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# Smart electrochemical sensor for some neurotransmitters using imprinted sol–gel films

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ABSTRACT

A hybrid sol–gel material formed by acid hydrolysis of a mixture of tetraethylorthosilicate (TEOS) and phenyltriethylorthosilicate (PTEOS) as functional monomers was imprinted by tyramine and dopamine as template molecules for the purpose of molecular recognition. Imprinted materials were spin coated as thin films on the surface of glassy carbon electrodes and then were characterized using cyclic voltammetry (CV). After extraction of the encapsulated molecules, imprinted films were tested in solutions of their templates and other molecules. Rebinding experiments were followed by electrochemical characterization using square wave voltammetry (SWV). Imprinted films showed higher affinities toward their template molecules compared to other structurally similar molecules especially for tyramine imprinted film. With the exception of tyramine and norepinephrine, the interference level did not exceed 5% for all compounds studied for dopamine-imprinted films. Tyramine-imprinted films however showed high affinity to tyramine with dopamine 40% interference. Some factors related to the rebinding ability process like pH of solution, concentration of template were studied. The sensing surface lifetime extended to 2 weeks with decay in response signal that ranged from 22%, 40% to 60% for dopamine, tyramine and norepinephrine, respectively. The standard deviation from the mean of measurements for the repeated experiments is 7.4%. Electrochemical impedance spectroscopy (EIS) measurements confirmed the results obtained by electrochemical measurements. Morphological characteristics of the imprinted thin films and their thickness were investigated using scanning electron microscope (SEM).



## 1. Introduction

Molecular imprinting has been widely studied and applied in the last two decades as an innovative tool for various technological and scientific fields. The first approach to molecular imprinting was not well known until 1949 when Dicky succeeded to create complementary molecular cavities for certain dye molecules. Dickey's silicates could be considered as the first molecularly imprinted material [1].

In general, molecular imprinting process involves the formation of molecular cavities inside the polymer matrix being imprinted which are of complementary structural, functional group orientation and geometrical features with respect to the molecule being imprinted. Specifically, the molecular cavities are created by the incorporation of the template molecule during the polymerization process in such a way that a three-dimensional network is formed around the molecule being imprinted. Upon the extraction of the molecular moiety (template) from the polymer matrix, molecular cavities with specific shape, size and electrostatic features, remain in the cross-linked host material [2–4]. The imprinted material can be used to rebind the template molecules with very high degree of selectivity.

Because of its unique properties, molecularly imprinted materials have been widely utilized for a lot of applications and in various fields. They were applied in high performance liquid chromatography [5], food analysis [6], capillary chromatography, solid phase extraction [7] and drug delivery systems [8].

One of the most important applications of the imprinting technique is the molecular recognition. Biosensors prepared by imprinting methods could introduce good solution for the recognition of a variety of biologically active molecules. Recognition mechanism of the molecules is largely similar to what happens in living organisms. In our bodies, there are a large number of different molecules, and cells, without which we cannot survive. These molecules are able to work cooperatively and integrally in such a way that certain functions are carried out very precisely and accurately. For example, the receptors on the surface of cell membranes bind hormones specifically and selectively. When the receptor binds a hormone, its conformation is changed and a message of the hormone is transferred in terms of a conformational change in such away that the function is achieved. In molecular recognition, the molecules being imprinted can rebind to their

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molecular cavities with very high degree of selectivity and specificity, so these materials may be named as artificial antibodies.

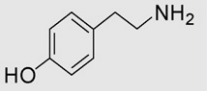
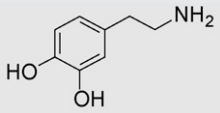
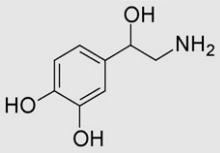
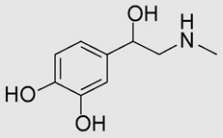
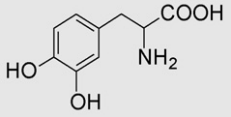
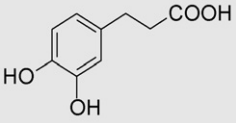
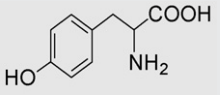
Sol–gel materials have been extensively used for the purposes of molecular imprinting. The imprinting of hybrid sol–gel materials has found great interest in the last few years [9–12] due to their unique properties. In this work, a hybrid sol–gel material synthesized by hydrolysis of a mixture of tetraethylorthosilicate (TEOS) and phenyltriethylorthosilicate (PTEOS) was molecularly imprinted with tyramine and dopamine for sensing applications in aqueous medium. Imprinted sol–gel materials were spin coated on the surfaces of glassy carbon electrodes at 2500 rpm for 45 s. The modified electrodes were electrochemically characterized using cyclic voltammetry for the imprinted molecules. Rebinding experiments were carried out by adsorption from solutions of the imprinted molecules and other test molecules followed by electrochemical characterization by SWV. Electrochemical impedance measurements were carried out to confirm the voltammetric results.

## 2. Experimental

### 2.1. Reagents

Tetraethylorthosilicate (TEOS) (>99%) and phenyltriethylorthosilicate (PTEOS) (>99%) were used as functional monomers for polymerization process. Dopamine (≥99%), tyramine (≥99%), dopa (≥99%), dopac (≥99.0%), tyrosine (≥99%), catechol (≥98%), epinephrine (≥99%), norepinephrine (≥99%), and catechol (≥98%) were used as template molecules in the imprinting process and as test molecules for rebinding experiments. Phosphate buffer solution (PBS) (pH 7.2 and 10 mmol $L^{-1}$) was prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate. 2-Ethoxyethanol and absolute ethyl alcohol were used as solvents for dissolving template molecules and monomers for polymerization process. Hydrochloric acid and distilled water were used for hydrolysis of functional monomers in polymerization process. All chemicals were supplied by Aldrich Chem Co. (Milwaukee, WI, USA). Chemicals were used as received. Table 1 contains the chemical structures of template molecules and test molecules being studied.

