Ni-NTA agarose), dopamine and S-adenosyl methionine (SAM), dihydroxyacetophenone, magnesium chloride hexahydrate (Fluka) and boric acid (Riedel-deHaën) were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). All chemicals were used as delivered from the producer without any additional purification.

The catechol-O-methyl transferase was recombinantly produced by BioTez GmbH, Berlin-Buch, Germany. For that, the s-COMT (Uniprot P21964-2) gene was amplified from cDNA clone plasmid (Sino Biological), cloned into pET22b and transformed into *E. coli* BL21 (DE3). One single clone was used for an overnight culture (lysogeny broth medium (LB), 50 µg/ml ampicillin) which was inoculated into LB medium with ampicillin. At an OD₆₀₀ of approx. 0.6 the protein expression was induced by β-D-thiogalactopyranoside (0.4 mmol/l). After 5 h the cells were disrupted by sonication and the recombinant COMT was purified using His-Bind resin (JenaBiosciences). The concentration was determined by BCA assay (ThermoFisher).

For all aqueous solutions purified water from the water system "ultra clear direct" (SG Water, Germany) was used.

Methods

Electrochemical Procedures

All voltammetric measurements for the characterization of the FTO electrodes and for the development of the electrochemical activity assay were carried out with the potentiostat CHI 1230B from CH Instruments, Inc. (USA) using the differential pulse voltammetry (DPV) mode. For the DPV measurements, a pulse period of 100 ms, a step potential of 5 mV, a pulse width of 50 ms, a pulse amplitude of 30 mV and a sampling width of 16.7 ms were applied. The DPV measurements of the single compounds were done in a potential range from -50 mV to $+500$ mV vs Ag/AgCl, 1 M KCl. DPV measurement in the presence of S-adenosyl methionine were carried out with an upper potential limit of $+350$ mV vs Ag/AgCl, 1 M KCl.

Before usage, the FTO electrodes were purified by sonication in acetone, isopropanol and ethanol – each solvent for 15 min – using the ultrasonic bath Elmasonic S40 H from the Elma Schmidbauer GmbH (Germany). For the electrochemical investigation via DPV a home-made flow cell with a three-electrode measuring arrangement consisting of the FTO working electrode with a surface area of 50 mm^2 , a platinum wire as counter electrode and an Ag/AgCl, 1 M KCl reference electrode (Microelectrodes Inc., USA) were used. Between two measurements the electrodes have been rinsed with 20 mM potassium phosphate buffer (pH 7.2) applying a flow rate of 350 $\mu\text{L}/\text{min}$. In parallel, for the regeneration of the electrodes 50 voltammetric cycles with a scan rate of 100 mV/s in a potential range from -300 mV to $+500$ mV vs Ag/AgCl, 1 M KCl were applied.

COMT Coupling onto Ni-NTA

According to the coupling protocol from Sigma Aldrich Chemie (Germany) 50 μL of the methanol-Ni-NTA-agarose suspension was centrifuged 1 min with 5000 g at room temperature. After discarding the supernatant, the Ni-NTA agarose was equilibrated in 50 mM sodium phosphate buffer (pH 8.0) containing 200 mM NaCl, followed by a centrifugation step at 5000 g for 1 min. Then the supernatant was removed and 200 μL of the His-tagged COMT-solution (600 $\mu\text{g}/\text{mL}$) were added to the Ni-NTA agarose and incubated for 40 min at RT. After a further centrifugation at 5000 g for 1 min the supernatant was removed and controlled by the spectroscopic activity assay in order to ensure that no active enzyme remained in the supernatant. The agarose pellet was resuspended and centrifuged again at 5000 g for 1 min. Finally, the agarose pellet was resuspended in 400 μL , 200 μL , 133 μL or 100 μL 20 mM potassium phosphate (pH 7.2) obtaining 4 different COMT-Ni-NTA agarose suspensions with 30 μg , 60 μg , 90 μg and 120 μg when 100 μL of these suspensions are used.

