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Ultrasensitive and selective detection of dopamine using cobalt-phthalocyanine nano-pillars based surface acoustic wave sensor

Najla FOURATI^{1,}, Mahamadou SEYDOU², Chouki ZERROUKI¹, Ajay SINGH³,
Soumen SAMANTA³, François MAUREL², Dinesh. K. ASWAL³, Mohamed CHEHIMI^{2,4}*

¹ SATIE, UMR 8029 CNRS-ENS Cachan-Cnam, 292 rue Saint Martin, 75003, Paris, France

² Université Paris Diderot, Sorbonne Paris Cité, ITODYS, UMR 7086, CNRS 15 rue J-A de Baïf, 75013 Paris, France

³ Technical Physics Division, Bhabha Atomic Research Center, Mumbai-400 085, India

⁴ Université Paris-Est Créteil, CNRS UMR 7182 ICMPE, 2-8 rue Henri Dunant, 94320 Thiais, France

Keywords: SAW sensor, Cobalt Phthalocyanine, Dopamine, Ascorbic acid, Chemical calculations.

Abstract

A highly selective and sensitive surface acoustic wave (SAW) sensor of dopamine (DA) has been developed by depositing cobalt phthalocyanine (CoPc) nano-pillars on gold coated sensing platform of SAW sensor. The developed biosensor presents a sensitivity of 1.6°/nM, has a low limit of detection (LOD) of order of 0.1 nM and imparts more selectivity towards DA, since the detection limit of the interfering ascorbic acid (AA) is as high as 1 mM.

* Corresponding author
Email :fourati@cnam.fr. Tel: +33 1 58 80 87 03

To understand the selectivity mechanisms of this sensor towards DA, density functional theory (DFT) based chemical calculations were carried out. Calculations suggest two different types of interactions: dative bond with a very strong character for DA-CoPc complexes, and significant ionic character in the case of AA-CoPc ones. The interaction energies, in liquid phase, were estimated to be equal to -81 kJ.mol^{-1} and -38 kJ.mol^{-1} for DA-CoPc and AA-CoPc complexes respectively, therefore accounting for the selective detection of DA over AA using tandem CoPc nano-pillars based SAW sensor device. This work demonstrates a simple and efficient design of SAW sensors employing thin nanostructured CoPc bio-molecular recognition layers for DA detection.

1. Introduction:

Dopamine (DA) is an important neurotransmitter in mammalian central nervous systems and the loss of DA-containing neurons may result in serious diseases such as schizophrenia and Parkinson's diseases ¹⁻³. However, there exist some challenges to measure DA under physiological conditions. The main ones are related to the coexistence of very low levels of DA with high levels of ascorbic acid (AA) in organisms. In fact, AA is not only present in brain, but it also releases during neuronal activity and by some psychoactive drugs ⁴. In the case of DA detection by commonly employed electrochemical methods, limitations are related to the fact that DA oxidized at almost the same potential as AA. Nevertheless, recent publications have highlighted several strategies to overcome this problem using new nanocomposites ⁵⁻¹⁰, magnetic particles ¹¹⁻¹⁶, conducting polymer ¹⁷⁻¹⁸, carbon nanotubes ¹⁹⁻²⁵ and molecularly imprinted polymers as active layers ²⁶⁻³⁰. However, the development of these electrochemical sensors requires yet large numbers of steps for fabrication. As alternatives to electrochemical methods,

one can design gravimetric sensors such as quartz crystal microbalance (QCM) and surface acoustic wave (SAW) sensors³¹⁻³². These methods could also be combined with electrochemistry for the fabrication of sensing layers³³⁻³⁴. To the best of our knowledge not much work has been done to detect dopamine using SAW sensors. The basic principle of SAW sensors is the detection of changes in the propagation characteristics of the surface acoustic waves which are caused by perturbations on the active surface of the device. The physical, chemical or biological variations, occurring on the sensor sensitive area, can be estimated from the output signal by measuring insertion loss, phase shift or oscillation frequency. Since frequency (or phase) can be measured with extremely high precision, hence test molecules / gas / liquids can often be detected at extremely low concentrations³⁴⁻⁴³. Still, one has to design efficient sensing layers for the target analytes and the approach might be simple or tedious. In the present work, we have functionalized the SAW sensor by metal phthalocynine (MPcs) thin films, as sensing layer. MPc thin layers have been widely used as recognition layers in gas sensors⁴⁴⁻⁴⁹. In the present work we demonstrate that cobalt phthalocyanine (CoPc) thin films, grown by molecular beam epitaxy on sensing platform of SAW sensors, can act as extremely selective and sensitive layers for the detection of DA. To the best of our knowledge this is first ever report on metal phthalocyanine based SAW sensor to detect a neurotransmitter in liquid media with lowest detection limit of 0.1 nM. The experimental results of CoPc based SAW sensor was supported by theoretical results obtained by chemical calculation based on the density functional theory (DFT).