**Table 1**
List of compounds studied in this work.

| Molecule | Structure | Molecular volume (Å$^3$) |
|---|---|---|
| Tyramine |  | 131.1 |
| Dopamine |  | 146.0 |
| Norepinephrine |  | 149.2 |
| Epinephrine |  | 166.1 |
| Dopa |  | 169.1 |
| Dopac |  | 157.4 |
| Tyrosine |  | 159.9 |

### 2.2. Equipments

Glassy carbon electrodes from BAS (USA) with 3 mm diameter were used as working electrodes. A platinum wire, 4 cm long with a diameter 0.5 mm, was used as counter electrode. All cell potentials were measured against saturated Ag/AgCl as a reference electrode. One compartment, three electrodes cell, made of glass (30 mL), fitted with gas bubbler was used for all electrochemical measurements. Electrochemical characterization including cyclic voltammetry (CV) and square wave voltammetry (SWV) were carried out using a BAS-100B electrochemical analyzer (Bioanalytical Systems, West Lafayette, USA). Electrochemical impedance spectroscopy (EIS) measurements were performed using a Gamry-750 system and a lock-in amplifier in conjunction with a personal computer used as data acquisition system and simulations. JEOL JSM-T330A scanning electron microscope (SEM) was used for all measurements.

### 2.3. Electrode preparation and procedures

#### 2.3.1. Imprinted film preparation

The electrodes were polished well using 0.05 μL alumina slurry on Buehler felt pads and rinsed thoroughly with deionized water and ethanol. Electrodes were then electrochemically activated before spin coating. Glassy carbon electrode was activated by polarization at +1.6 V for 60 s and at −1.6 V for the same period. Electrodes were then cycled between +1.0 and −0.2 till a stable CV was obtained [13,14]. Then, 400 μL (1.791 mmol) tetraethylorthosilicate were mixed with 65 μL (0.351 mmol) phenyltriethylorthosilicate in 3.0 mL of 2-ethoxyethanol, and then solution was stirred till a homogeneous is obtained. This was followed by adding 100 μL of 0.1 mol $L^{-1}$ HCl solution drop-wise with continuous stirring, and 90 μL $H_2O$ were added in the same manner. The mixture was stirred gently for 2.5 h. A 1.0 mL portion of the prepared sol–gel mixture was mixed with 300 μL of 0.1 mol $L^{-1}$ tyramine or 350 μL of 0.05 mol $L^{-1}$ dopamine in order to prepare tyramine- or dopamine-imprinted surfaces, respectively. The resulting mixtures were stirred for another 2 h. The imprinted sol–gel materials were spin coated on the surfaces of glassy carbon electrodes at spinning rate 2500 rpm for 45 s. The electrodes were allowed to dry overnight, and then they were electrochemically characterized by cyclic voltammetry (CV) between −200 mV and +1000 mV at a scan rate 100 mV/s. Template molecules were extracted form the imprinted film by repeated CV and the decay in current response was followed as indicator for the extraction procedure. Electrodes were allowed to dry overnight before rebinding experiments were carried out.

#### 2.3.2. Rebinding experiments

Modified electrodes were dipped for 15 min in rebinding solutions for different test molecules of concentration 50 μmol $L^{-1}$ each, and then followed by electrochemical characterization with

square wave voltammetry. The incubation time for rebinding experiments was selected in such a way that high sensitivity and stability of the current response was obtained.

## 3. Results and discussion

### 3.1. Sol–gel synthesis and characterization

Tetraalkylorthosilicates monomers are hydrolyzed in the presence of either acid of base catalyst. Acid catalyst was the best to be used for a lot of applications especially for the design of sensing elements [15–18]. The hydrolysis step is followed by a condensation step of the hydrolyzed monomers. It was reported that strong concentrated acids produces highly cracked and slightly shrunken aerogels. On the other hand, very dilute acids results in monolithic and well shrunken aerogels [19]. In this work silica gel material was polymerized using dilute hydrochloric acid (0.1 mol $L^{-1}$). Condensation between completely hydrolyzed monomers occurs by the elimination of water while incompletely hydrolyzed monomers may condense by elimination of alcohol molecules.

In order to obtain the best imprinted material with good physicochemical properties, water content has to be adjusted in the start mixture before synthesis step. The ratio of water and alkyl orthosilicates monomers was determined experimentally to be 4.5:1 (water:silane). This ratio was found to be sufficient for complete hydrolysis of all monomer units before the start of condensation step which was necessary to enhance the process of incorporation of template molecules into the sol–gel material before the growth of the polymeric networks around them.

The use of hybrid sol–gel matrix is very crucial factor for material design. A hybrid monomer solution composed of TEOS and PTEOS was hydrolyzed using 0.1 mol $L^{-1}$ HCl solution. The phenyl group inserted in the sol–gel material is of great importance. This group allows for non-polar interaction with the imprinted molecules which all carry benzene rings so it could act as a Pi–Pi interaction center with the template molecules through further steps. Also, it was reported that a combination of functional monomers enhances and promotes the rebinding ability of template molecules to the imprinted films [20–22].

The modified electrodes coated with the imprinted sol–gel films were characterized electrochemically using CV. An applied potential window between −0.2 V and +1.0 V at scan rate of potential 100 mV/s, was sufficient to cover the oxidation potentials of all the template and test molecules rebinding to the imprinted film. Cyclic voltammetry characterization was carried out in PBS (10 mM and pH 7.2). Fig. 1(A and B) illustrates the CVs of sol–gel films imprinted with tyramine and dopamine, respectively.

The extraction process of template molecules was carried out through repeated CV in phosphate buffer solution followed by dipping in PBS only for a relatively short period of time (ca. 1.0 h) to confirm the complete extraction of the molecules from the imprinted films. Repeated cycling between −0.2 V and +1.0 V, at scan rate 100 mV/s, was applied to the surface till complete disappearance of the template current response was attained.

