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"Polydopamine" (Dopamine-Eumelanin) Coatings as a Concept for an Integrated Experimental Surface Science Course for Graduate Students

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Abstract

"Polydopamine" coatings have been introduced about 8 years ago as a versatile coating technology allowing for the surface modification of almost all kinds of materials (even Teflon). This is a kind of "holly Graal" in surface chemistry particularly if one considers that all the chemistry can be performed in aqueous solutions, hence in environmentally friendly conditions. In addition to many potential applications afforded by such coatings, their physicochemical properties, their reactivity and their yet unknown deposition mechanism are an extraordinary opportunity for chemistry students at the master level to unify their knowledge of surface chemistry concepts and to be faced with real research problems both at the fundamental and applied levels. In this article, a series of experiments will be presented that can be performed using a UV-vis spectrophotometer and a single electrochemical workstation by master students in a typical time frame of a four-five days lab course to get a global knowledge in surface chemistry (at the solid-solution interface). Some thickness measurements by means of ellipsometry and some contact angle measurements may complement this experimental course.

Keywords: Surface Science, Interfacial Electrochemistry, Contact Angles, Film Permeability, Electrochemical Impedance Spectroscopy, Master Students, Experimental Course.

Introduction

Surface science is one of the major fields of chemistry owing to practical applications as the development of catalysts, the protection against corrosion, the adhesion of dyes to a substrate in painting processes, the control of adhesion processes after the implantation of a biomaterial (for instance a hip prosthesis) in a living organism, etc. The major aim is to control the composition as well as the surface topography of the interface between the considered material and its environment. This will allow a fine control of the atoms available at the interface and hence of the surface tension of the material – environment interface. This surface tension is the free energy required to create an interface of unit surface area between the material and its environment at constant temperature and pressure [1].

In many cases it is important to hide the atoms present at the materials surface, because their reactivity maybe too high, hence to coat it with a "protecting" layer of atoms or molecules. This layer should at least be of monomolecular thickness (hence a monolayer), without uncovered domains on the surface. In addition such a coating should be robust by strongly adhering on the substrate for a prolonged period of time. The history of surface technology is marked by the empirical development of coating methods allowing to functionalize interfaces with such homogeneous and robust coatings, painting being a significant example. More sophisticated technologies allow to get monomolecular thick coatings, such as vacuum deposition technologies (molecular beam epitaxy, sputtering, etc…), Langmuir-Blodgett deposition, grafting of self-assembled monolayers on the surface of noble metals like gold, silver and platinum; electrostatic self-assembly on the surface of charged interfaces (among which charged oxides) [2-6]. All these technologies are rather material specific, among other possible drawbacks. It is hence of the highest interest to find versatile and universal coating technologies. Inspiration from nature was and still is extremely useful for such a goal: for instance after having understood the reversible adhesion mechanism of the Gecko lizard it was possible to develop new kinds of adhesives compatible with a wet environment [7]. The way mussels like *Mytilus Californianus* adhere to solid substrates in wet conditions and under strong shear stresses has also inspired the development of a new coating technology [8]. Indeed the most significant proteins of the mussel foot, at the foot-substrate interface, called *mefp*5 proteins, are rich in lysine residues as well as in L-dopa, a catechol containing group. The terminology "Catechol" holds for 1,2-dihydroxybenzene. Such groups undergo oxidation to quinones in the presence of an oxidant like the oxygen dissolved in sea water. The catechol groups are responsible for the strong adhesion of the mussel to almost all kind of substrates through a combination of hydrogen bonds and coordination with metal ions. Based on this knowledge, the group of professor Ph. Messersmith showed that "polydopamine" films can be deposited on the surface of almost

all kinds of materials (metals, oxides, polymers) to build up an homogeneous film of about 40-45 nm in thickness [9]. Note that we put the term "polydopamine" in brackets because it means implicitely that this material is a polymer. Recent experimental evidence tends to show that it is rather a supramolecular aggregate [10]. But herein we do not wish to enter in such considerations and prefer to focus on a material in the form of a coating made by oxidation of dopamine and which is of the highest interest for teaching purposes.

It is the aim of this article to show that investigating the deposition of such coatings and some of their basic properties constitutes an excellent subject for an integrated experimental course in (wet) surface chemistry. This course is dedicated to master students because it requires good basic knowledge in physical chemistry (basics in electrochemistry are mandatory, and good knowledge in optics maybe useful).

Materials and methods

Chemicals: "polydopamine" films and or particles will be synthesized using dopamine as the catechol amine source (Sigma Aldrich, ref. H 8502). Electrochemical probes used to measure the permeability of the "polydopamine" films are potassium hexacyanoferrate ($K_4Fe(CN)_6$, for instance ref. P3987 from Sigma-Aldrich), ruthenium (II) hexamine ($Ru(NH_3)6Cl_2$, for instance ref. 303690 from Sigma-Aldrich) and ferrocene methanol ($C_{11}H_{12}FeO$, for instance ref. 335061 from Sigma-Aldrich). The reactions will be performed in the presence of Tris(hydroxymethyl) aminomethane buffer (Sigma-Aldrich, ref. T1503 for instance) to stabilize the pH at around 8.5 during the experiments.

In cases where the instructor wishes the students to measure the surface energy of the polydopamine coatings, the following solvents should be used: distilled water, iodomethane and n-hexane.