Spectroscopic Activity Assay

For the verification of the results from the electrochemical activity determination a spectroscopic assay after Borchardt^[53] was applied. This test is the actual one used for the determination of COMT activity from enzyme producers and providers. As substrate dihydroxyacetophenone is used which has a significant smaller K_M -value than dopamine (8 μM vs. 200–260 μM).^[54–56] For this assay the COMT was incubated in 500 μL test solution containing 100 μM dihydroxyacetophenone, 1 mM S-adenosyl-methionine, 4 mM di-

thiothreitol and 1.2 mM magnesium chloride for 60 min at 37 °C. After stopping the reaction by adding 500 μL 0.4 M sodium borate the absorption at 344 nm was recorded. In the blank the S-adenosyl-methionine was replaced by water. The substrate conversion in nmol was obtained by multiplying the difference of the absorption at 344 nm with 63.1. This factor considers the extinction coefficient of dihydroxyacetophenone and its O-methylated products as well as the ratio of the two formed products.^[53]

Electrochemical Activity Assay

The assay composition for the electrochemical activity determination was derived from the spectroscopic assay. Since dopamine instead of dihydroxyacetophenone is used, the higher K_M -value of the COMT for this substrate has to be regarded. Hence, 500 μL of the assay solution, containing 1 mM dopamine, 1 mM S-adenosyl methionine (SAM), 400 μM dithiothreitol (DTT) and 120 μM magnesium chloride were incubated with different amounts of the COMT (bound onto Ni-NTA agarose) for 25 min and 50 min at 37 °C. After the separation of the Ni-NTA agarose bound COMT, the assay solution was led into the flow cell and the dopamine signal was recorded by DPV while the flow was stopped (Figure 2).

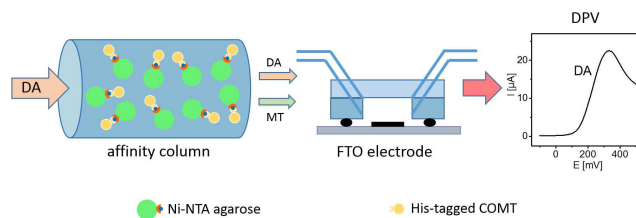


Figure 2. Principle of the stop-flow-system for the activity determination of the captured COMT by electrochemical dopamine detection.

2. Results and Discussion

The sensorial detection system for the enzyme activity focuses on a flow system which allows a selective detection of one reaction partner of the COMT-catalyzed reaction. The enzyme is intended to be separated from the biological matrix by an affinity column which shall be coupled to the sensorial detection unit (see Figure 2). The focus of the present study is however, not on the enzyme capturing from real samples such as blood, but the development of an electrochemical detection system allowing a quantification of the COMT activity. Thus, the investigations start with the electrode material and the analysis of factors which may influence the dopamine signal and comprises finally the detection of different activities of the COMT enzyme.

2.1. Basic Characterization of Dopamine Sensing at FTO Electrodes

First, a suitable electrode material for the electrochemical determination of COMT activity has to be found. With this respect not only the electrochemical behavior of the substrate

of the COMT, dopamine, but also the conversion product, methoxytyramine, are examined at a fluorine doped tin oxide (FTO) electrode. For these investigations differential pulse voltammetry has been used in order to reduce the charging current and to improve the voltammetric signal of dopamine oxidation. Additionally, for the sensorial application of the proposed enzyme activity test, the method can be easily integrated in simple potentiostats without raising significant costs.

The voltammogram of the inset in Figure 3 shows the oxidation current for a 1 mM dopamine solution with a peak