The mechanism of the extraction of template molecules by repeated cycling can be understood in terms of oxidation–reduction properties of the neurotransmitter molecules. For example;

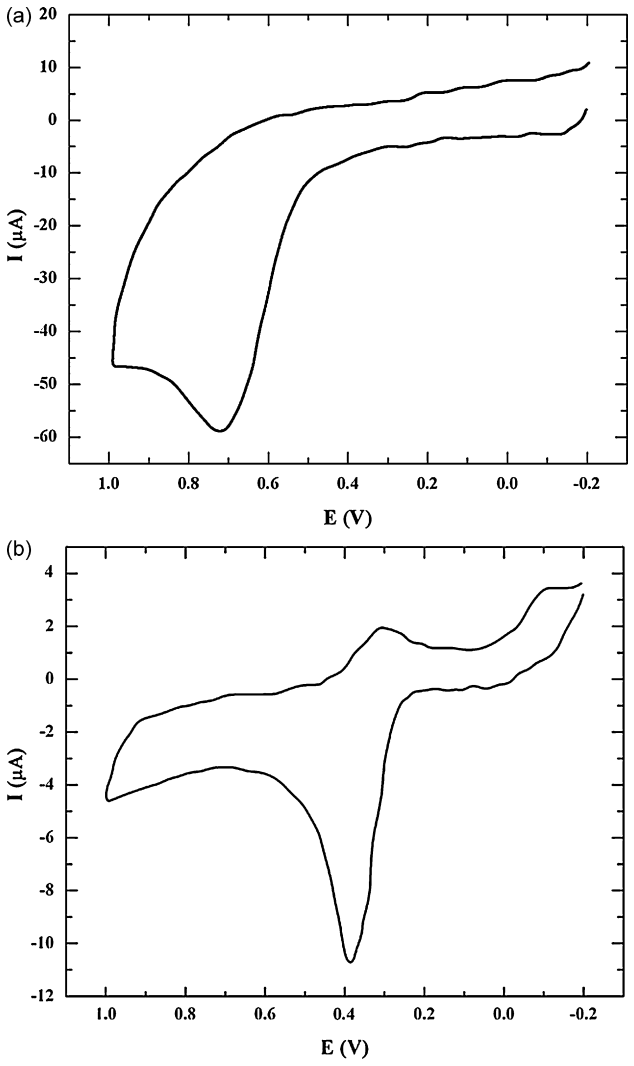
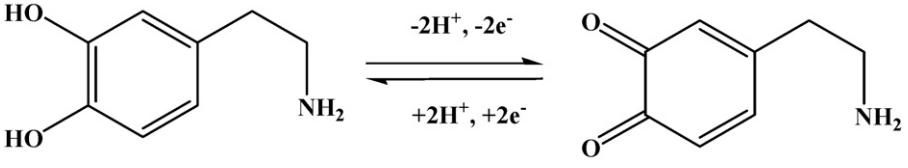



**Fig. 1.** Cyclic voltammograms of tyramine (A) and dopamine (B) molecularly imprinted sol–gel films spin coated on the surface of glassy carbon electrodes cycled in PBS (pH 7.2 and 10 mmol $L^{-1}$) versus Ag/AgCl reference electrode.

dopamine is electrochemically oxidized by losing two electrons and two protons. Oxidation reaction was found to be quasi-reversible process; as a result of this behavior, the structure of the molecules is changed during its oxidation process. The change in chemical structure makes the template molecule less bound to the molecular cavity (ca. the transformation of the two hydroxyl groups that affect the extent of hydrogen bonding) which in turn helps its extraction out of the molecular cavity inside the solution with repeated cycling.




### 3.2. Molecular recognition and selectivity of the imprinted films

#### 3.2.1. Tyramine-imprinted film

Tyramine (p-hydroxy-phenylethylamine) is naturally occurring in animals and plants. In foods, it is produced via decarboxylation of amino acid tyrosine, so considerable amounts in food stuffs indicate potentially an expired product. The molecule increases the release

of neurotransmitters stored in the body. To the best of our knowledge, this is the first time that tyramine is molecularly imprinted. Rebinding experiments for tyramine-imprinted film were carried out from template solution of different molecules of concentration 50 μmol $L^{-1}$ prepared in PBS. Rebinding experiments as mentioned in the above section were followed by electrochemical characterization using SWV. Tyramine molecularly imprinted films showed a preferential adsorption for their imprinted molecules compared to other molecules. The high affinity of the imprinted films toward its template was attributed to the fact that the molecular cavities created inside these films are correctly sized and shaped and appropriately functionalized in the way that they bind these molecules or allow the passage of any moiety which may be analogous in size or geometry into them [23–25]. It is also perceived that the recognition ability of molecularly imprinted materials comes from the functional group orientation inside the molecular cavities created during polymerization which enhances the ability of these template molecules to interact well with these molecular cavities. As shown in Fig. 2A tyramine-imprinted film showed relatively high current response for tyramine compared to other molecules. Although dopamine has larger molecular size than tyramine, it is found to be partially adsorbed into the surface imprinted with tyramine. The appreciable interference of dopamine into the tyramine cavities is largely attributed to the similarity of their functionality beside the fact that the molecular size of dopamine is not extremely larger than tyramine. On the other hand, norepinephrine showed relatively smaller interference to tyramine-imprinted film compared to dopamine. This is certainly due to larger molecular size of norepinephrine and the extra hydroxyl group which changed the average electron density around the molecule and in turn decreased the possibility of electrostatic interaction with the molecular cavity oriented functional groups. As illustrated in the same figure other molecules including epinephrine, dopa, dopac, tyrosine, and catechol showed almost no rebinding ability with respect to tyramine-imprinted film. All molecules except catechol are characterized by larger molecular size in addition to relatively different functionality. It is interesting to notice that although catechol is smaller in size compared to tyramine, it could not rebind to the imprinted film to any extent. This is attributed mainly to the fact that catechol is missing the amino group present in tyramine which inhibit its electrostatic interaction with the imprinted film. Therefore, we can conclude that the selectivity of imprinted films depends on the size of template molecule and its ability for electrostatic interaction with the functional groups oriented inside these cavities during polymerization. Moreover, it is important to mention that all measurements were carried out using three independent electrodes to ensure reproducibility of the results.