In all cases the substrates to deposit "polydopamine" can be glass slides but preferentially quartz slides (owing to their transparency between 200 and 300 nm for characterization of the polydopamine films by UV-vis spectroscopy). Even if quartz slides are expensive, they can be cleaned and reused for further experiments. The working electrodes for the cyclic voltammetry experiments can be made from amorphous carbon, but semi-conducting ITO (indium tin oxide) electrodes are preferred because they allow to perform simultaneously electrochemical and spectroscopic characterization. However ITO is expensive and may not be accessible to all experimental labs.

For standard experimental labs, the required instrumentation is:

1. A double beam UV vis spectrophotometer. The experiments presented herein were performed on mc^2 spectrophotometer from Safas (Monaco).
2. An electrochemical workstation fitted with a variable frequency generator to perform some electrochemical impedance spectroscopy. The experiments presented in this paper were performed with a CHI 604B workstation (CH Instruments, Austin, Texas, USA).
3. Eventually, an ellipsometer (an Auto SE spectroscopic ellipsometer from Horiba was used herein, but a single wavelength apparatus can also be used) and a contact angle goniometer (an OCA goniometer was used in this investigation) may be useful to measure the thickness of the films and their surface energy, respectively.

Description of the experiments

"Polydopamine" films will be produced by the students using aerated dopamine solutions. The absorption spectra of the coatings deposited on quartz slides should be measured after different reaction times. The coatings obtained at the end of the kinetics can be characterized by contact angle goniometry. If no contact angle goniometer is available, the experiments can be of qualitative nature, ie by observing the shape made from small droplets (a few µL) of different liquids on the "polydopamine" coatings.

The students will first start to read some basic papers about eumelanin and the deposition of polydopamine films [9,11]. This will typically last over the first morning. They will then prepare the required chemicals: Tris buffer at 50 mM and adjust its pH to 8.5 with hydrochloric acid (typically at 1.0 M) and prepare some aliquots of dopamine. The dopamine hydrochloride powder should be added to the Tris buffer just before the beginning of the film deposition experiments. The recommended concentration in dopamine is of 2 $mg.mL^{-1}$ (ie 10.6 mM). But experiments can be performed at other concentrations if the students wish to investigate the influence of dopamine concentration on the kinetics of the "polydopamine" film growth. More details about such experiments can be found in the literature [12].

The samples to be coated, namely quartz, ITO or silicon slides should be cleaned with a surfactant solution (sodium dodecyl sulfate at 10 mM, or a commercial soap solution like Decon at 1 %), followed with intensive water rinse, rinse with hydrochloric acid, and finally (if possible) with an oxygen or UV-ozone plasma (to remove all traces of organic compounds). These cleaning steps should also be applied to the beaker in which the solution will take place and should be performed just before the beginning of the coating experiment. The instructor should discuss with the students about the importance of specific cleaning methods in surface chemistry.

If amorphous carbon electrodes are to be used for cyclic voltametry (CV) and electrochemical impedance spectroscopy (EIS), these electrodes should be carefully polished with diamond pastes (typically with grain sizes of 1 µm and 100 nm) and sonicated in the presence of water (2 sonication steps at 35 kHz during 2 min in the presence of fresh distilled water, the beaker containing the electrodes being immersed in an ultrasonic bath). This is time consuming, but extremely didactic. The students will be able to check the quality of their cleaning. Indeed the kinetics of electron transfer between the surface of the working electrode and the electroactive probes (potassium hexacyanoferrate is well suited in aqueous solutions) depends markedly on the presence of contaminants on the surface of the working electrode. In the absence of such contaminants, the oxidation and reduction peaks of redox probes implying the exchange of one electron should be spaced by $2.303xRT/F = 0.059$ V at T= 298 K (R is the gas constant and F the Faraday, the charge carried by one mole of electrons) in conditions of a reversible redox process. In addition, the oxidation and reduction waves are equal in amplitude. Practically if the oxidation and reduction waves are separated by less than 80 mV, the electrode cleaning can be considered as satisfactory, otherwise the electrode should not be used for the coating process with polydopamine [13].

The cleaned substrates (quartz slides, ITO slides, silicon slides and/or amorphous carbon electrodes) should be glued on the wall of the beaker with a double faced scotch tape. The rectangular shaped

substrates should be hold vertically (ie glued on the vertical walls of the beaker) in order to avoid deposition of particles by means of sedimentation. Such particles will appear during the course of the dopamine oxidation and polydopamine self-assembly.

At time $t = 0$, the freshly dissolved dopamine solution (in the presence of the Tris buffer) should be added in the beaker containing the glued adsorption substrates. At least 8-10 substrates should be used in order to follow the deposition kinetics. The quartz slides will be used for spectroscopic characterization and the silicon slides for thickness measurements (if an ellipsometer is available). Two beakers should be used for that aim, one in which the substrates for UV-vis spectroscopy and ellipsometry and one in which substrates electrochemistry will be immobilized.

During the whole kinetics, the solution should be vigorously shaken (typically 300-400 rpm under magnetic stirring) and the reaction vessel coated with an aluminum foil (some small holes should be perforated through it to allow fast exchange with ambient air). The optimal temperature for the experiment is 25 °C (the students should be recalled that temperature control is of tremendous importance in chemical kinetics).

Before the first measurement aiming to characterize the coating (typically after half an hour of reaction), the students should perform some control experiments to understand why the kinetics are performed in the above described conditions (ambient air and pH 8.5). To that aim they should dissolve dopamine in distilled water (pH of about 6): no color change will be observed even after prolonged time. In a second control they will be asked to make some argon or nitrogen to bubble (for about 10 min) through the Tris buffer (pH = 8.5) before addition of dopamine. An alternative to that would be to put the buffer in a vacuum pump for a few minutes before addition of the dopamine powder. Again no color change will be apparent in strong contrast with the dopamine solution in Tris buffer and in the presence of ambient air (essentially nitrogen + oxygen).