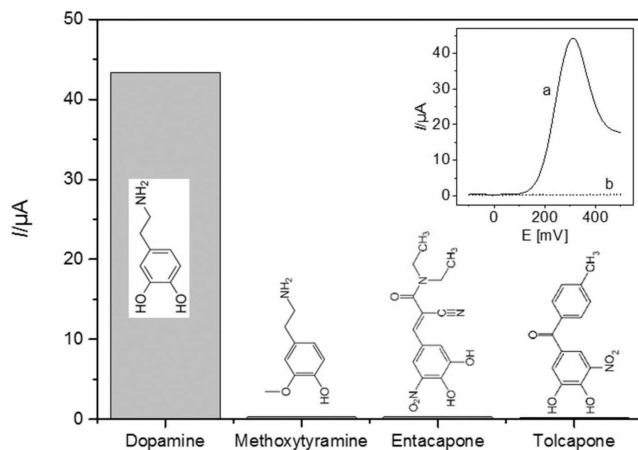


Figure 3. Peak currents of substrate, product, and two inhibitors from the human COMT measured by using DPV with a FTO working electrode at a potential of 320 mV vs. Ag/AgCl, 1 M KCl, applying a concentration of 1 mM for each compound. Inset: DP voltammograms of 1 mM dopamine (a) and the product of COMT reaction: methoxytyramine (b) in 20 mM potassium phosphate, pH 7.2.

current at 320 mV vs. Ag/AgCl, 1 M KCl. For methoxytyramine no current signal can be observed in the potential range of dopamine oxidation. That means FTO allows a clear discrimination between substrate and product of the COMT reaction. Hence, in principle an activity determination of the decrease of the dopamine concentration caused by the enzymatic conversion.

Considering that the electrochemical activity assay should also be applicable when the enzyme is inhibited, the DPV signals of the inhibitors of the COMT have to be discriminated from the dopamine signal. Subsequently, the voltammetric behavior of entacapone and tolcapone have been investigated by DPV in the potential range applied for the dopamine detection. The determined current signals at a potential of 320 mV vs Ag/AgCl, 1 M KCl demonstrate clearly that nearly no current signal from methoxytyramine, entacapone and tolcapone can be observed in the range of dopamine oxidation (< 1%) – even when high concentrations as 1 mM are applied.

Furthermore, for a reliable detection also the stability of measuring signal for a defined analyte concentration has to be examined. Due to formation of polymerization products from electrochemically oxidized dopamine, the current signal often

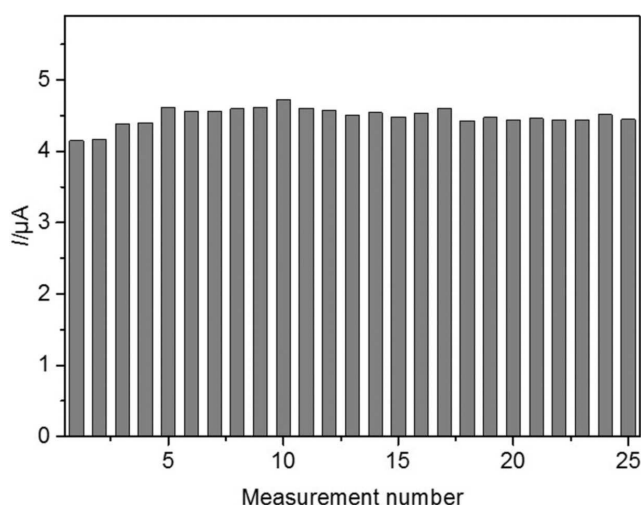


Figure 4. Peak currents of 25 consecutive DPV measurements with a dopamine concentration of 200 μM (in 20 mM potassium phosphate, pH 7.2) at an FTO electrode at a potential of 320 mV vs. Ag/AgCl.

decreases during consecutive measurements at most electrode materials applied for the electrochemical dopamine detection. Hence, the stability of the dopamine signal during a sequence of 25 DPV measurements has been examined. At FTO no decrease of the dopamine signal can be observed after these consecutive measurements (Figure 4). It has to be mentioned here, that a regeneration of the FTO electrode in between the measurements by buffer flow and cyclic voltammetry (50 cycles) is necessary in order to avoid the deposition of reaction products of the electrochemical neurotransmitter oxidation.

Evaluating the results of the pre-investigations above it can be concluded that essential requirements for reliable dopamine detection via DPV can be met. Hence, FTO could be a suitable electrode material for an activity assay for the COMT via voltammetric dopamine measurement.