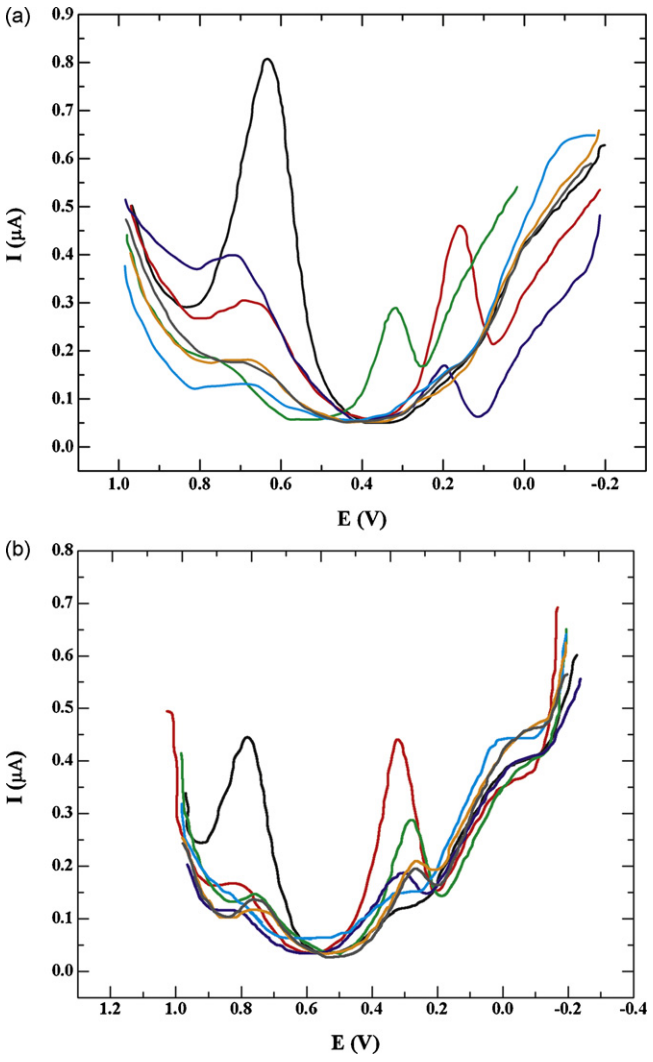



**Fig. 2.** (A) Square wave voltammograms of tyramine and other test molecules with respect to tyramine-imprinted film. Rebinding experiments are carried out by dipping electrodes in 50 μmol $L^{-1}$ solutions of the tested molecules prepared in PBS: tyramine (—), dopamine (—), norepinephrine (—), epinephrine (—), tyrosine (—), dopa (—), dopac (—). (B) Square wave voltammograms of dopamine and other test molecules with respect to dopamine imprinted film. Rebinding experiments are carried out by dipping electrodes in 50 μmol $L^{-1}$ solutions of the tested molecules prepared in PBS: tyramine (—), dopamine (—), norepinephrine (—), epinephrine (—), tyrosine (—), dopa (—), dopac (—).

### 3.2.2. Dopamine imprinted film

Dopamine is a neurotransmitter found in various animals whether vertebrates or invertebrates. It is produced in various areas in the brain and activates five types of receptors. As illustrated in Fig. 2B, current response after rebinding tyramine to dopamine-imprinted film is relatively high when compared to the current response after dopamine rebinding to its imprinted film. This is due to the smaller molecular size of tyramine which facilitates the diffusion of tyramine into the dopamine imprinted cavities. Although norepinephrine is larger in molecular size than dopamine, it gives relatively high rebinding current response. This is mainly due to the similarity of functional groups of both molecules which implies very similar electron density and as a result of this, strong electrostatic interactions arise between them and the molecular cavities oriented functional groups. Also, it is observed from the results that norepinephrine interferes with dopamine-imprinted film nearly to the same percentage of interference of dopamine to tyramine-imprinted film. Other molecules interfere to dopamine film with less than 5% as depicted in Fig. 2B.

It is to be mentioned here that non-imprinted sol–gel films were prepared following the same procedure as the imprinted films but without the addition of the template solution to the sol mixture during the polymerization process and were tested for rebinding of different test molecules. Percentage adsorption to non-imprinted films is negligible which confirms the fact that rebinding process mainly takes place into the imprinted surface sites.

### 3.3. Binary mixture analysis

One important challenge for the effective use of imprinted materials in molecular recognition processes is the ability of the imprinted film for selective recognition of imprinted template in the presence of other molecules. As an example dopamine molecularly imprinted film was tested for selective adsorption

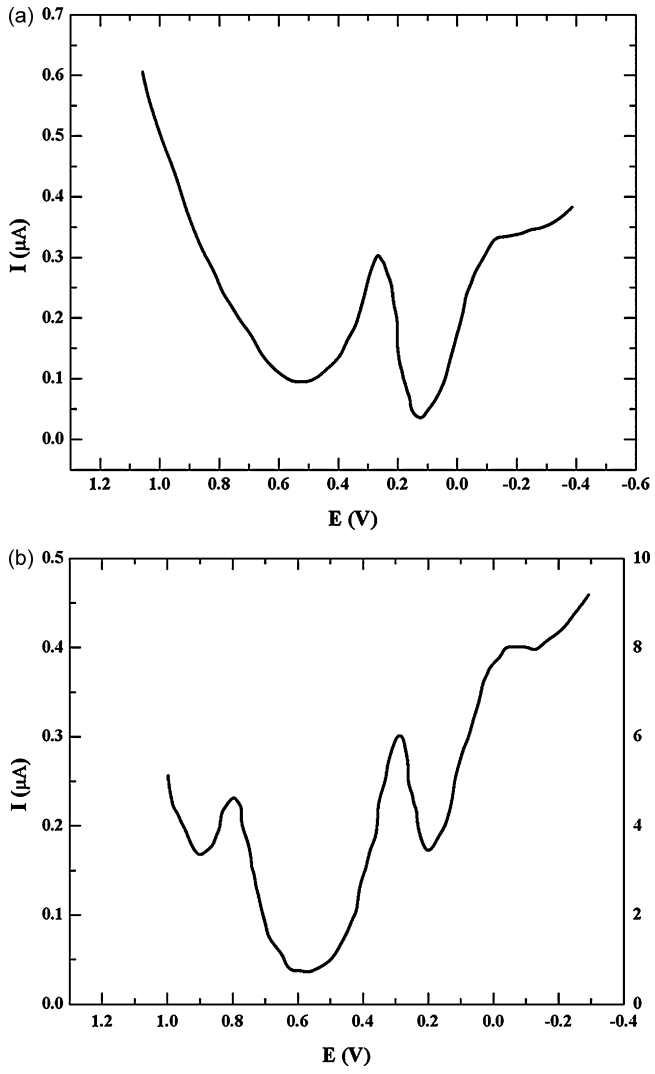



**Fig. 3.** SWV signal characteristic for dopamine imprinted film after dipping in (A) binary mixture of dopamine and tyrosine and (B) binary mixture of dopamine and tyramine. Rebinding binary solutions are 50 μmol $L^{-1}$ with respect to template molecules.