This means that the *control over pH and the presence of oxygen* is mandatory to perform the deposition in these experimental conditions. The students can now discuss, based on the knowledge of the oxidation reaction of dopamine:

$$\text{Dopamine} \Leftrightarrow \text{dopaminequinone} + 2H^+ + 2e^- \quad (1)$$

The other redox partner is dissolved oxygen in the present experimental conditions:

$$O_2 + 4H^+ + 4e \Leftrightarrow 2H_20 \quad (2)$$

This asks the question if other oxidants than oxygen may be used. The answer is of course yes and the students should have a look on a standard redox potential table to select some possible oxidants. A look in the literature will show that similar (but not necessarily identical) coatings can be produced using Cu^{2+} or periodate ions [14,15].

Ellipsometry

Ellipsometry experiments

The students will measure the ellipsometric angles (ψ and Δ) along the major axis of the silicon slides coated with "polydopamine" films at least at 5 different positions to get average values and to evaluate the lateral homogeneity of the coatings. They will treat the data in the framework of an homogeneous and isotropic layer model. The silicon slide will be considered as a semi-infinite medium and the spontaneously grown silicon oxide layer will be treated as an homogeneous film of 2 nm thickness with a refractive index of 1.465 at a wavelength of 632.8 nm (most of the single wavelength ellipsometers are fitted with a Helium-Neon laser). The "polydopamine" film will be treated as a medium with a complex refractive index of 1.73-0.02i. A justification for this particular value of the refractive index may be found in the next paragraph.

Determination of the refractive index of "polydopamine"

To calculate the refractive index of melanin Nm from the one measured for the solution N and the one of the solvent Ns the following relationship is used:

$$N = \theta N_m + (1-\theta) N_s. \quad (3)$$

where Θ is the volume fraction of "polydopamine" and can be calculated as its concentration C divided by its density p Thus

$$N = N_s + \frac{N_m - N_S}{\rho} C = N_s + \frac{dN}{dC} C \Rightarrow N_m = \rho \frac{dN}{dC} + N_s \quad (4)$$

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The density of "polydopamine" is measured by dissolving 1.607 g of dopamine in 5.0 mL of a 1 M sodium hydroxide aqueous solution. After one day of reaction the volume of the black solution is measured. The density of the formed melanin is given by the ratio of the initial mass to the difference in volume provided the mass of "polydopamine" is identical to the initial dopamine mass and evaporation of the solvent is negligible. By this way a density of (1.2 ± 0.1) g.cm^{-3} is obtained being in agreement with theoretical (1.3 g.cm^{-3}) and experimental (1.27 g.cm^{-3}) values for synthetic melanin [Additional References: 1,2].

It is found that the refractive index of "polydopamine" depends only weakly on the time of polymerisation at pH 8.5 for time intervals from 0.5 to 48 h. For two hours at pH 8.5 the real and imaginary part of refractive index are calculated as $n = 1.73 \pm 0.05$ and $k = 0.027 \pm 0.002$ at a wavelength of 589 nm. At 632.8 nm the imaginary part is $k = 0.022 \pm 0.002$.

The cyclic voltammetry (CV) and the electrochemical impedance spectroscopy (EIS) experiments will be performed in the presence of 0.15 M NaCl solutions containing 1mM $K_4Fe(CN)_6$ as a redox probe. Ruthenium hexamine and ferrocene methanol can also be used.

Electrochemical measurements

Cyclic voltametry and impedance spectroscopy

The DC potential will be maintained at +0.25 V (close to the standard redox potential of $Fe(CN)_6^{3-}$ / $Fe(CN)_6^{4-}$) versus Ag/AgCl in a traditional 3 electrode set up, also used for the CV experiments. An AC wave will be superimposed on the DC signal. Its amplitude can be set at 5 mV, and the frequency of this AC signal can be changed from 10^5 to 10^{-2} Hz. Note that this makes an EIS experiment to

last over about one hour. Ideally, the students should perform EIS experiments for coatings resulting from 5min, 1 h, 6h and 24 h of deposition from an aerated dopamine solution (in the presence of 50 mM Tris buffer at 50 mM, pH = 8.5). This will typically be done during the second day of the experimental course. As for the ellipsometry experiments, these experiments should be treated in a theoretical framework which will be detailed in the next section. CV experiments will be performed on the same coatings as those used for the EIS experiments. The same NaCl + 1 mM $K_4Fe(CN)_6$ solution will be used as well as NaCl+1mM Ferrocene methanol and NaCl+1mM $Ru(II)(NH_3)_6Cl_2$. These last experiments are aimed to check the influence of the redox probe's charge on the resulting CV curves. Typically the CV curves will be performed between -0.2 and 0.6 V vs Ag/AgCl at a scan rate of 50 mM.s^{-1}. Another reference electrode, like a saturated calomel electrode may be used.

Calculation of the impedance of the circuit represented in Figure 3A

The electronic circuit represented in Figure 3A is made from the association in series of *R1* and of the association of *R2* and *C2*. Hence, using the association law of impedances in series:

$$Z_T(\omega) = R_1 + Z_2(\omega) \qquad (5)$$

Where ω is the angular frequency (ω=2πf), ZT(ω) the total impedance of the circuit and Z2(ω) the impedance resulting from the association of the resistance *R2* and the capacitance *C2* in parallel.