2.2. Influence of Components of the Enzyme Activity Assay on the Dopamine Signal

Some essential components for the development of an activity assay for the COMT can be obtained from the spectroscopic activity assay according to Borchardt.^[53] Beside the information on the suitable co-substrate concentration, also the optimal pH value, the incubation temperature, the concentrations of the co-factor magnesium and the reducing agent dithiothreitol have been derived. For the application of all the essential ingredients in a voltammetric activity assay, their redox properties in the range of the electrochemical dopamine oxidation have to be analyzed. Hence, all compounds are examined via DPV with the final concentrations in the activity assay. The voltammograms in Figure 5 demonstrate clearly that none of the additional assay components – 1 mM SAM, 4 mM DTT and 1.2 mM MgCl₂ – show any redox signal in the potential range up to 500 mV vs. Ag/AgCl, 1 M KCl.

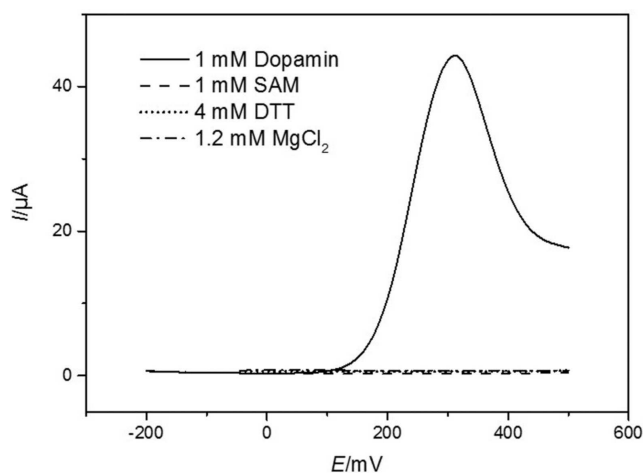


Figure 5. Voltammetric behavior of essential components for the activity assay at an FTO electrode during DPV measurements in 20 mM potassium phosphate, pH 7.2, applying a potential up to 500 mV vs. Ag/AgCl, 1 M KCl (SAM: S-adenosyl-methionine, DTT: dithiothreitol and magnesium chloride).

After these promising results the dopamine detection via DPV has been analyzed in the presence of the co-substrate SAM. Here a significant decrease of the DPV-signal for a defined dopamine concentration after several consecutive measurements can be revealed, even though the same parameters for the DPV-measurement of dopamine have been applied as before (Figure 6 a). Obviously, reactions products from oxidized dopamine and SAM lead to a partial passivation of the electrode surface. This would however, hinder a reliable dopamine concentration analysis. A successful strategy in order to avoid this loss of signal intensity can be provided by reducing the upper potential limit during the voltammetric dopamine measurement to 350 mV vs Ag/AgCl, 1 M KCl. The result of such a measurement is given in Figure 6b – clearly demonstrating that in this diminished potential range a reliable dopamine detection is feasible.

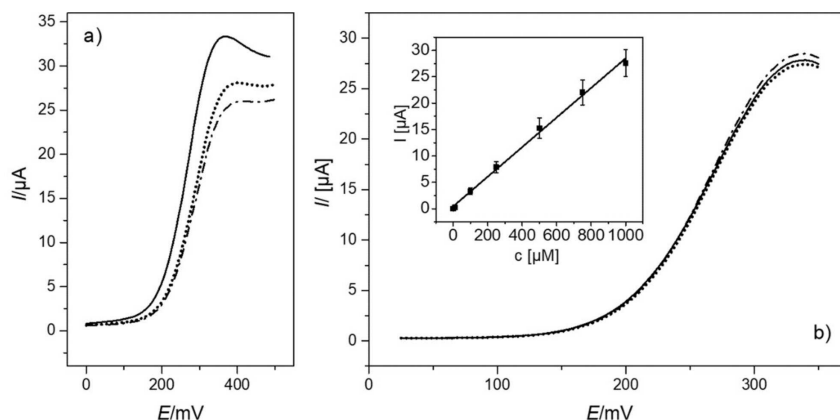


Figure 6. Consecutive dopamine measurements (1 mM) in the presence of S-adenosyl methionine (1 mM). First DPV (solid line), second DPV (dotted line), third DPV (dashed/dotted line). (a) Decrease of the DPV signal, applying an end potential of 500 mV vs. Ag/AgCl, 1 M KCl. (b) Behavior of the DPV signal after the reduction of the upper potential limit to 350 mV vs. Ag/AgCl, 1 M KCl. Inset: dependency of the peak current at 320 mV vs. Ag/AgCl, 1 M KCl on the dopamine concentration, measured via DPV at an FTO electrode. (in 20 mM potassium phosphate, pH 7.2; $n = 3$)