(recognition) of a binary mixture of equal concentrations of tyrosine and dopamine (50 μmol $L^{-1}$). Dopamine-imprinted film showed high selectivity for dopamine in presence of tyrosine. As illustrated in Fig. 3A, dopamine showed relatively high current response at 250 mV while tyrosine showed almost no current response after the rebinding process. On the other hand, when the imprinted film was tested in a binary mixture of tyramine and dopamine, tyramine could rebind to the imprinted film and showed relatively appreciable current response at 780 mV with respect to dopamine imprinted film as shown in Fig. 3B. The results of rebinding experiments in binary mixture of dopamine and tyramine are similar to those obtained previously (cf. Section 3.2) in single rebinding runs. Thus, with the exception of tyramine that showed appreciable interference, dopamine imprinted film showed excellent selective recognition for dopamine in presence of other interfering molecules.

### 3.4. Effect of pH of the template solution on rebinding process

Virtually all drug-like molecules are weak acids or bases. This means that they contain at least one site that can reversibly disas-

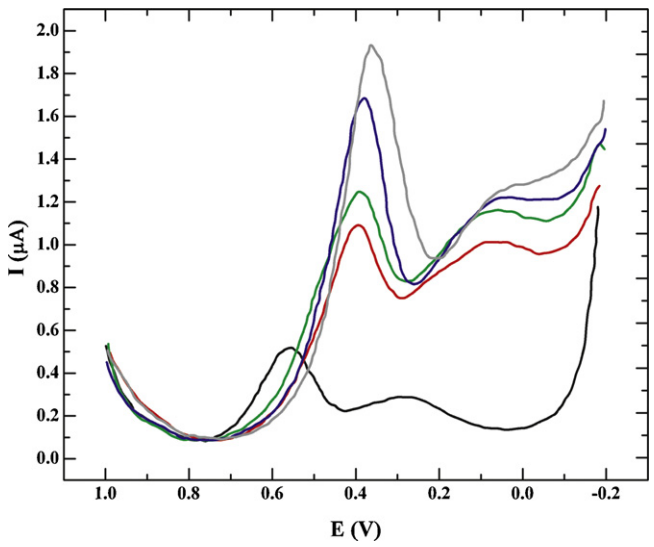



**Fig. 4.** SWVs of dopamine imprinted film after dipping in 100 μmol $L^{-1}$ of dopamine solutions at different values of pH: 1.0 (—), 3.0 (—), 5.0 (—), 7.0 (—), 9.0 (—).

sociate or associate a proton (a hydrogen ion) to form a negatively charged anion or a positively charged cation. Molecules that disassociate protons are acids, and those that associate protons are bases. The reversibility means that a sample is always in equilibrium with some fraction protonated and the rest deprotonated.

$$HA \Leftrightarrow H^+ + A^- \text{or} HB \Leftrightarrow H^+ + B^-$$

By varying the availability of protons, i.e. the acidity of the media, the balance of the equilibrium can be shifted. Alternatively, the p*K*a-values of a site can be thought of the pH at which the protonated and deprotonated fractions are equal. Experiments were carried out from different pH solutions on dopamine imprinted surface. All pH measurements were carried out from 100 μmol $L^{-1}$ with respect to dopamine. As illustrated in Fig. 4, the current response increases with the increase in pH value. At pH-values lower than p$Ka_1$ (8.57), the protonated form of dopamine predominates than the unprotonated zwitter ion form. On the other hand, at pH-values higher than p$Ka_2$ (10.08) the deprotonated (phenoxide ion) form is higher than unprotonated zwitter ion. Between p$Ka_1$ and p$Ka_2$, the zwitter ion form predominates over the charged form [26]. This argument clarifies the fact that the protonated dopamine molecule is less interacting with the oriented functional groups in the molecular cavities while the neutral form is best fitted to the imprinted sites so by increasing the pH values current response enhances.

On the other hand, there was an increase in the oxidation potential value of the adsorbed species on the imprinted surface with the increase of pH value. This result could be explained from the fact that, positively charged molecules due to protonation of dopamine at lower pH values needs higher polarization potential (overvoltage) for oxidation. This is because the average electronic charge on the molecules is lowered, therefore it is more difficult to withdraw electrons at such a lower potential, while at higher pH values the molecule is either neutral or negatively charged which increases its electro-oxidation. This study was not extended to pH values higher than 9 because the glassy silicon film could decompose under the effect of strong alkaline medium.

### 3.5. Sensitivity versus electrode selectivity and lifetime

One important factor regarding the molecularly imprinted materials is the stability of the geometry of molecular cavities cre-

**Table 2**
Comparison of the rebinding results of dopamine imprinted film at the beginning and at the end of 2 weeks. Adsorption experiments were carried out from 50 μM solution with respect to dopamine prepared in PBS.

| Molecule | $I$ ($10^{-7}$ A) Immediately after preparation | $I$ ($10^{-7}$ A) After 2 weeks | Percentage of decrease in current response signal (%) |
|---|---|---|---|
| Dopamine | 3.35 | 2.59 | 22.3 |
| Tyramine | 3.01 | 1.82 | 40 |
| Norepinephrine | 1.93 | 0.71 | 63.5 |

ated inside the sol–gel material which in turn affects both the selectivity and the sensitivity of the imprinted material for template molecules on the long term. Dopamine imprinted films were tested in dopamine solution and in solutions of the other molecules being studied, then the electrodes were left in a dry place for relatively long period (ca. 2 weeks). The electrodes were tested again in dopamine solution and in solutions of other molecules at the end of the 2 weeks for rebinding process in order to test the selectivity and stability of the response. Measurements were carried out using three independent glassy carbon electrodes prepared in the same way to ensure repeatability of measurements. The standard deviation from the mean of measurements for the repeated experiments is 7.4%.