With $C_2(\omega) = \frac{j}{\omega . C_2}$ and j^2=-1, one gets:

$$\frac{1}{Z_2(\omega)} = \frac{1}{R_2} - j.\omega.C_2 \qquad (6)$$

And hence:

$$Z_T(\omega) = R_1 + \frac{1}{1/R_2 - j.\omega.C_2} \qquad (7)$$

Manipulation of complex numbers allows then the separation of the previous equation in a real and an imaginary part:

$$Z_T(\omega) = R_1 + \frac{R_2}{1+(\omega.R_2.C_2)^2} - j.\frac{\omega.R_2.C_2}{1+(\omega.R_2.C_2)^2} = Z'(\omega) - j.Z''(\omega) \qquad (8)$$

An analysis of (8) shows that the total impedance of the equivalent circuit represented in Figure 3A is a real number for ω=0 and for ω+∞. The whole curve is a semi-circle of radius $R_2/2$ and shifted from the origin by *R1*.

A typical Z"(ω) versus Z'(ω) plot is given in Figure 3A with: *R1*=100 Ω, R2= 1000 Ω and C_2= 50 x 10^{-6} F.

Concerning the contact angle measurements, substrates put in the presence of an aerated dopamine solution for 24h will be used. Solvent droplets of 2-5 µL will be delivered and the contact angles of these droplets with the substrates will be measured. Too large droplets have to be avoided because their shape will be modified by the gravitational force. Contact angle goniometry will typically be performed during the third day of this experimental course.

Another day is required for the students to write a report or to present and discuss their results.

Results and Discussions

Absorption spectra of the "polydopamine" coatings can be found in Figure 1. The students will observe that the coating is deposited not only on the surface of the quartz substrate but also on the whole beaker. If the solution is not well stirred a gradient of color, related to a gradient in film thickness will be observed. This gradient is related to the fact that oxygen is consumed during the oxidation of dopamine (equation (2)). Hence if the stirring speed is not high enough, the reaction kinetics will be faster close to the solution/air interface than in the bulk of the solution. In addition the solution undergoes a color change: from initially transparent, to pink after a few minutes, to brown and finally black. After around 1 day some sediment will also be found on the bottom of the reaction vessel. This means that some colloids or aggregates of significant size are formed. The spectrum of the solution can eventually be followed as a function of time (Figure 2) and compared to the spectra of the coatings (Figure 1). It appears that even after one week of contact with the oxygen from air, the spectra of the solution still contains the signature of un-oxidized dopamine (peak at λ = 280 nm) in addition to a continuous broadband absorption which is responsible for its black color. At the same time, the spectra of the coating contains trace of the peak at 280 nm, even after intensive rinse with water before measurement of the absorption spectrum. This means that the film does incorporate dopamine or closely related molecules [17].

The absorption values at a typical wavelength of 589 nm (the yellow sodium line) can then be extracted from the whole adsorption spectra and plotted as a function of time (Figure 1). This gives an idea of the amount of material deposited on the quartz slides (on both faces of it) and hence of the deposition kinetics. This kinetics can be fitted according to:

$$A(t)=A_{max}.[1-\exp(-k1.t)] \qquad (9)$$

Where A_{max} and k1 are the maximal absorbance at the end of the deposition kinetics and the kinetic rate constant, respectively. A_{max} and k_1 are the fitting constants. The fits were performed with Sigma Plot 11 (Jandel) but other softwares (like Origin or Kaleidagraph) can be used. The data in Figure 1 yield: A_{max}= (0.21 ± 0.03) and k1= (0.25 ± 0.03) h-1. Note that the given standard deviations correspond to a statistical error obtained from the fitting procedure and not to an experimental error. To that aim the students may be asked (depending on the available time) to measure the absorption spectrum of 3 (or more) quartz slides coated under identical conditions and for a given reaction time.

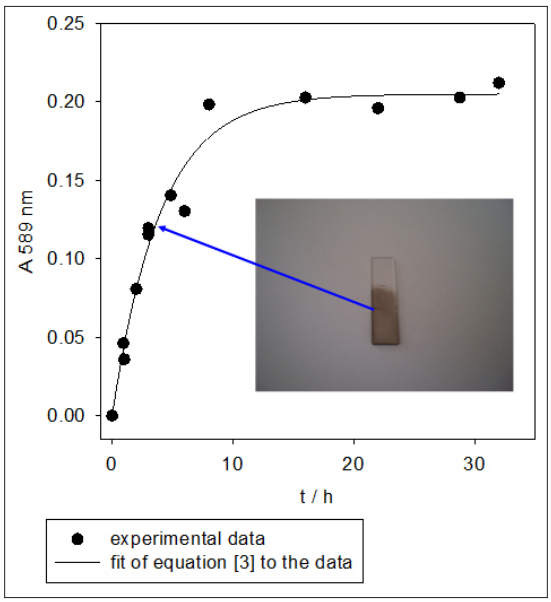

Figure 1: Evolution of the absorbance at λ=589 nm of "polydopamine" coatings as obtained from absorption spectra as those displayed in Figure 1 (initial dopamine concentration: 2mg.mL-1 in the presence of 50 mM Tris buffer at pH = 8.5). Each point corresponds to an independent quartz slide. The full line corresponds to a fit of equation (3) to the experimental data. The inset is a picture of the quartz slide put in contact with the aerated dopamine solution during 5 h.

