AFM and SEM Characterization of Chemically Modified Electrodes Based on 5-[(azulen-1-yl) methylene]-2-thioxothiazolidin-4-one

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*Preparation and microscopy characterization of polymer modified glassy carbon electrodes based on (5-[(azulen-1-yl) methylene]-2-thioxothiazolidin-4-one (**L**) were reported. Atomic Force Microscopy was used to investigate the morphological and mechanical properties of the deposited polyL films onto glassy carbon. The topography images of the analyzed samples exhibited the presence of some columnar shape features onto the layer surfaces. The surface roughness of the layers deposited at constant charge calculated from topography images, increased with the more positive applied potential for controlled potential electrolysis. At different charges, the roughness parameter showed the same behavior for the layers obtained applying a constant potential without having a noticeable influence on the adhesion properties on the substrate. Analysis using scanning electron microscopy shows a relatively uniform surface arrangement of the polymer and the presence of some clusters which are disturbing the planarity. PolyL chemically modified electrodes have been used for heavy metal ions detection with best results for lead.*

Keywords: 5-[(azulen-1-yl) methylene]-2-thioxothiazolidin-4-one, modified electrodes, Atomic Force Microscopy, Scanning Electron Microscopy, lead(II) detection.

Azulenes have a five-member cyclic moiety (electron-rich) connected with a seven-member cyclic moiety (electron-poor). According to Ungureanu et al. [1, 2], azulene derivatives present an irreversible electrochemical character in the anodic domain, a quasi-reversible character in the cathodic domain, and they can lead to polymeric films either by oxidation or reduction; these films can be used to create electrochemical sensors.

One of the most recent methods for analytical detection of heavy metals from water samples is by using electrochemical sensors based on azulene derivatives films [3]; this method gives comparable results to other recent investigated methods based on iron oxide/graphene composite [4], or bismuth nanoparticle-porous carbon paste [5].

This paper concerns the preparation and AFM and SEM characterization of modified electrodes based on (5-[(azulen-1-yl) methylene]-2-thioxothiazolidin-4-one (**L**). The electrochemical behavior of the ligand has been studied in detail by Diacu et al. [6]. Modified electrodes based on polyL films have been prepared according to recent literature in view of heavy metals recognition, using the complexing properties of polyL films.

Previous electrochemical investigations using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and rotating disk electrode voltammetry (RDE) for **L** in 0.1M TBAP in CH_3CN [6] have permitted the establishment of the main processes which characterize the electrochemical behavior of **L**. The anodic processes are examined and the following conclusions concerning the film formation by electrooxidation have been drawn:
- two oxidation processes occur in DPV at close potentials (2 anodic peaks *a1* and *a2*) are seen in DPV, as shown in Fig. 1; they are found to be both irreversible oxidations leading to the coverage of the electrode surface with insulating films [6]; Successive CV scans led to the decrease of the currents, as shown in figure 2;

- the shape of RDE anodic current in the potential range corresponding to *a2* (which does not have the expected form of a wave, but appears as a peak in figure 1) indicates the electrode blocking; RDE current drops suddenly to values close to zero at potentials more positive than *a2*, and the electrode is passivated over a large range of potential. This drop is characteristic for the coverage electrode with an insulating film.

Due to the complexing properties of **L** versus heavy metals [6], polyL films present also the ability to bind metal ions. They have been tested *vs* lead (II) cations. Examination of polyL films by SEM and AFM had the aim to explain some properties (morphology, roughness, etc) that influence their complexing properties.

Experimental part
The azulene derivative was synthesized according to the previous described methods [7]. Acetonitrile (CH_3CN) and tetra-*n*-butylammonium perchlorate (TBAP) from Fluka were used as received for the solvent and supporting electrolyte.

The electrochemical characterization has been carried out using a PGSTAT 12 AUTOLAB potentiostat connected to a three-electrode cell. The working electrode was a glassy carbon disk (3 mm diameter). The active surface was polished before each determination with diamond paste (1µm) and cleaned with acetonitrile. Ag/10 mM $AgNO_3$ in 0.1M TBAP, CH_3CN was used as reference electrode and a platinum wire was used as auxiliary electrode.

The experiments were performed by CV, DPV and RDE. CV curves were usually recorded at 0.1 V/s, or at different scan rates till 1.0 V/s. DPV curves were recorded at 0.01 V/s with a pulse height of 0.025 V and a step time of 0.2 s. RDE curves were recorded at 0.01 V/s. The electrode potential was referred to the potential of the ferrocene/

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ferrocenium redox couple (Fc/Fc+) which in our experimental conditions was +0.07 V. The determinations were performed at 25°C under argon atmosphere.