Another aspect for a reliable detection of the COMT activity is the defined dependency of the measuring signal on the dopamine concentration in the presence of the co-substrate SAM - in the concentration range used for enzyme activity analysis. The K_M value of the soluble COMT is reported to be about 200 μM to 260 μM dopamine.^[55,56] Hence, for the activity determination dopamine concentrations of up to 1 mM have to be applied in order to ensure saturation conditions. With this respect, the dependency of the voltammetric signal from the dopamine concentration has been examined with a SAM concentration of 1 mM present in solution. The results of these investigations reveal a linear dependency of the current signal on the dopamine amount even when applying high concentrations as 1 mM (inset of Figure 6 b). The R^2 -value can be determined with 0.996 indicating the high linearity of the signal-concentration function. This behavior provides a good basis for an exact measurement of the dopamine concentration, which is essential for the determination of the enzymatic reaction rate.

After the identification of these conditions, the redox signal for a defined dopamine concentration has been studied not only in the presence of SAM, but also in the presence of the other two essential assay components DTT and MgCl_2 . The investigations reveal that the presence of each essential component has a significant influence on the dopamine DPV-signal (Figure 7). The presence of 4 mM DTT lead to an increase of the dopamine signal and a shift of the redox peak towards lower potentials. The behavior can be explained by a chemical redox cycling allowing a chemical reduction of the oxidized dopamine back to the original dopamine molecule thus, enhancing the oxidation signal.^[57] In contrast, the presence of 1.2 mM MgCl_2 leads to a diminished dopamine oxidation peak current of about 20 percent and a small potential shift of the peak maximum to higher values of about +15 mV.

DTT and MgCl_2 are essential components for the detection of COMT activity since experiments without them reveal a significantly decreased enzyme activity, i.e. only less than 10 percent remain. However, by using a drastically reduced

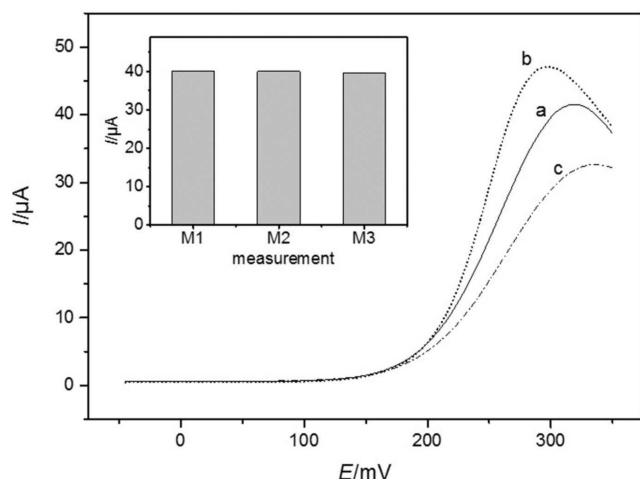


Figure 7. Influence of the additional assay components on the DPV signal for 1 mM dopamine: a) 1 mM dopamine, b) 1 mM dopamine and 4 mM DTT c) 1 mM dopamine and 1.2 mM MgCl_2 . Inset: stability of the DPV-signal at 320 mV (vs Ag/AgCl, 1 M) of 1 mM dopamine in the presence of 1 mM SAM and the significantly reduced amount of the essential assay components DTT (400 μM) and MgCl_2 (120 μM) during consecutive measurements (M1–M3) (in 20 mM potassium phosphate, pH 7.2).