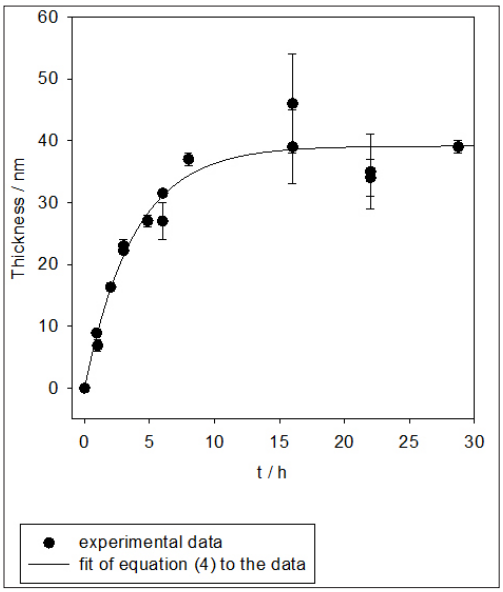

Figure 2: Evolution of the thickness of "polydopamine" coatings as calculated from ellipsometry measurements performed on coated silicon slides (initial dopamine concentration: 2mg.mL^{-1} in the presence of 50 mM Tris buffer at pH = 8.5). Each point corresponds to an independent silicon slide on which 5 measurements were done to get an average thickness value and the error bars correspond to one standard deviation. The full line corresponds to a fit of equation (4) to the experimental data.

If an ellipsometer is available in the experimental room, the same reaction batch used to coat the quartz slides can be used to coat silicon slides (the substrate has to be highly reflective to investigate the change in polarization of linearly polarized light upon reflection). The teacher should put some theoretical description about ellipsometry at the disposal of the students, such a reference [16].

A typical adsorption kinetics is displayed in Figure 2. Each experimental point corresponds to the measurement of an independent film. The indicated thickness value corresponds to the average of 5 ellipsometry measurements and the error bar to one standard deviation. At first glance it appears that the absorption kinetics obtained by ellipsometry has similar shape as the kinetics obtained from absorbance measurements (Figure 1). Hence it seems natural to fit equation (4) to the experimental data:

$$d(t) = d_{max}.\,[1-\exp(-k_2.t)] \qquad (10)$$

where dmax and k2, the fitting parameters, correspond to the thickness of the coating at the end of the kinetics and the rate constant. Note that we distinguish, at purpose, k_2 in equation (4) from k_1 in equation (3). This is because two different properties (at first glance) of the coatings are measured by UV-vis spectroscopy and by ellipsometry. From the experiments displayed in Figure 2, we find: d_{max}= (39.1 ± 1.30) nm and k_2= 0.264 ± 0.03 h^{-1}. The maximal thickness value obtained herein is very close to that obtained by Messersmith et al on silicon [9].

Note that k_1 is equal to k_2 within the experimental error. This is of course expected since, even if both experimental techniques are different, they allow to follow the same phenomenon. Anyway, the students should be aware that one optical parameter of the coating, the refractive index is a complex number,

$$n(\lambda) = no(\lambda) - j.k(\lambda) \qquad (11)$$

Where the imaginary part of the refractive index, k (λ), is directly proportional to the molar absorbance A of the film having a thickness d:

$$k = \frac{A\lambda}{4\pi \mathrm{h}\ d.} \qquad (12)$$

This means that the optical properties of the films as probed by ellipsometry and by means of absorption spectroscopy are not independent from each other. The students should be able to understand the physical basis of the derivation of equation (6).

Derivation of equation (12), determination of the refractive index of "polydopamine" in solution, calculation of the impedance of the circuit represented in Figure 3A, additional figures.

The measured absorbance A is transformed into the imaginary part of the refractive index, k , by the following calculations.
A is given by:

$$A = \log\left(\frac{I_0}{I}\right) \qquad (13)$$

with the incident intensity I_0 and the detected intensity I. The Lambert-Beer law links these variables to the extinction coefficient ε and the concentration C of the absorbing species as well as the optical path length d:

$$I = I_0 \exp(-\varepsilon C d) \Rightarrow \varepsilon C = \frac{\log(I_0/I)}{d\log(e)} = \frac{A}{d\log(e)} \quad (14)$$

An electromagnetic wave of angular frequency ω and absolute value K of its wavevector with

$$K = \omega (n + ik) \quad (15)$$

and propagating a distance d in the time t can be described by its electric field vector E:

$$\vec{E} = \vec{E}_0 \exp[i(Kd - \omega t)] = \vec{E}_0 \exp\left[i\omega\left(\frac{nd}{c} - t\right)\right]\exp\left(-\frac{\omega k}{c}d\right) \quad (16)$$

c denotes the vacuum speed of light. Since the intensity is proportional to the square of the absolute value of the electric field vector it follows that:

$$I = I_0 \exp\left(-\frac{2\omega k}{c}d\right) = I_0 \exp\left(-\frac{4\pi k}{\lambda}d\right). \quad (17)$$

λ is the wavelength of the absorbed light. Comparison with the rt-Beer law leads to

$$\varepsilon C = \frac{4\pi k}{\lambda} \Rightarrow k = \frac{\varepsilon C \lambda}{4\pi} = \frac{A\lambda}{4\pi \log(e) d.} \quad (18)$$

The coatings deposited on the quartz slides (and/or) on the silicon slides at the steady state of the deposition kinetics may be used (if the apparatus is available) for contact angle goniometry. This will allow for the determination of the surface energy of the polydopamine coatings. This is fundamental because it is directly related to the adhesion energy of the considered material [1,17].