2. Experimental

2.1. Chemicals

Dopamine (DA), cobalt phthalocyanine (CoPc), ascorbic acid (AA), Phosphate buffered saline (PBS) BioReagent pH 7.4 for molecular biology, H₂SO₄ (95%) and H₂O₂ (30%) were procured from Sigma Aldrich. DA and AA were used as received.

2.2. Details of SAW sensor fabrication

The developed sensor consists of a dual delay lines fabricated on a 36° YX-LiTaO₃ piezoelectric substrate. Sensitive area as well as the interdigital transducers (IDTs) were realized by evaporation of (20/80) nm Cr/Au layers. The IDTs were photolithographically patterned with a periodicity of $\lambda = 40 \mu\text{m}$ which corresponds to an operating frequency of about 104 MHz (Figure 1(a)). Each electrode consists of 30 double finger pairs, minimizing thus triple transit interferences. The measurement setup consists of a CoPc-coated SAW sensor, a Kalrez® flow cell deposited on the sensing region, to which is associated a PMMA cover including inlets and outlets (Figure 1(b)) connected to a peristaltic pump, and a HP8711C network analyzer. Before depositing CoPc films, SAW devices were immersed for 30 min into a piranha solution (1:1 (v/v) 98% H₂SO₄/30% H₂O₂), then rinsed with ultra-pure water three times and immediately dipped in a methanol solution. This procedure is followed to obtain a clean and pre-activated surface, suitable for deposition of CoPc films. Finally the SAW devices were dried using N₂ gas and immediately loaded into the molecular beam epitaxy vacuum chamber (base vacuum $\sim 2 \times 10^{-8}$ mbar) for deposition of 50 nm thick CoPc films. The growth temperature and deposition rate were respectively 50°C and 2 Å.s⁻¹. After that, CoPc coated SAW devices were taken out of vacuum chamber for the further characterization.

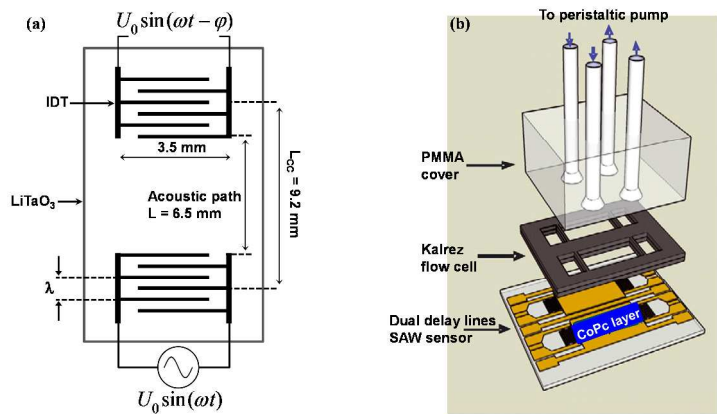


Figure 1. Schematic representation of (a) LiTaO₃ surface acoustic wave sensor
(b) Parts of the measurement setup for use in liquid media

2.3. AFM measurements

AFM measurements were carried out with a Nanosurf easyScan 2 Flex system in the dynamic force mode, with cantilever’s resonance frequency of about 165 kHz. Commercially available tips (ACLA silicon AFM probes from AppNANO) with curvature radius ~ 6 nm were used. The different scans were performed under ambient conditions.

3. Calculations method

Chemical calculations were performed based on the density functional theory. The minimum structures, the electronic and free energies, corresponding to the isolated and complex stable conformers, were calculated at the B3LYP/6-311+G* level of theory⁵⁰. The tight termination criteria (Berny algorithm) optimization procedure as implemented in Gaussian09 was used. The geometries were fully optimized at the B3LYP/GEN level by using 6-311+G(d) basis set for H, C, N, O atoms and LANL2DZ basis set for Co atom in the gas phase. The analyses of vibrational frequencies indicated that optimized structures of complexes were at stationary points corresponding to local minima without imaginary frequencies. The basis set superposition error

is not corrected here, so interactions energies are overstated. Solvent (water) effect is taken into account by Polarizable Continuum Model⁵¹ (PCM) with Kohn Sham united atomic topologies cavities (UAKS) as implemented in Gaussian09⁵². Free energies of complex in solution were derived by mean of thermodynamic cycle applied to systems in gas phase with correction for solvation energies. Stabilization or formation energies $\Delta E_s = E_{A-B} - (E_A + E_B)$ were calculated as the difference between the energy of relaxed complex E_{A-B} and the sum of energies of isolated systems relaxed separately. In our case, E_{A-B} is the energy of complex CoPc and DA or AA, E_A is the energy of AA or DA and E_B is the energy of CoPc. As we compare here energy differences between complexes, the basis set superposition error was not corrected.