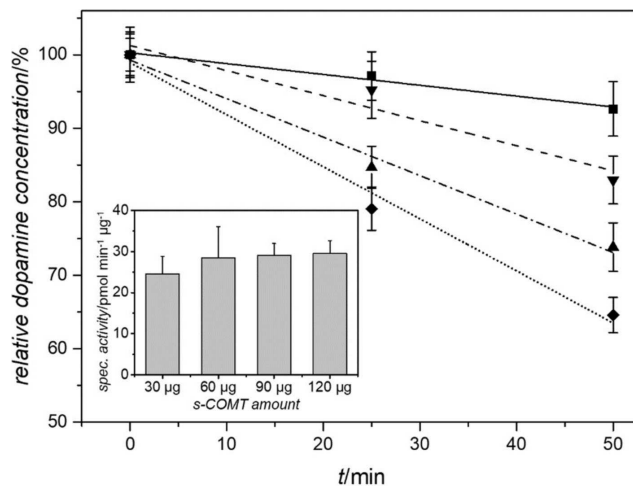


Figure 9. Electrochemical determination of the dopamine concentration after incubation of the developed test solution with 30 μg (square), 60 μg (triangle tip down), 90 μg (triangle tip up) and 120 μg (rhombus) COMT for 0 min, 25 min and 50 min. Inset: specific activity of the COMT, calculated by the data from the developed electrochemical activity assay for 4 different enzyme amounts. ($n = 3$)

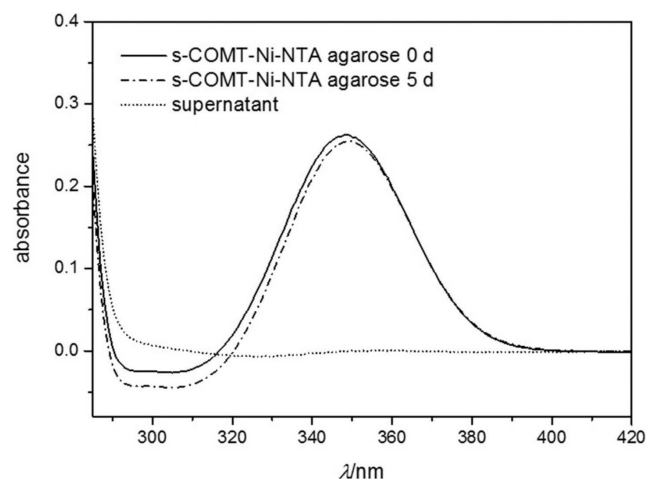


Figure 8. Absorption spectra from the activity assay according to Borchardt.^[53] The spectra of the test solutions were recorded after 60 min at 37 °C applying 50 μl from the supernatant after COMT coupling onto Ni-NTA-agarose (dotted line), 50 μl fresh prepared COMT-Ni-NTA agarose (solid line) and 50 μl COMT-Ni-NTA agarose stored for 5 d at 4 °C (dashed/dotted line). The absorbance of the O-methylated products 3-hydroxy-4-methoxyacetophenone and 4-hydroxy-3-methoxyacetophenone is detected here.

amount of DTT and MgCl_2 (400 μM and 120 μM) and testing the COMT activity with the optical assay, it is found, that the activity of COMT can be fully retained. When applying these conditions for the electrochemical analysis of the dopamine signal, it is found that the height of the current signal is nearly stable (inset of Figure 7). The drastic effects of DTT and MgCl_2 can be almost completely avoided.

2.3. Verification of Electrochemical COMT Activity Detection

With the adapted potential window (< 350 mV vs. Ag/AgCl, 1 M KCl) and the new assay composition (400 μM DTT, 120 μM MgCl_2) a stable dopamine detection can be ensured and thus, feasibility of the electrochemical activity determination of the COMT has been studied. For this purpose His-tagged COMT has been bound to Ni-NTA agarose and incubated for different times at 37 °C in the adopted activity assay mix. After coupling of the COMT onto Ni-NTA agarose no activity can be detected in the supernatant indicating the complete enzyme amount has been bound. The activity of the agarose bound enzyme is stable for a time period of several days as could be shown by spectroscopic activity measurements (Figure 8). This experimental format has been chosen to be close on the assay format as illustrated in Figure 2.