Measuring the contact angle of 3 solvents (water, iodomethane and n-hexane) and solving the Lifschitz equations (these equations are solved with the software associated with most commercial contact angle goniometers), one gets a polar, γp , and an apolar part, γa, of the film's surface energy. We found $\gamma p \approx \gamma a \approx$ 20-25 mJ. m-2. The values obtained for the "polydopamine" coatings should be compared with tabulated values of standard materials [18]. "Polydopamine" appears as a pretty hydrophilic material which is not surprising owing to its composition rich in catechol and quinone groups [9]. The hydrophilic character of the coating can be seen qualitatively, even without a goniometer, because water droplets spread much more on "polydopamine" than iodomethane and n-hexane droplets.

At this moment, the students have a good knowledge about the deposition kinetics of "polydopamine" coatings and about their surface energy. But what is the morphology of the coatings, are they "true films"? By "true films" one means homogeneous coatings without uncoated regions on the surface. The experimental techniques used up to now to characterize the coatings, namely UV-vis spectroscopy, ellipsometry and contact angle goniometry are not suited to answer such a question because they probe the coatings at a scale corresponding to the size of the light probe (a few 100 µm) or the size of the solvent droplets deposited on the coatings (typically ~ 1 mm for a droplet of a few µL in volume). Atomic force microscopy (AFM) is ideally suited for that aim but is out of the scope of this experimental course. The students can find surface topographies as well as thickness determinations of the melanin coatings in the literature [9]. It comes out that the coatings are homogeneous and continuous after about 2 h of reaction, hence "true films" at the scale probed by AFM, namely the scale of the radius of curvature of the tip (usually ~ 10 nm). One can however not exclude the presence of "nanochannels" crossing the whole thickness of the coating and not observable with the used cantilever, just because the tip is not able to sense such small asperities. The presence of those may have important consequences on the film properties, for instance the transport of ions, etc.

Electrochemistry is ideally suited to address such questions: it is able to probe the transport of small solutes from the solution to the surface of an electrode where charge-discharge phenomena occur. In addition when using regular working electrodes, the area of the coatings is of a few mm² (or more). Hence electrochemistry allows to probe the homogeneity and/or the permeability for a given probe of a coating on macroscopic areas (which is not yet routine in AFM experiments).

"Polydopamine" coatings can hence be prepared on ITO or amorphous carbon electrodes according to the previously described method and their impedance spectra (for instance in the presence of 100 mM NaCl and 1 mM potassium hexacyanoferrate as a redox probe) can be measured after different deposition times (the deposition kinetics is already known, see Figures 1 and 2). A good description of impedance spectroscopy can be found in reference but a more detailed description can be found in the book of Bard and Faulkner or in that written by Gileadi [19-21].

These impedance spectra may be fitted with an equation accounting for the impedance of an electronic circuit equivalent to the coating in contact with an electrolyte solution. The students can easily understand that the film has to be described at least with a capacity (accounting for the charge accumulation at the interfaces) and with a resistance for the electron transfer. In addition, the solution in contact with the coating has a given conductivity and hence a finite resistance, which connects the films on the working electrode with the counter-electrode (in order to close the electric circuit). The minimal circuit describing these phenomena is given in Figure 3A. The formula allowing to calculate the impedance of such an association of the solution resistance in series with a parallel association of the film capacity (C1 in Figure 3A) and its resistance to electron transport (R1 in Figure 3A) can be found in the SI and relies simply on the association laws of impedances in series and in parallel.

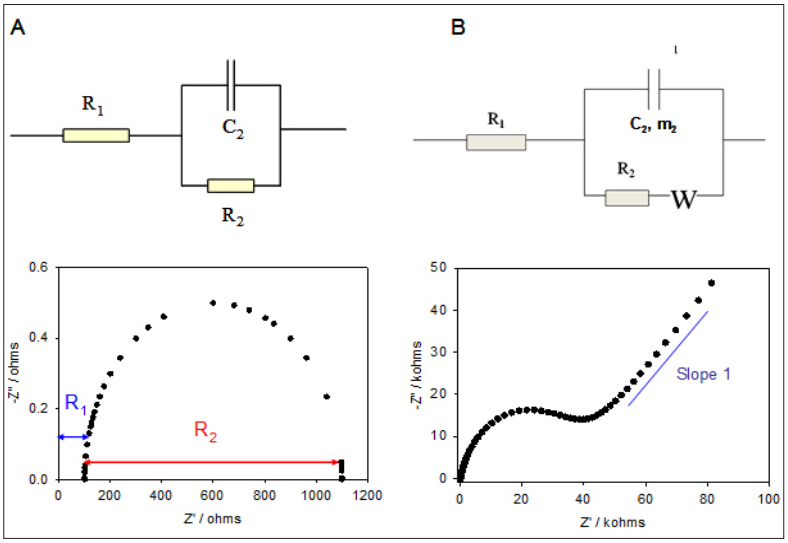

Figure 3: Typical equivalent circuits (top row) used to model the impedance spectra of thin coatings in presence of an electrolyte solution and typical model impedance spectra (botton row) resulting from such models. R_1 and R_2 represent the electrical resistance of the solution and the coating respectively whereas C_1 is the capacitance of the coating. In part A, the coating is not permeable to electroactive species, meaning that the redox process occur only at the coating solution interface whereas in part B, the diffusion of the redox species form the solution to the electrode surface trough the coating is taken into account. This needs to add an impedance element W (see equation (7)) in series with R_2. The equation allowing to plot the imaginary part of the impedance –Z" versus its real part Z' is established in the Supporting information for the equivalent circuit of part A. In part B, the impedance corresponding to the capacitance can be modified with a power law of the frequency, with an additional fitting parameter m_2, yielding to a so called "constant phase element". For a pure capacitor one gets m_2=0.5.