Current response for different test solutions was found to decrease after 2 weeks of drying with varying percentages. The most interesting point here is the incremental decrease in current response ascending from dopamine down to catechol. It was found that dopamine current response after 2 weeks, decreased by 22%. Tyramine signal decreased by 40% and norepinephrine by 63.5% of the original current response signal as indicated in Table 2. Rebinding current response disappeared completely for the remaining of the group of molecules being tested. It is concluded that the selectivity of the imprinted film increases appreciably for imprinted film if compared to other molecules, by increasing the time between drying and measurement.

Moreover, although the selectivity of the imprinted film toward its template compared to other structurally related molecules is enhanced, there is an appreciable decrease in the current response of the template itself. In other words meanwhile the selectivity of imprinted films toward its template molecule increases, the sensitivity of the imprinted films decreases. More extensive studies may help in the future to achieve a compromise between selectivity and sensitivity of molecularly imprinted materials.

### 3.6. The effect of concentration of the template molecule on the rebinding ability

In this part, we studied the relationship between the concentration of template molecule in the solution from which the template rebinds to the imprinted film, and the electrochemical current response after rebinding to the film. The imprinted films showed fairly linear rebinding response over the concentration range from 100 μmol $L^{-1}$ down to 10 μmol $L^{-1}$ as illustrated in Fig. 5. Beyond this concentration range; over the 100 μmol $L^{-1}$ limit deviation from linearity was observed, while below 10 μmol $L^{-1}$ the imprinted films are not sensitive for the template molecules. The linearity of the process confirms again that the rebinding process occurs specifically on selected sites created during the imprinted process in this concentration region.

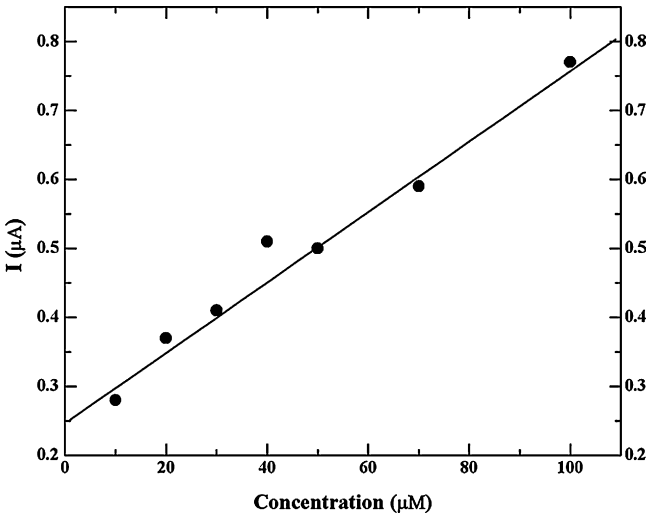



**Fig. 5.** Current response (SWV) of dopamine imprinted film as a function of the concentration of dopamine in the rebinding solution.

### 3.7. Electrochemical impedance characterization of the imprinted film

Impedance measurements were carried out over a frequency range from 0.5 Hz to $10^5$ Hz with an applied AC voltage of 5 mV. Excitation DC voltages were predetermined using cyclic voltammetry for the molecules being tested against the imprinted films. Work was carried out at DC potentials 330 mV for dopamine, 720 mV for tyramine, and 550 mV for tyrosine. Previous studies showed that the imprinted film swells upon the association with their template molecules, which in turn enhances their electrical permittivity with respect to the electrolyte [27,28]. As a result of this, the electron transfer process is facilitated across the double layer. In case of dopamine-imprinted film that was incubated in dopamine solution the swelling effect of the imprinted film occurs due to rebinding of the template to its film. Fig. 6A illustrates Nyquist plots of dopamine films in case of incubation in dopamine solution and in PBS, respectively. Lines in Nyquist plot represent the calculated and fitted data for the equivalent circuit components while symbols represent the experimental data. Triangle symbols in the diagrams represent the signal after incubation of the imprinted film into its template solution while the square symbols represent the impedance signal of the imprinted film after incubation in buffer solution (buffer background).

Dopamine and tyramine-imprinted films were incubated in a solution containing a different molecule like tyrosine which showed no rebinding affinity to the imprinted film. The slope of Nyquist plots in both cases did not change appreciably for the incu-

**Table 3A**
EIS results of the equivalent circuit for dopamine imprinted film (a) incubated in 50 μM dopamine solution prepared in PBS (b) incubated in PBS. EIS work was carried out over a frequency range 0.5 Hz to 100 kHz at AC voltage with an excitation DC voltage 330 mV.

| Impedance component | $R_p$ ($10^5$ Ω $cm^{-2}$) | $R_u$ ($10^3$ Ω $cm^{-2}$) | $C_f$ ($10^{-6}$ F $cm^{-2}$) | $W$ ($10^5$ Ω $s^{-1/2}$) | $CPE$ ($10^6$ F $cm^{-2}$) | $n$ |
|---|---|---|---|---|---|---|
| (a) | 0.0254 | 0.6030 | 6.3260 | 2.3670 | 1.9270 | 0.898 |
| (b) | 3.0640 | 1.0800 | 3.1090 | 8.8520 | 6.5020 | 0.902 |

**Table 3B**
EIS results of the equivalent circuit for dopamine imprinted film (a) incubated in 50 μM tyrosine solution prepared in PBS (b) incubated in PBS buffer. EIS work was done over a frequency range 0.5 Hz to 100 kHz at AC voltage with an excitation DC voltage 550 mV.

| Impedance component | $R_p$ ($10^4$ Ω cm$^{-2}$) | $R_u$ ($10^2$ Ω cm$^{-2}$) | $C_f$ ($10^{-6}$ F cm$^{-2}$) | $W$ ($10^5$ Ω s$^{-1/2}$) | $CPE$ ($10^6$ F cm$^{-2}$) | $n$ |
|---|---|---|---|---|---|---|
| (a) | 4.6110 | 6.831 | 1.095 | 5.4470 | 2.6900 | 0.952 |
| (b) | 4.4790 | 6.762 | 1.279 | 5.4670 | 3.0460 | 0.9616 |