The electrochemical experiments for heavy metals ions detection in aqueous solutions were carried out using another three electrode cell (transfer cell). The working electrode was a modified electrode with poly**L**, deposited on glassy carbon disk (3 mm diameter). The reference electrode was Ag/AgCl, 3M KCl, a platinum wire was used as auxiliary electrode and 0.1M buffer acetate ($pH = 5.5$) solution was used as supporting electrolyte. The recognition experiments were performed at 25°C and under argon atmosphere. The acetate buffer solution was prepared from 0.2M acetic acid and 0.2M sodium acetate solutions. The salts containing metal cations were purchased from Sigma Aldrich and Fluka: mercury(II) acetate - Sigma Aldrich, cadmium nitrate tetrahydrate - Sigma Aldrich, copper(II) acetate monohydrate - Fluka, lead(II) nitrate - Sigma Aldrich. Heavy metals solutions with concentrations of 10^{-4} and 10^{-6} mol/ L, were prepared by successive dilution from 10^{-2} M stock solutions.

Scanning, Microscopy (SEM) measurements using Hitachi SU 8230 equipment were carried out in a low acceleration voltage (1 kV) in order to perform SEM analysis without damaging the sample.

Atomic Force Microscopy (AFM) technique has been successfully used to study the surface properties of the polymer modified glassy carbon.Topography, roughness and adhesion properties of the prepared polymer films were investigated via a multimode atomic force microscope (NTEGRA System, NT-MDT). The AFM measurements were performed in contact mode using a commercially available cone-shaped tip from monocrystalline silicon (having a radius of approximately 10 nm) mounted on a cantilever with a stiffness of about 0.26 N/m. All images (512 x 512 lines) were acquired in ambient conditions with a 0.3 Hz scan rate. The Root Mean Square (RMS) roughness parameter was calculated from the acquired topographic images using processing software.

The adhesion properties were determined from the 'pull off' region of the acquired force-distance curves. All the force-distance curves were measured applying a constant force between the tip and the sample. The adhesion force (F_{ad}) was calculated as $F = k \cdot \Delta z$, where k is the cantilever spring constant and Δz is the displacement.

Results and discussions
Selection of the preparation conditions for L films
Poly**L** films were obtained by successive scanning or by controlled potential electrolysis (CPE) in millimolar solutions of **L** in the domain of the anodic processes identified by CV, as it has been shown previously for other azulenes derivative [3]. Evidence for the films formation on the electrode surface was obtained by transferring the modified electrodes in millimolar ferrocene solutions in 0.1M TBAP, CH₃CN. The signal for ferrocene on the modified electrodes is very different from that recorded in the same solution on bare electrode. Both anodic and cathodic peaks are diminished in intensity and shifted to higher potentials, indicating a thick insulating layer film was formed on the electrode [6]. The film formation and the coverage of the electrode with an insulating layer shown in DPV curves were confirmed also by electrochemical impedance spectroscopy [8].

In order to have control over the number of complexing sites in the film the procedure to modify the glassy carbon electrode was established as follows:
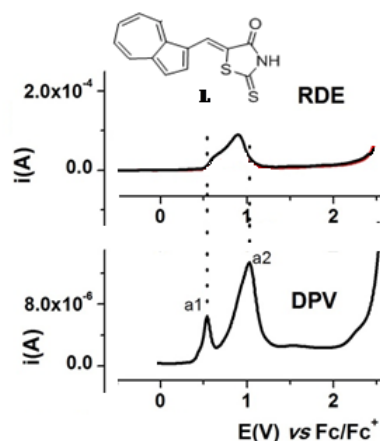

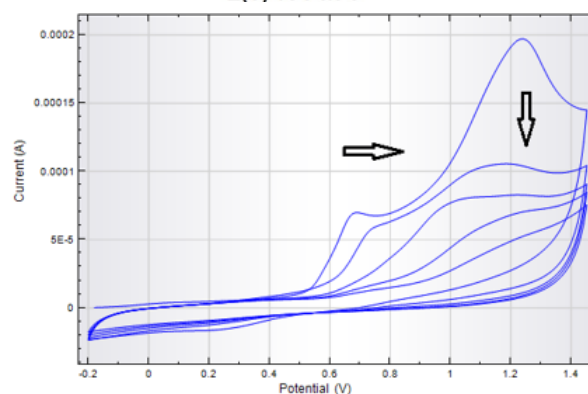Fig.1. DPV and RDE (at 1000 rpm) curves on glassy carbon (3mm in diameter) for [**L**] = 0.5 mM in 0.1M TBAP, CH₃CN