In part A the simulation parameters were: R1=100 Ω, R2= 1000 Ω and C_2= 50 x 10^{-6} F.

In the case of "polydopamine" coatings, this simple circuit does not allow to fit the impedance spectra correctly, particularly in the domain of the high Z' and Z" values (which correspond to measurements performed in the low frequency domain). Figure 4 represents, in the form of a so called Nyquist plot, the impedance spectra of "polydopamine" coatings obtained after 5 min of contact with a freshly prepared dopamine solution (at 2 mg.mL^{-1} in the presence of 50 mM Tris buffer at pH = 8.5). Such a coating should have an average thickness of about 0.9 nm according to equation (10) and using the fitting parameters obtained by fitting this equation to the data in Figure 2. The students will see that the EIS impedance spectra of "polydopamine" coatings are significantly different from that of the pristine amorphous carbon electrode even after short deposition times. A reasonable fit of the impedance spectrum can only be obtained with the aid of an equivalent Randless circuit (Figure 3B).

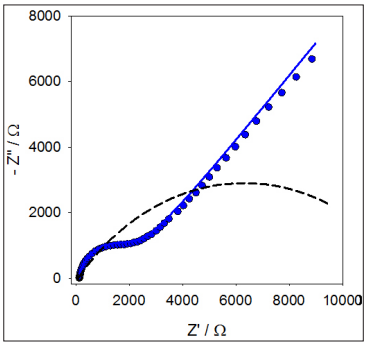

Figure 4: Nyquist plots obtained from impedance spectra of "polydopamine" coatings deposited on amorphous carbon electrodes during 5 min of agitation of dopamine solutions (2 mg.mL^{-1} in the presence of 50 mM Tris buffer). The spectra were recorded at a constant potential of 0.25 V vs Ag/AgCl in the presence of 100 mM NaCl + 1 mM $K_4Fe(CN)_6$. The DC signal was superimposed with an alternative potential of 5 mV. The frequency of this AC signal was varied from 10^5 to 10^{-2} Hz, with 12 measurements per frequency decade.

In the Randless circuit, the impedance related to the transport of the redox probe to the electrode surface accross the "polydopamine" film is explicitely taken into account, in the form of a "Warburg impedance", Z_w. This contribution should be important at low frequencies, when the redox probes have enough time to diffuse to or away from the interface. Z_w is described according to equation (7) [19,20]:

$$Z_W = \frac{\sigma}{\omega^{1/2}} - j.\frac{\sigma}{\omega^{1/2}} \qquad (19)$$

Where σ depends on the concentration and diffusion coefficient of the redox probe in its oxidized and reduced form (see equation (16.23) from reference 20). It appears from equation (19) that the contribution of this "Warburg impedance" to the whole impedance of the circuit equivalent to the investigated electrochemical system is a straight line of slope one in the complex impedance plane (-Z" versus Z' representation, the so called Nyquist plot). Such a straight line is immediately apparent in experimental curves as those represented in Figure 4 for polydopamine films having reached a critical thickness.

Of course it has to be assumed that the film thickness is the same (in given experimental conditions) on ITO, on amorphous carbon, on glass or on silicon. This is usually a highly hazardous assumption,

but it seems to hold true for "polydopamine" films [9].

It will appear that the modulus of the impedance increases rapidly with the thickness of the coatings (Figure 3). After one hour of reaction between the amorphous carbon electrode and the same aerated dopamine solution the coating should reach a thickness of about 9 nm, according to equation (4). It appears clearly that the amplitude of the electrochemical impedance (Figure 3, part B) is dramatically increased when the average thickness of the coating is increased from about 0.9 nm (5 min of reaction) to about 9 nm (1 h of reaction).

The impedance spectra strongly suggests that the "polydopamine" coatings are highly impermeable to $Fe(CN)_6^{4-}$ anions as soon as the coating reaches a thickness of a few nanometers, far below the maximal thickness that can be obtained (39-40 nm, Figure 2) in these experimental conditions. Indeed CV experiments show that the thicker the "polydopamine" coatings are the more impermeable they become not only for the negatively charged $Fe(CN)_6^{4-}$ ions (Figure 4) but also for the positively charged $Ru(NH_3)^{2+}$ and for the neutral ferrocene methanol molecule.

Typically, since the time of the experimental course is limited, the instructor should ask some group of students to perform experiments with different redox probes put in contact with the obtained "polydopamine" films. Concerning the cationic ruthenium (II) hexamine and the neutral ferrocene methanol probes, similar experimental CV curves as those shown in Figure 4 can be obtained (data not shown). The results will be qualitatively the same for all the three redox probes ($Fe(CN)_6^{4-}$, $Ru(NH_3)^{2+}$ and ferrocene methanol): the films will become impermeable (Figure 5) to all redox probes as soon as a critical thickness will be reached.

The value of this critical thickness depends however on the nature of the redox probe. The "polydopamine" films will become impermeable to the cationic probe only for a critical thickness higher than about 35 nm, whereas the impermeability threshold for ferrocene methanol will be reached for a thickness of about 20 nm. In the case of hexacyanoferrate anions, the films are totally impermeable as soon as they are thicker than about 5 nm (Figure 5).