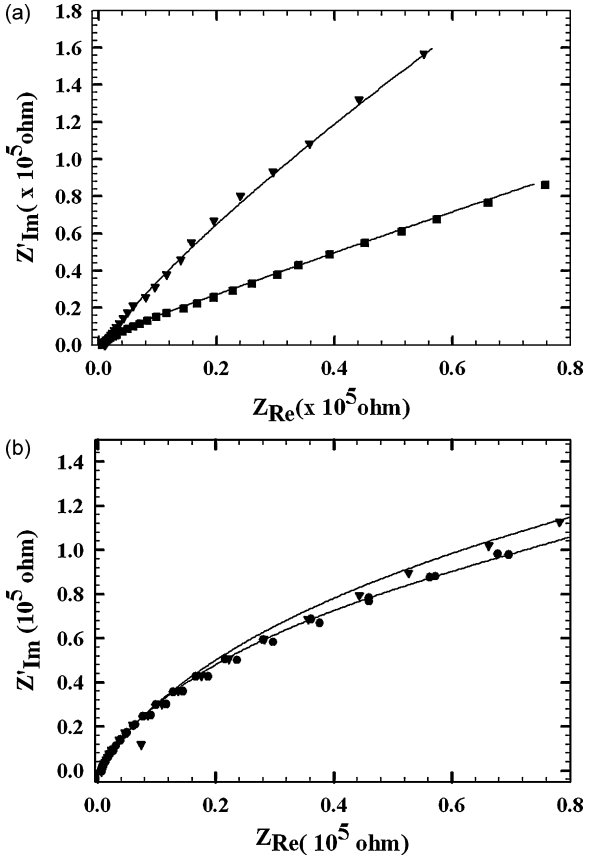



**Fig. 6.** (A) Nyquist plots of dopamine imprinted films tested in dopamine solution compared to the signal after testing in PBS. (B) Nyquist plots of dopamine imprinted films tested in tyrosine solution compared to the signal after testing in PBS.

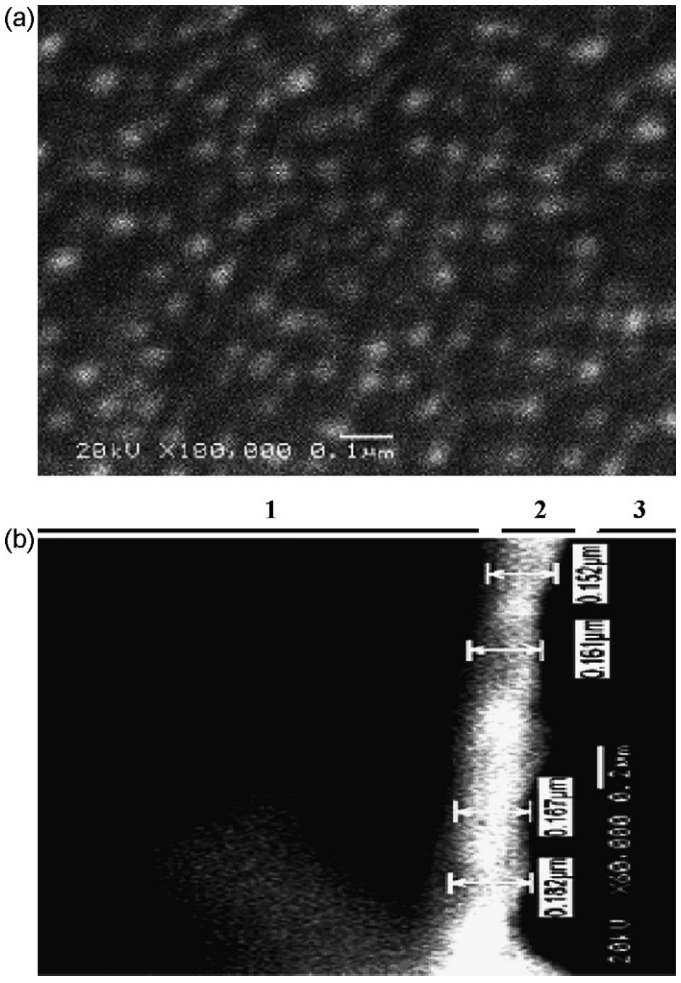



**Fig. 8.** Scanning electron microscope image of (A) sol–gel material used in the imprinting processes and (B) the side image of the sol–gel film coated on the surface of glassy carbon electrode. (1) Substrate edge, (2) sol–gel film and (3) air.

bation of the imprinted films in tyrosine solution compared to the incubation in PBS for the same period of time which is shown in Fig. 6B. This result indicated that tyrosine did not rebind into the molecular cavities of either tyramine or dopamine. From the foregoing results, it is confirmed again that imprinted sites are able to rebind their template molecules while molecules of largely different molecular structures and shape are unable to rebind.

The fitted electrochemical equivalent circuit is formed of five components connected in a specific way that they fit the impedance measurements carried out across the interface between the imprinted film coated on the electrode surface and the solution. Equivalent circuit designed for the molecularly imprinted film is indicated in Fig. 7. This circuit is formed of five components. $R_u$ is the solution resistance, $R_p$ is polarization resistance, $C_f$ is capacitance component while $CPE$ is another capacitance component called constant phase element. Constant phase element is more accurate component in describing the capacitance behavior of the double layer. $W$ is Warburg component which describe the diffusion behavior of electro-active species with respect to the electrode surface.