4. Results and discussion

4.1. Morphology and structure of CoPc films grown on sensing platform of SAW sensor.

A typical AFM image of a gold sensing area of the SAW sensor reveals a granular morphology with average grain size of order of 100 nm (Figure 2(a)). These grains are protruding out, as revealed by the three dimensional (3D) high magnification image (Figure.2(b)). The two dimensional (2D) AFM image of a 50 nm thick CoPc film, grown over the SAW sensing area, shows that a CoPc layer grows up as quasi-regularly spaced spherical grains with an average size of about 100 nm (Figure 2(c)). The 3D magnified view reveals that quasi-periodically spaced spherical grains are protruding out of the substrate plane like nano-pillars whose height vary between 60 and 130 nm whereas spacing vary between 300 and 650 nm (Figure 2(d)). The surface roughness of these CoPc films was found to be equal to 32.7 nm (in the spatial frequency range: 2.10^{-2} - $20.5 \mu\text{m}^{-1}$).

Notice that such nano-pillars kind of morphology with an extended surface area may be highly suitable for sensing applications.

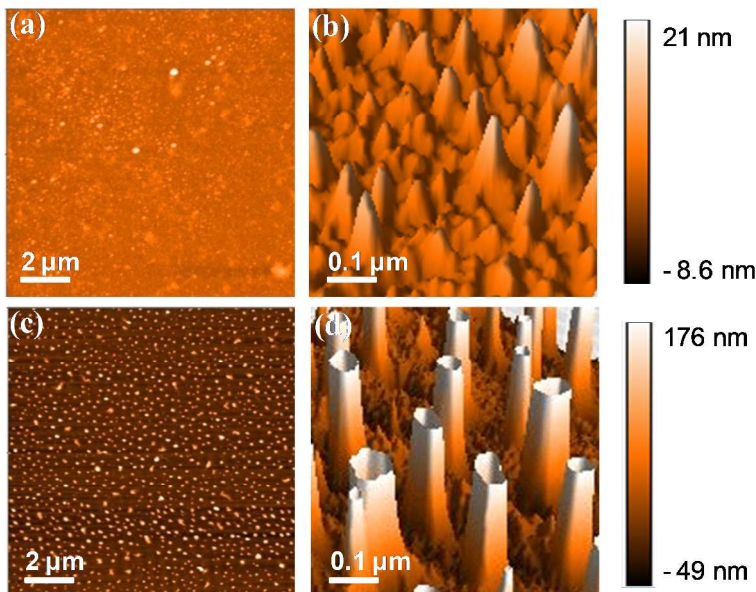


Figure 2. AFM images of (a) 2D scan of gold sensing area of the SAW sensor, (b) the corresponding 3D high magnification image, (c) 2D scan of 50 nm thick CoPc film grown over the gold sensing platform of the SAW sensor, (d) 3D view of the high magnification image of CoPc films.

Now we discuss the origin of such well separated nano-pillars morphology of the CoPc films over the gold coated sensing platform of the SAW sensor. The growth mechanisms of organic films on inorganic substrates are complicated phenomena, because of the non-covalent interactions at the interface, that are, molecule–molecule and molecule–substrate. These two interactions allow molecules to adsorb on the substrate surface in edge on or face on configurations. Moreover, as the properties of thin films are very sensitive to the substrate nature, layers of a same material and a same thickness may present different physical/morphological properties when the substrate is modified. Most studies agree on the fact that the first growth process leads to the appearance of isolated islets, on the substrate surface, which raise independently up to a critical thickness, beyond which the islands coalesce and the

thin film becomes continuous. This thickness depends on the nature of the material and the substrate, the speed of deposition, the temperature..., and varies typically from 10 nm to 20 nm⁵³⁻⁵⁴. For the particular case of metallic phthalocyanine, previous studies⁵⁵⁻⁵⁶ have shown that up to a critical thickness (till which molecule-substrate interaction dominates over molecule-molecule interaction), metallic phthalocyanine thin films growth initially takes place via face on configuration over the gold surface (and edge on configuration at higher thicknesses). The reported value of critical thickness for CoPc over gold surface is ~ 10 nm. Based on these reports, it can be speculated that initially CoPc grows up in “face on” configuration on the nano-spherical grains of gold and then grain growth is more favorable perpendicular to the substrate plane. Notice that even if the used high vacuum evaporation can contribute to this particular growth, we think that the key factor is rather the substrate surface. Indeed, in previous works using high vacuum evaporation of CoPc on flexible polymeric substrate, no comparable structures were observed by atomic force microscopy⁵⁷.

In Figure. 3, we have represented a cross section of AFM images of the SAW sensing area before and after the deposition of a 50 nm thick CoPc film. Higher and less numerous peaks appear in the case of a CoPc film cross-section (Figure. 3(b)) compared to that of a bare gold surface (Figure. 3(a)). To highlight the previous idea, we have considered two other statistical parameters to characterize the previous images of Figure. 2: the maximum surface peak height S_p and the maximum surface valley depth S_v . For the bare gold surface, we founded $S_p = 41$ nm and $S_v = -6$ nm. That means that the bare surface consists mainly of peaks, which constitute a favorable condition for CoPc growth perpendicularly to the substrate plane. After the deposition of a CoPc film, S_p and S_v were founded equal to 474 nm and - 45 nm respectively. The substrate

surface features have thus been reproduced and amplified. This is also in line with our assumption that the dominant peaks were the seat of the first molecule-substrate interaction.