Fig. 2. Successive CV curves during the formation of poly**L** films by scanning

Step 1. Preparation of poly**L** films in millimolar solutions of **L** in 0.1M TBAP, CH₃CN, followed by cleaning in acetonitrile. This step has been performed either by potentiostatic method (CPE at a potential more positive than that of the *a1* peak in DPV curve given in fig. 1) or by potentiodynamic method (by scanning the potential in the anodic range, between -0.2 V and a positive limit, bigger than *a1* potential, as seen in figure 2). In the last procedure, the film formation could be followed during successive cycles, as the peak potential is slowly moving toward more positive values – as indicated by the horizontal arrow in figure 2, and the current is decreasing stepwise - as indicated by the vertical arrow in figure 2, but it is more difficult to control the thickness of the film.

Step 2. Equilibration of the modified electrode obtained in *Step 1* in aqueous medium in 0.1M acetate buffer at *p*H 5.5, by cycling it during 15 CV cycles in potential scan domain between -0.9 V and +0.6 V *vs* Ag/AgCl.

Step 3. Overoxidation of the film obtained in *Step 2* in 0.1M acetate buffer at *p*H 5.5, by cycling the electrode in five CV cycles between -0.2 V to +1.2 V.

After *Step 3* the modified electrode is ready for a recognition experiment. It concerns in the following steps:

Step 4. Accumulation of heavy metal ions, when the modified electrode is introduced in the assay solutions containing heavy metals ions, and it is maintained for 10 min under magnetic stirring.

Step 5. Ions reduction, when a potential of -1.2V was applied for 120 s in view of reduction of the metal ions accumulated in the film.

Step 6. Anodic stripping, when the electrode was polarized toward anodic potentials with low scan rate (0.01 V/s). The stripping currents for the metals dissolution are recorded during this scan. The peaks for Cd, Pb, Cu, and Hg dissolution appears successively. The stripping currents have been evaluated for each condition and examined in relationship with each varied parameter.

Sample	Potential for CPE (V)	Charge for CPE (mC)	Remarks
P1	0.7 V	3.2 mC	CME step 1
P2	1.0 V	3.2 mC	CME step 1
P3	1.45 V	3.2 mC	CME step 1
P4	1.45 V	2.4 mC	CME step 1
P5	1.45 V	1.6 mC	CME step 1
P6	1.45 V	0.8 mC	CME step 1
P7	1.45 V	1.6 mC	CME step 1 + step 2 + step 3 + step 5 + step 6 (in 10^{-4}M aqueous solution of Pb(II))
P8	1.45 V	1.6 mC	CME step 1+ step 2 + step 3
P9	5 cycles between -0.2V si +1.45V	3.2 mC	CME prepared by scanning the potential (5 cycles between -0.2V and +1.45V using an anodic charge of 3.2 mC

Table 1
MAIN CHARACTERISTICS OF THE MODIFIED ELECTRODES (CME) PREPARATION FOR THEIR USE IN LEAD (II) DETECTION

The surface morphology of the prepared samples has been examined in order to see the influence of each parameter on the evaluated property. The varied electrolysis parameters during the preparation of CME for Pb(II) recognition could be seen in table 1 (evidenced by different fonts) as follows: applied potential in CPE for the samples P1-P3 (bold letters) electropolymerization charge in CPE for the samples P3-P6 (italic bold letters), steps of preparation (samples P6 and P8), presence of lead in the film (samples P8 and P7), method of preparation (CPE or scanning, for samples P3 and P9, respectively).

The poly**L** modified electrodes obtained by CPE at different electropolymerization charges or electrode potentials have been checked in ferrocene solutions; the obtained curves indicate the coverage of the electrode with films, because the ferrocene signal is modified, comparing with the bare electrode. The coverage has been tested at three potential values: 0.7 V, 1 V, 1.45 V, 1.7 V [6], and it increased for the films prepared from 0.7 V till 1.45 V, and then it decreased. That is why the samples P1-P3, examined by SEM and AFM, were prepared at 0.7 V, 1 V, 1.45 V, respectively. Also, the influence of the charge between 0.2 mC and 0.8 mC was examined by SEM and AFM images.