When the bound COMT has been incubated for the time period of 25 min and 50 min with the whole assay mix including dopamine, the determination of dopamine concentration via DPV reveals a clear and time-dependent decrease of the voltammetric signal (Figure 9).

This indicates that an activity measurement of the COMT can be realized at FTO under the identified conditions. In order to show that this electrochemical assay can be used for samples with different activities, four assays with different enzyme amounts have been investigated. The results shown in Figure 9 demonstrate that the decrease of the determined dopamine concentration depends clearly on the applied COMT amount used for the dopamine conversion. The activity of the COMT calculated by the electrochemically determined dopamine concentration correlates closely to the enzyme amount used for incubation. For the different enzyme amounts of 30 μg , 60 μg , 90 μg and 120 μg reaction rates of 0.74 nmol/min, 1.7 nmol/min, 2.6 nmol/min and 3.5 nmol/min can be determined. From

these data the specific activity can be calculated with about $0.029 \mu\text{mol}/(\text{min} \cdot \mu\text{g})$ for the higher enzyme amounts and $0.025 \mu\text{mol}/(\text{min} \cdot \mu\text{g})$ for $30 \mu\text{g}$ COMT (inset of Figure 9).

Comparative activity measurements with an enzyme amount of $30 \mu\text{g}$ Ni-NTA-agarose bound COMT via the spectroscopic assay according to Borchardt yield an activity of $0.33 \text{ nmol}/\text{min}$. That means, the reaction rate determined by the electrochemical assay is more than twice higher. The significant lower activity value from the spectroscopic assay can be explained by the application of the substrate dihydroxyacetophenone, which is not the natural one for the COMT. This prevents a direct comparison of both activity values. However, the reaction rate of the COMT determined by the developed electrochemical activity assay should be more relevant for evaluation of the natural enzyme activity with its physiological function as dopamine-converting enzyme and also its inhibition by specific drugs.

When comparing this newly developed method for COMT activity determination with earlier studies based on chromatography or electrophoresis, this sensor development allows the analysis of the substrate of the COMT in the presence of the product and all essential components without a previous separation step. Thus, it avoids the use of sophisticated instrumentation. The electrochemical detection of the COMT activity could be a convenient technique with comparatively low costs if it is combined with an isolation of the enzyme from real samples via an affinity column.

3. Conclusions

At FTO electrodes not only the substrate and the product of the COMT reaction can be discriminated by a DPV-measurement, it is also possible to exclude measuring signals from two important enzyme inhibitors, which are used to regulate the activity of the COMT in medicine. Furthermore, the dopamine signal reveals a high stability after repeated measurements.

Despite these advantageous characteristics of FTO as electrode material for the dopamine detection, components, which are necessary for the accomplishment of an activity assay, are disturbing a reliable dopamine detection significantly. Even though the single essential assay components such as the co-substrate SAM and the reducing agent DTT and the salt MgCl_2 show no activity in the range of dopamine oxidation, the addition of each compound has a significant influence on the strength of the dopamine signal. However, in this study ways have been found to overcome these limitations. After the adjustment of the potential window for the DPV measurement ($< 350 \text{ mV}$ vs. Ag/AgCl) and the adaption of the concentration of essential assay components ($400 \mu\text{M}$ DTT, $120 \mu\text{M}$ MgCl_2) a reproducible detection of dopamine concentrations can be achieved without compromising the enzyme activity determination.

With the identified parameters, the activity of different enzyme amounts can be determined by analyzing the enzymatic consumption of dopamine by voltammetric detection. The results reveal, that the dopamine concentration clearly

depends on the time period the reaction mixture is in contact with the COMT and the amount of enzyme applied for the study. This demonstrates, that an electrochemical determination of the COMT activity can be realized via DPV at FTO electrodes. Due to the application of dopamine as COMT substrate – in contrast to dihydroxyacetophenone for the commercially available optical activity assay – the results of this new developed COMT activity assay should be more relevant for medical applications. This developed assay allows activity measurements similar to spectrophotometric methods, but in a sensorial format with rather inexpensive equipment.

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Conflict of Interest

The authors declare no conflict of interest.

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