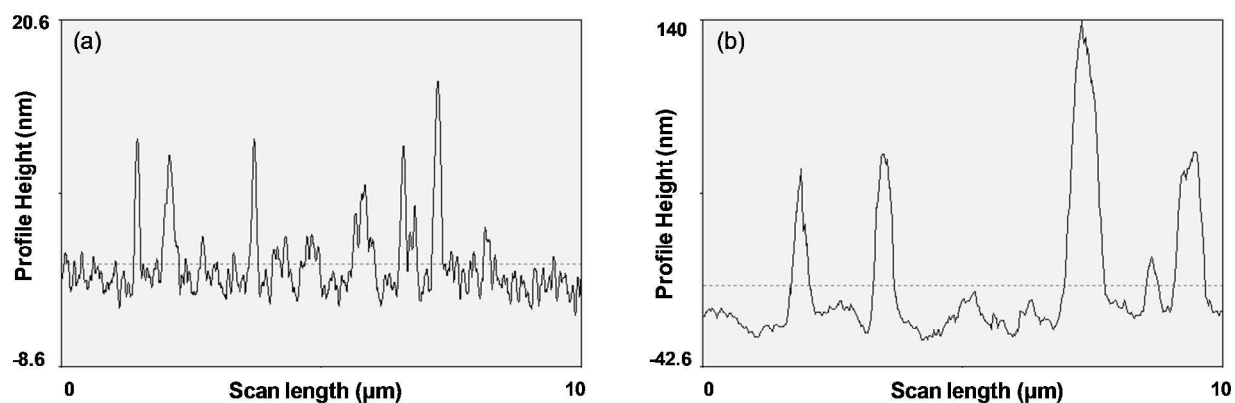


Figure 3. Cross section of AFM images sensing area of the SAW sensor

(a) bare gold (b) after the deposition of 50 nm thick CoPc film

4.2. Sensor performance evaluation

The sensing area of the CoPc SAW sensor was first rinsed with a phosphate buffered saline (PBS) solution, and then dopamine solution was injected. The typical response curve (i.e. phase shift versus time) after the injection of two successive concentrations [0.1 nM] of DA is shown in Figure 4 (a). It appears that, within uncertainties, phase shift values are comparable after exposures to the same DA concentration. The repeatability of the developed sensor is of order of 14%. From Figure 4 (a) it can also be seen that these sensors exhibit a response time of about 500 s, a value which is in the same order of magnitude as other SAW sensors applications [35]. Sensitivity of the developed sensor, calculated from the slope of the phase/concentration curve, was found equal to 1.6°/nM. This value is sufficiently high to foreshadow further easy detection of DA with concentrations in the sub-nanomolar range. The lowest detection limit

(LDL) of ~ 0.1 nM is significantly lower than 26 nM characteristic of living systems⁵⁸, making thus disease diagnosis, using the present SAW sensor, possible in further developments.

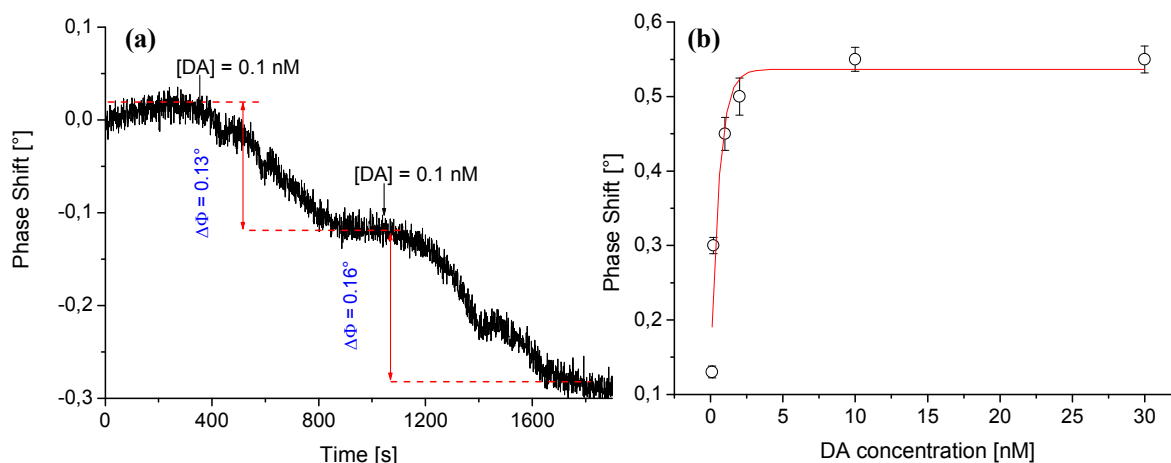


Figure 4. (a) Phase variations versus time after two successive injections of 0.1 nM of a DA solution (b) Phase shifts as function of DA concentration.