Tables 3A and 3B lists the best fitting components calculated for the equivalent circuit for dopamine after testing in dopamine and in

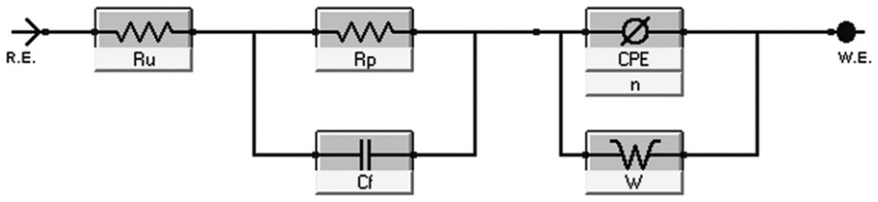



**Fig. 7.** Electrochemical equivalent circuit for EIS measurements.

tyrosine solutions, respectively. The circuit components decreased in case of incubation in dopamine solution compared to incubation in PBS. On the other hand these components did not change appreciably in case of incubation in tyrosine which confirms the experimental results described in Fig. 6(A and B).

### 3.8. Surface characterization

Sol–gel material synthesized for imprinting was characterized using scanning electron microscope at magnification power 100,000. The sol–gel particles were distributed very homogeneously and uniformly on the surface of substrate. The average particle size was found to be in the range of 38–45 nm as indicated in Fig. 8A. In this work, molecularly imprinted thin films were made using spin coating with spinning rate 2500 rpm as an optimized time in such away that it could allow the highest adsorption (recognition) capacity of the template molecules. The uniformity of the particles is mainly attributed to carrying spinning process at this high speed. Thin films were then allowed to dry to give a chance for complete polymerization of free hydroxyl groups on the film surface which may increase non-specific adsorption toward different molecular species by increasing the probability of H-bonding between the amino and hydroxyl groups of catecholamine compounds and these free hydroxyl groups.

We determined the average film thickness using side imaging of the coated electrode by scanning electron microscope [29] as illustrated in Fig. 8B. The film thickness ranged between 152 nm and 180 nm with an average value of 165 nm. The sensitivity as noticed in the high current responses of the modified electrode is highly affected by the film thickness. Thin films facilitate the diffusion of the template inside the molecular cavities compared to relatively thicker films [9].

## 4. Conclusions

Tyramine and dopamine molecules were imprinted successfully in a hybrid sol–gel material and then were spin coated as thin films on the surfaces of glassy carbon electrodes. The modified electrodes were used for sensing of the template molecules electrochemically. Selectivity of the electrodes for dopamine or tyramine was found to depend largely on the shape and size of the molecular cavity created during polymerization of sol–gel material in addition to the functional group orientation inside the cavities with respect to the template molecules.

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## References

[1] F.H. Dickey, Natl. Acad. Sci. U.S.A. 35 (1949) 227.
[2] N.K. Raman, M.T. Anderson, C.J. Brinker, Chem. Mater. 8 (1996) 1682.
[3] S. Mann, S.L. Burkett, S.A. Davis, C.E. Fowler, N.H. Medelson, S.D. Sims, D. Walsh, N.T. Whilton, Chem. Mater. 9 (1997) 2300.
[4] D. Kriz, O. Ramstrom, K. Mosbach, Anal. Chem. (1997) 345A.
[5] Q. Fu, H. Sanbe, C. Kagawa, K.K. Kunimoto, J. Haginaka, Anal. Chem. 75 (2003) 191.
[6] O. Ramstrom, K. Skuder, J. Haines, P. Patel, O. Bruggeman, J. Agric. Food Chem. 49 (2001) 2105.
[7] P. Spegel, L. Schweitz, S. Nilsson, Anal. Chem. 75 (2003) 6608–6613.
[8] A.G. Mayes, M.J. Whitcombe, Adv. Drug Deliv. Rev. 57 (2005) 1742–1778.
[9] S. Firenman-Shoresh, I. Turyan, D. Mandler, D. Avir, S. Marx, Langmuir 22 (2005) 7842.
[10] T. Shiomi, M. Matsui, M. Mizukami, K. Sakaguchi, Biomaterials 26 (2005) 5564.
[11] Z. Zhang, Y. Long, L. Nie, S. Yao, Biosens. Bioelectron. 21 (2006) 1244.
[12] R.G. de Costa, F. Augusto, J. Chromatogr. A 1114 (2006) 216.
[13] I. Turyan, D. Mandler, Anal. Chem. 69 (1997) 894.
[14] G. Shustak, S. Marx, I. Turyan, D. Mandler, Electrocatalysis 15 (2003) 398.
[15] M.E. Brown, D.A. Puleo, Chem. Eng. J. 137 (2008) 97.
[16] K. Lee, R.R. Itharaju, D.A. Puleo, Acta Biomater. 3 (2007) 515.
[17] X. Jiang, W. Tian, C. Zhao, H. Zhang, N. Liu, Talanta 72 (2007) 119–125.
[18] X. Jiang, C. Zaho, N. Jiang, H. Zhang, M. Liu, Food Chem. 108 (2008) 1061.
[19] A.V. Rao, G.M. Pajonk, N.N. Parvathy, J. Mater. Sci. 29 (1994) 1807.
[20] S. Marx, Z. Liron, Chem. Mater. 13 (2002) 3624.
[21] S. Fireman-Shoresh, D. Avnir, S. Marx, Chem. Mater. 15 (2003) 3607.
[22] S. Marx, A. Zaltsman, Int. J. Environ. Anal. Chem. 83 (2003) 671.
[23] K. Farrington, F. Regan, Biosens. Bioelectron. 22 (2007) 1138.
[24] D. Silvestri, N. Barbani, C. Cristallini, P. Giusti, G. Ciardelli, J. Membr. Sci. 282 (2006) 284.
[25] Oliver K. Castell, Christopher J. Allender, David A. Barrowb, Biosens. Bioelectron. 22 (2006) 526.
[26] M.T.I.W. Schüsler VanHees, G.M.J. Beijersbergen Van Henegouwen, M.F.J. Driever, Pharm. Weekbl. [Sci.] 5 (1983) 102.
[27] M. Watanabe, T. Akahoshi, Y. Tabata, D. Nakayama, J. Am. Chem. Soc. 120 (1998) 5577.
[28] J. Matsui, K. Akamatsu, S. Nishiguchi, D. Miyoshi, H. Nawafune, K. Tamaki, N. Sugimoto, Anal. Chem. 76 (2004) 1310.
[29] H.-J. Liang, T.-R. Ling, Rick F. Jo, J.-C. Choub, Anal. Chim. Acta 542 (2005) 83.