Scanning Electron Microscopy results
The polymer topography images acquired by SEM at different magnifications are seen in figure 3a (8k), and figure 3. b. (25k).
SEM images (fig.3) show a relatively uniform arrangement of the polymer matrix surface. On the sample's surface the polymer makes some clusters that deviate from planarity of the sample.
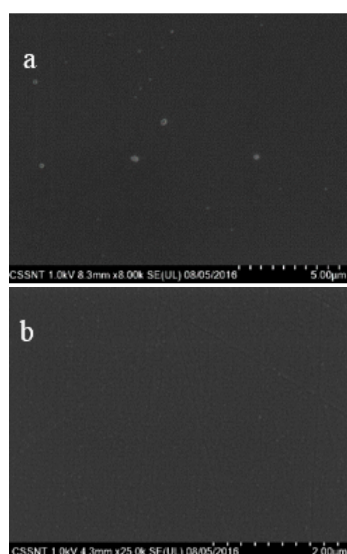

Fig. 3. Secondary electron SEM images with polymer surface at two magnifications (a) x8000; (b) x25000

Also the surface has some mechanical defects (scratches of the surface area) due to the sample preparation process. SEM results are in according with Atomic Force Microscopy results presented below.

Atomic Force Microscopy results
The poly**L** films of the P1, P2, and P3 samples were deposited on glassy carbon electrodes (GCEs) by CPE using constant electropolymerization charge and different potentials (according to the step 1, described above). Figure 4 shows the AFM results obtained for P1, P2, and P3 polymer modified GCEs. As it can be noticed in the displayed topography images, the electropolymerization potential has an influence on the studied surfaces, leading to the formation of some columnar shape features. As it

P1	P2	P3
RMS = 2.069 nm	RMS = 2.574 nm	RMS = 4.036 nm
$\overline{F_{ad}}$ = 25,6 nN	$\overline{F_{ad}}$ = 21.05 nN	$\overline{F_{ad}}$ = 24.03 nN

Fig. 4. Topography (10 X 10 μm), RMS roughness, and the mean adhesion force values obtained for P1, P2, and P3 polymer modified GCEs prepared at constant electropolymerization charge (3.2 mC) and different electrode potentials (P1 = 0.7V, P2 = 1.9 V, P3 = 1.45 V)
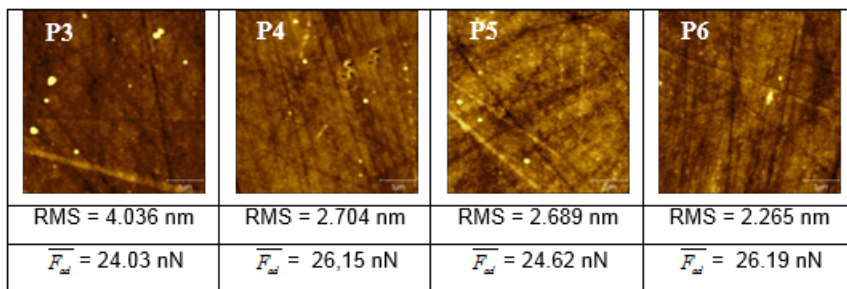
Fig. 5. Topography (10 X 10 μm), RMS roughness, and the mean adhesion force values obtained for P3, P4, P5, and P6 polymer modified GCEs prepared at constant electropolymerization potential (1.45 V) using different charges (P3 = 3.2 mC, P4 = 2.4 mC, P5 = 1.6 mC, P6 = 0.8 mC)

P3	P4	P5	P6
RMS = 4.036 nm	RMS = 2.704 nm	RMS = 2.689 nm	RMS = 2.265 nm
$\overline{F_{ad}}$ = 24.03 nN	$\overline{F_{ad}}$ = 26,15 nN	$\overline{F_{ad}}$ = 24.62 nN	$\overline{F_{ad}}$ = 26.19 nN

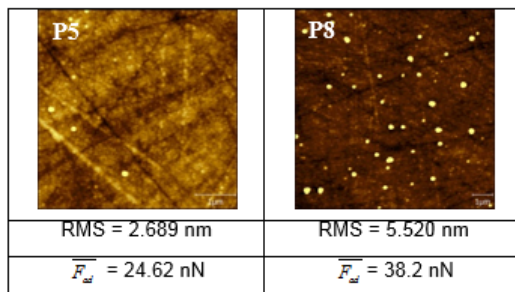| P5	P8
RMS = 2.689 nm	RMS = 5.520 nm
$\overline{F_{ad}}$ = 24.62 nN	$\overline{F_{ad}}$ = 38.2 nN

Fig. 6. Topography (10 X 10 μm), RMS roughness, and the mean adhesion force value results for P5, and P8 polymer modified GCEs obtained using different steps of preparation (table 1)