Phase shift variations ($\Delta\Phi$) versus DA concentration (C), plotted in Figure 4(b), can be fitted with the “One site binding” equation: $\Delta\Phi = \frac{B \times C}{K + C}$, where $B = 0.558 \pm 0.018$ [°] is the maximum sensor’s response to the specific binding (CoPc-DA), reached when all the interaction sites are blocked (due to full coverage of the sensing layer); and $K = 0.227 \pm 0.038$ [nM] represents the equilibrium binding constant.

4.3. Selectivity test

Useful biosensors must be selective in addition to being sensitive. To investigate selectivity, the developed SAW sensor was exposed to ascorbic acid solutions. From Figure 5, it can be seen that, for concentrations of AA in the range $[10^{-6} - 10^{-4}]$ M, no noticeable changes in the phase

shift were recorded. The sensor starts giving response at an AA concentration of 10^{-3} M: a phase shift of order of 0.19° is observed. The CoPc films based SAW sensors are thus highly selective to DA in comparison to AA.

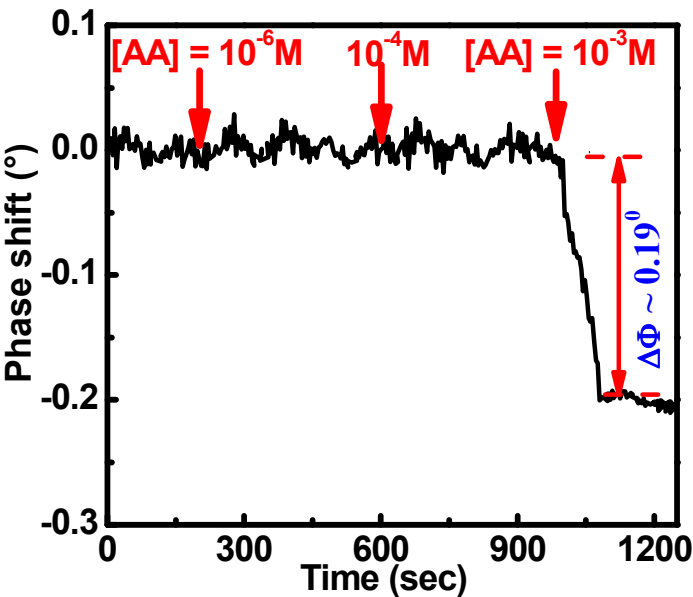


Figure 5. Response of the CoPc based SAW sensor for different AA concentrations.

5. Chemical calculations

5.1. Isolated systems conformational landscape

In order to explore the potential energy landscape of DA and AA, we have applied an optimized scan with a pitch of 10° , from -180° to 180° , on the main dihedral angle, denoted θ , which defines the tail position relative to the ring (Figure. 6). The corresponding potential energy landscape, for both DA and AA, released at B3LYP/6-31G* is also presented in Figure. 6. The most stable conformers, corresponding to the minima of energy, were fully optimized with the large and diffuse basis set 6-311+G* and selected to complex with the template.

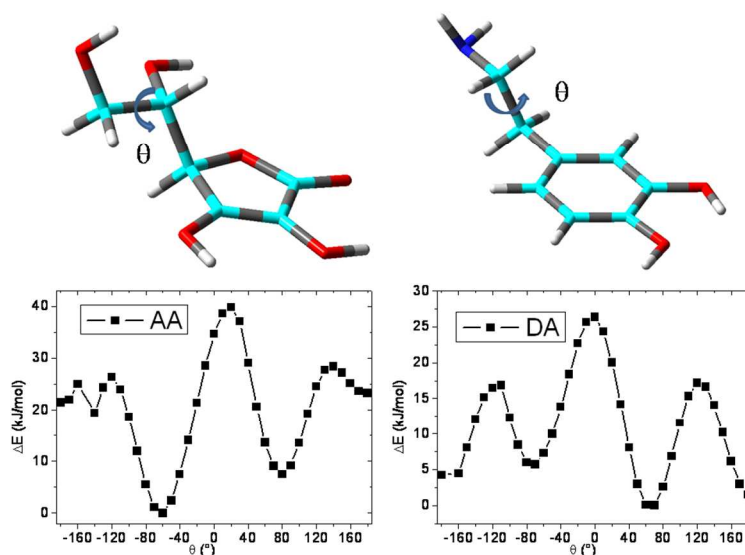


Figure 6. Structures and potential energy landscape of AA (left) and DA (right) in function of dihedral angle θ at the B3LYP/6-31G* level of calculation.

5.1.1. dopamine

The systematic conformational search realised at B3LYP/6-31G* shows the existence of four minima. All of them were fully optimized at the improvement calculation level (B3LYP/6-311+G*). Results show that the increase in basis set size changes the relative conformational energies and reduces the conformational landscape from fourth to three stable conformers.

Using the large basis set 6-311+G*, the most stable conformer is DAI, with a final torsion angle of 60° . DAII and DAIII are less stable than DAI by 0.60 and 6.04 kJ.mol⁻¹ respectively (Table. 1). The optimized dihedral angle θ values are -178° and -72° for DAII and DAIII respectively.