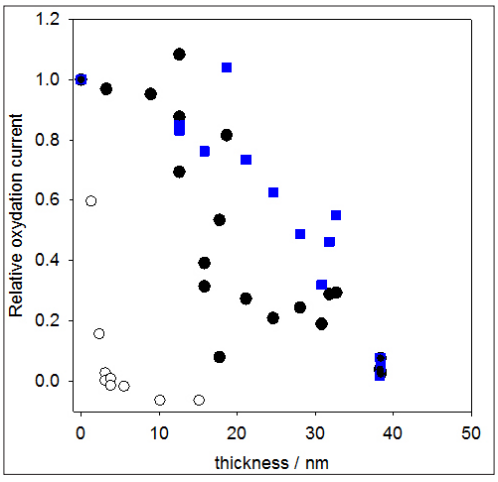

Figure 5: Variation of the relative oxidation current of potassium hexacyanoferrate (○ 1 mM in the presence of 100 mM NaCl), of ruthenium (II) hexamine (●, 1 mM in the presence of 100 mM NaCl), and of ferrocene methanol (■ , 1 mM in the presence of 100 mM NaCl) in the presence of "polydopamine" coatings deposited for various times (and hence various thickness, cf equation (10) on amorphous carbon electrodes. For each redox probe, the relative oxidation current is defined as the ratio of the oxidation peak current in the presence of the "polydopamine" coating by the oxidation peak current measured on the pristine carbon electrode. The oxidation currents were corrected from the capacitive currents (measured in the same electrolyte solution but in the absence of the redox probe). Each point corresponds to an individual electrode coated with "polydopamine".

Note that the same films as those used for the investigations with $K_4Fe(CN)_6$ solutions can be used for the experiments performed with ruthenium (II) hexamine and ferrocene methanol because the probes are not retained in the coatings after intensive rinse with the 100 mM NaCl electrolyte. This fact can be easily checked by means of CV: after rinse with 100 mM NaCl during a few minutes, the oxidation and reduction currents cannot be distinguished from the capacitive currents.

For a given film thickness, the permeability of the "polydopamine" coatings, quantified here as the relative oxidation current for a given redox probe, is hence in the following ranking: $Fe(CN)_6^{4-} < Ru(NH_3)_6^{2+} < C_{11}H_{12}FeO$.

A discussion can then be started among the students (and with the supervisor): what is the reason for the difference in the film thickness allowing to totally suppress the access of the redox probes to the electrode surface? This could obviously be related to the relative size of the redox probe with respect to the average size of the pores and channels present in the "polydopamine" film (data that are not yet available) or to a Donnan exclusion phenomenon. It appears that the redox probes investigated herein have similar hydrodynamic diameters (about 1 nm). Hence the most reasonable assumption is that the difference in the critical film thickness required to "extinguish" the oxidation-reduction currents of the different redox probes is due a selective exclusion of negatively charged probes by the "polydopamine" film. This coating is negatively charged in this pH conditions (about 6 for a 100 mM NaCl solution containing 1 mM of the investigated redox probe) [21].

Additional points of interest that can be discussed at the end of the experimental course

In Figures 1 and 2, the students fitted model equations (equations (3) and (4)) to their experimental data. This may always be subjective. They could be asked to plot the residuals, ie the difference of the calculated and the experimental value, as a function of time and to calculate χ^2 values. Then to see if the model equations are adapted to describe the data, the students may be asked to fit the sum of two exponentials to the experimental data. This requires 4 fitting parameters. In this case equation (3) will be replaced by:

$$A(t)= A_{1max}.\ [1-\exp(-k_1.t)] + A_{2max}.[1-\exp(-k_2.t)] \qquad (20)$$

This may lead to an over modelling of the data. The question is to know if the quality of the fit is significantly improved with respect to equation 3. To answer such a question the students have interest to look at specialized textbooks in statistics. In this particular case, they can demonstrate that the use of equation (20) does not improve the quality of the fit. Hence equation (20) would correspond to an over modelling of the data.

Feedback of the students to this experimental course

The experiments described herein have been performed by a group of 6 master students (divided in three groups of two) at the Université de Strasbourg. They did not encounter major difficulties in reproducing, within ± 10 %, experimental data as those displayed in Figures 1, 2 and 4. The students were however faced to real challenges in the interpretation of the data, particularly in understanding the relationships, through equation (6), between the absorption spectra and the film thickness calculated by ellipsometry. Most of the students were also faced with EIS spectroscopy for the first time and learned how the shape of the Nyquist plot (Figure 3) allows to help in the selection of the model to be used to fit the experimental data.

They all understood the need to approach surface science problems form a multidisciplinary perspective using a collection of complementary characterization tools.

Conclusion

"Polydopamine" films are easily deposited on almost all kinds of materials and their deposition kinetics can be easily followed by means of UV-visible spectroscopy and electrochemical methods like electrochemical impedance spectroscopy. These two methods can be complemented with ellipsometry and contact angle goniometry to get the film thickness and its surface energy components, respectively. Students at the master level will have great benefit to use complementary surface characterization methods to get information not only on the kinetics of the film growth (cf equations (3) and (4)) but also on their optical and electrochemical properties. The students should be asked about the reason why such "polydopamine" films are able to deposit on almost all kinds of materials with similar growth regimes. A look in the literature will provide some information by taking into account the analogy between the chemistry of "polydopamine" and that of mefp proteins. But this is only an assumption at the moment and this should induce the students to feel how close they are to real fundamental research problems. Indeed, polyaniline also deposits, like "polydopamine", on the surface of the beakers from aniline and oxidant containing solutions [22,23].

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