Recently, conformational landscape of dopamine has been investigated by experimental rotational spectra and theoretical calculations through a systematic variation of the dihedral angles that determines the conformation of the side chains and OH group position of

dopamine⁵⁹. These previous results suggested the presence of seven conformers for the neurotransmitter; however the most stable ones are those we found by means of B3LYP/6-311+G*level (see Table 1).

Table 1: Dihedral angles, relative energies of conformers compared to the lowest energy conformations of DA and AA calculated at the B3LYP/6-311+G* level.

Molecule	DA			AA			
Conformer	III	I	II	II	I	III	IV
Dihedral Angle θ (°)	-72	60	-178	180	-62	81	172
ΔE (kJ.mol ⁻¹)	6.04	0.00	0.60	2.26	0.00	7.55	21.62

5.1.2. Ascorbic acid

The L-ascorbic acid used in experiment can adopt two tautomeric forms depending on protonated sites. A previous study showed that the most stable tautomer is that for which the two oxygen in beta position relative to the oxygen atom of the ring, are protonated⁶⁰.

According to the flexible tail, different conformers could be present. Results, presented in Figure 6, show the existence of four stable conformers (which were optimized with a large basis set 6-311+G*) and that the most stable one (AAI) has a final torsion angle of -62°. AAII, AAIII and AAIV are less stable than AAI by 2.26, 7.55 and 21.62 kJ.mol⁻¹ respectively (Table 1). These results are in a good agreement with those of Yadav et al⁶¹.

5.1.3. Cobalt phthalocyanine (CoPc)

Calculations have been done for different symmetries with LANL2DZ on Co and 6311+G* basis set on the over atoms. We found that the most stable configuration is planar with D_{4h} symmetry, in agreement with previous observations where it was shown that for the three-fold symmetry of the Au (111) surface, the symmetry of the adsorbed planar metallophthalocyanine (MPc) molecules is reduced from D_{4h} to C_{2v} ⁶².

5.2. Complex formation

The conformers of both DA and AA were attached to CoPc by chemical intuition in order to establish metal nitrogen or oxygen ionic covalent bond and hydrogen bond interactions. The complexes were fully optimized at B3LYP/6-311+G* level and normal mode vibration frequencies were computed to ensure that they are really local minima. The results are reported in Figure 7.

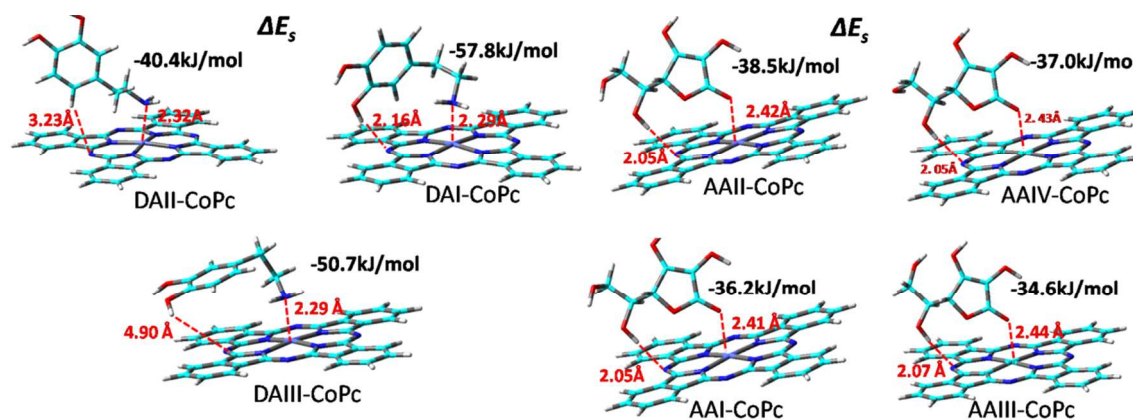


Figure 7. Structures of stable conformers of DA-CoPc and AA-CoPc complexes (the formation energies and geometrical parameters $d_{H...N}$ and $d_{Co-N(O)}$ are inserted).

The most stable configuration for dopamine corresponds to the case where nitrogen atoms are placed above cobalt atom to form Co-N dative bond. The Co...O interaction was also interrogated by placing oxygen atom above Co. The obtained complexes are not stable and the geometry changes drastically. The most stable structure is that for which one strong hydrogen bond takes place between one OH group of dopamine and the nitrogen of phthalocyanine ($d_{H...NPc} = 2.16 \text{ \AA}$) in addition to the dative bond ($\text{Co-N} = 2.29 \text{ \AA}$) (Figure 6). In our case, the most stable complex is DAI-CoPc, for which the formation energy was found equal to -58 and -81 kJ.mol^{-1} in gas and liquid phases respectively. It is noteworthy that the Co-N dative length value is in a good agreement with the experimental and theoretical values obtained in ⁶³⁻⁶⁴. The two other structures are less stable with formation energies of -51 and -40 kJ.mol^{-1} in gas phase. In the DAIII-CoPc complex, dopamine conformer is linked essentially by Co-N dative bond in addition to a weak π - π stacking. The comparison of DAI-CoPc and DAIII-CoPc complex leads to evaluate the Co-N bond formation energy. The dihedral angle values are not modified significantly by adsorption. They derived from isolated values of $+11^\circ$, 0° and -6° for DAIII, DAII and DAI respectively. Our results are in line with the previous work of Ponce et al. ⁶⁵ who have investigated, by both experimental and theoretical studies, the formation of SAMs involving cobalt phthalocyanines anchored by 4-aminothiophenol and 4-mercatopyridine to the Au (111) surface. They found dative bond lengths of 2.06 and 2.39 $\text{\AA}$ for 4-mercatopyridine and 4-aminothiophenol respectively.

In the case of AA, the most stable AA-Pc complex corresponds to that where a dative bond takes place between the oxygen of carbonyl group and cobalt atom. The complexes are stabilized also by one hydrogen bond between the OH group of the tail and one nitrogen atom of phthalocyanine as shown in Figure 7. The formation energies of these complexes are much

closed and vary from -34 to -38 kJ.mol⁻¹. Our values are however higher than those obtained by Sun et al.⁶⁶ for oxygen molecule adsorption on CoPc (-27.7 kJ.mol⁻¹). This difference could account for the presence of hydrogen bond between AA and CoPc. By taking into account the solvation influence, the formation energy decreases down to -60 kJ.mol⁻¹ for the AAIL-CoPc conformation. As the energies of formation, the length of the dative bonds and the hydrogen bonds vary slightly between conformations. The Co-O dative length varies from 2.41 to 2.44 Å, while the hydrogen bond length varies in the range 2.05 - 2.07 Å. The dihedral angles of AA conformers are not significantly modified by the complex formation. They remain unchanged for all conformers except for AAIL where a shift of 4° was recorded.

Comparing the two molecules of interest, we can observe that CoPc affinity is more important for DA than AA with more than 20 kJ.mol⁻¹ in both gas and aqueous phases. This result is in concordance with metal ligand bond length. In the case of DA, the Co-N bond length (2.29 Å) matches the sum of covalent radii of N and Co (2.01 Å). For AA, the Co-O (2.40 Å) is larger than the sum of O and Co covalent radii (1.99 Å). These results argue for the formation of a dative bond between Co and N, whilst C and O are expected to develop a rather ionic bond. The hydrogen bonds lengths H...NCoPc for DA and AA complexes are 2.16 and 2.05 Å respectively. The six-coordinate Co complexes were investigated by adding two molecules DA or AA around CoPc. Formation energies per molecule, in gas phase, were found equal to -48 and -33 kJ.mol⁻¹ for DA and AA complexes respectively. It may be noted that in both cases the formation energy decreases proving that the penta-coordinate is more stable than the six-coordinate CoPc complexes. The lengths of dative CO-N(O) bonds increase from penta-coordinate values of about 0.14 (0.10) Å and hydrogen bond OH...NPc changes to 2.04 Å.

The Mulliken charges on cobalt, nitrogen of dopamine and oxygen of AA carbonyl group are gathered in Table 2. Results show an important charge transfer between cobalt atom and oxygen or nitrogen atom of AA and DA. This charge transfer is more important for AA (0.45 e⁻) than DA (0.25 e⁻). This observation proves that the ionic character is more pronounced for Co-O in AAIL-CoPc. It should be noted that part of cobalt charge was transferred to its neighbouring nitrogen denoted NPc in Table 2.

Table 2 :Formation energies, dative and hydrogen bonds length and Mulliken atomic charges on nitrogen (qN), oxygen (qO), cobalt(qCo) and nitrogen of DAI, AAIL, CoPc, DAI-CoPc and AAIL-CoPc.

Isolated or complex	ΔEs (kJ.mol ⁻¹)	Co...N(O) (Å)	d H....N (Å)	qN (O) (a.u)	qCo (a.u)	qNPc (a.u)
DAI	-	-	-	-0.63	-	-
AAIL	-	-	-	-0.33	-	-
CoPc	-	-	-	-	0.98	0.06
DAI-CoPc.	-57.8	2.29	2.16	-0.02	0.73	0.08-0.27
AAIL-CoPc.	-38.5	2.43	2.05	0.06	0.530	0.16-0.38

Results of frontier molecular orbital's around the highest occupied orbital (HOMO) and the lowest unoccupied orbital (LUMO) analysis are summarized in Figure 8. For DA, the occupied orbital HOMO-2 corresponds to π orbital essentially localized on nitrogen atom. For CoPc, the unoccupied LUMO+2 orbital correspond to dz² of cobalt atom. For AA, the occupied HOMO-2

orbital represents the lone pair π orbital localized in oxygen atom. The difference of energy between these frontier orbitals is low and may allow an overlap between them. In addition to hydrogen bonds and ionic part, the interaction between AA or DA and CoPc is dominated by the overlap between the unoccupied LUMO +2 (d_z^2) of CoPc and the occupied HOMO-2 (π) orbitals of DA or AA. The products of this interaction are the bonding orbital HOMO-19 and the antibonding LUMO+2 in the DA-complex and HOMO-21 and LUMO+1 for the AA one. Similar types of interaction were found in previous study carried out on NO molecule adsorption on CoPc⁶⁷. In the case of AA, and because of hybridization, one can observe that the position of the lone pair orbital cannot allow maximizing the overlap with d_z^2 . This could explain the low energy of formation. In contrast to AA, the occupied π orbital, located on N atom of DA, takes place perfectly above Co and gives better recovery with d_z^2 of CoPc. This may explain why the dative bond is stronger for DA compared to AA.

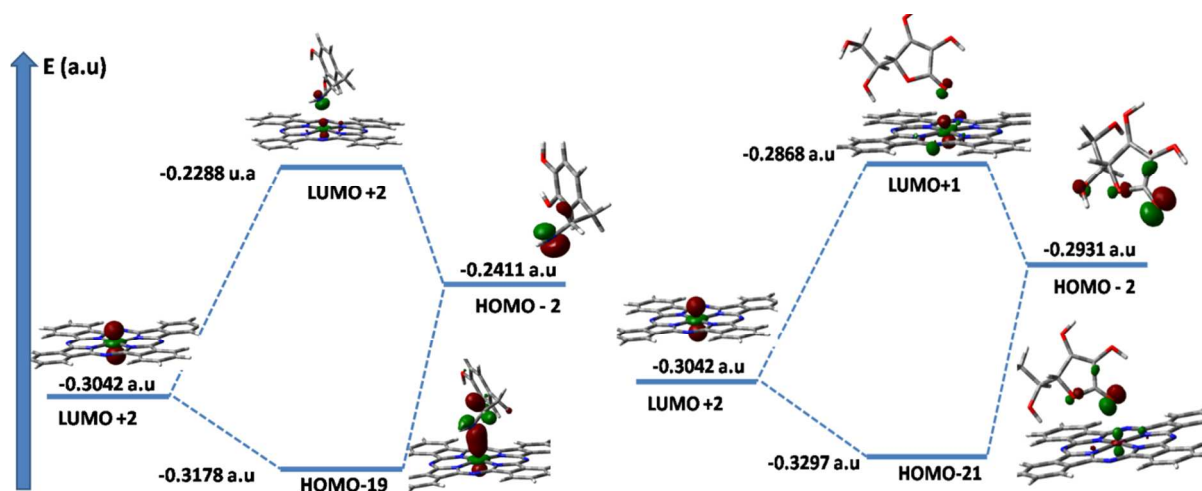


Figure 8. Frontier molecular orbitals overlap between CoPc and DA and AA.

6. Sensing mechanism

Based on the theoretical calculations results, we propose that the interaction between DA and CoPc is mediated by the formation of dative bond between N (from DA) and Co. In the formation of dative bond the lone pair of N gets transferred to $3d^7$ orbital of Co^{2+} . In case of AA and CoPc interaction is due to the formation of ionic bond between O (from AA) and Co. Further deep understanding of interaction can be understood by molecular orbital picture. For DA, the occupied orbital HOMO-2 corresponds to π orbital essentially localized on nitrogen atom. For CoPc, the unoccupied LUMO+2 orbital correspond to d_z^2 of cobalt atom. For AA, the occupied HOMO-2 orbital represents the lone pair π orbital localized in oxygen atom. The interaction between AA or DA and CoPc can be understood by the overlap between the unoccupied LUMO+2 (d_z^2) of CoPc and the occupied HOMO-2 (π) orbitals of DA or AA. In the case of AA, because of hybridization, one can observe that the position of the lone pair orbital cannot allow maximizing the overlap with d_z^2 of CoPc. In contrast to AA, the occupied π orbital, located on N atom of DA, takes place perfectly above Co and gives better overlap with d_z^2 of CoPc. This explains why CoPc SAW sensors respond to lower concentration of DA as compared to AA.

7. Conclusion

A surface acoustic wave sensor coated with a thin layer of cobalt (II) phthalocyanine has been developed for selective detection of dopamine. Results show that this device has an excellent dopamine sensing capability in the sub-nanomolar range, including a reasonably fast response, and low detection limit of 0.1 nM. The CoPc nano-pillars based SAW sensors were found to be highly selective towards dopamine in comparison to ascorbic acid. Chemical calculations, based

on the density functional theory (DFT), were performed in order to understand the high affinity of CoPc to dopamine in comparison to ascorbic acid. Results show the formation of a dative bond with a very strong character for dopamine-CoPc complex, while the analysis of Mulliken charges and frontier orbitals highlighted a significant ionic character in the case of ascorbic acid. The agreement between theoretical calculations and experimental results are not only encouraging but pave also the way to more important developments of metal phthalocyanine-functionalized SAW sensors in applications where there is a demand for high sensitivity and extremely low limits of detection, associated to great selectivity.

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Graphical Abstract