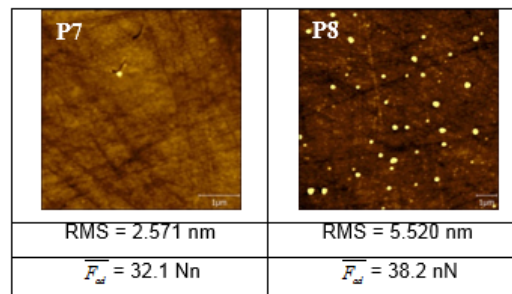| P7	P8
RMS = 2.571 nm	RMS = 5.520 nm
$\overline{F_{ad}}$ = 32.1 Nn	$\overline{F_{ad}}$ = 38.2 nN

Fig. 7. Topography (10 X 10 μm), RMS roughness, and the mean adhesion force value obtained for P7 and P8 polymer modified GCEs obtained using different steps of preparation (table 1)

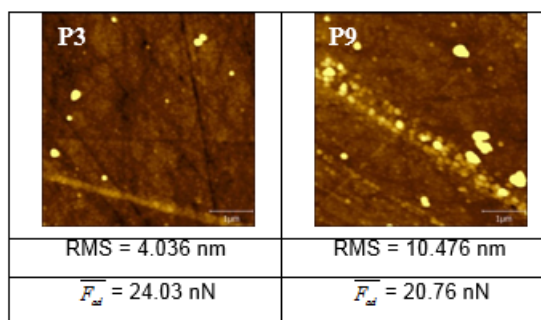| P3	P9
RMS = 4.036 nm	RMS = 10.476 nm
$\overline{F_{ad}}$ = 24.03 nN	$\overline{F_{ad}}$ = 20.76 nN

Fig. 8. Topography (10 X 10 μm), RMS roughness, and the mean adhesion force value obtained for P3, and P9 polymer modified GCEs prepared using different methods (table 1)

was expected, the presence of these columnar shape features over the polymer surfaces are increasing the surface roughness parameter, calculated from the acquired topography images, so the RMS roughness is increasing with the more positive applied potential for CPE.

The polyL films of the P3, P4, P5 and P6 GCEs were deposited by CPE using constant electropolymerization potential and different charges (according to the step 1, described above). Figure 5 reveals the obtained AFM results for P3, P4, P5, and P6 polymer modified GCEs. By keeping constant the potential for CPE and varying the charge, the deposited layers show to the same behaviour as the samples presented previously, in figure 4.

An overview of the obtained results presented in both figure 4 and figure 5 reveal the decisive role of the polymerization parameters (potential/charge) in the morphological and roughness properties of the surfaces. However, the variation of these electropolymerization parameters did not have a noticeable influence on the adhesive properties of these surfaces.

The polyL films of the samples shown in figure 6, P5 and P8 were obtained using the same electropolymerization parameters. In addition, P5 sample was subjected to some extra treatments: equilibration of the modified electrode (according to the step 2) and overoxidation of the deposited polymer film (according to the step 3). These treatments seem to have a significant impact on the morphological properties of the deposited layer, leading to a considerable decrease of the number of columnar shape features in P5 vs P8, as revealed by the acquired topographic images, and confirmed by the smaller roughness value of P5 than P8. The mean adhesion force value determined for the polymer surface for P5 GCE (2.62 nN) is noticeably decreased in comparison with P8 GCE (38.2 nN).

The topographic images in figure 7 show the significant influence of the accumulation step in Pb aqueous solution onto the investigated surface. The immersion of the modified electrode into 10^{-4} Pb aqueous solution lead to the decrease of the mean adhesion force and RMS roughness (for sample P7 in respect to P8). The P7 film immersed in Pb aqueous solution has a lower RMS adhesion force value (32 nN) than that of P8 film (38.2 nN), probably due to the Pb contribution.

Figure 8 illustrates the differences between the methods of preparation of complexing modified electrodes. P9 was prepared using the potentiodynamic method, which consists in 5 cycles of scanning between -0.2 V ÷ +1.45 V, while P3 was prepared by potentiostatic method (CPE at the electropolymerization potential of 1.45 V). The potentiodynamic method leads to obtain the roughest surface (RMS roughness = 10.476 nm) from all the prepared sample surfaces.

Recognition experiments using polyL complexing modified electrodes

Experiments using modified electrodes with polyL have been performed for recognition of heavy metal ions (Cd, Pb, Cu, Hg). One stripping curve obtained after accumulation and reduction steps is given in figure 9. The peaks for Cd, Pb, Cu, and Hg dissolution appear successively at -0.805, -0.524, -0.074, +0.206 V, respectively. The stripping current for Pb is by far the highest among the other peaks, showing a bigger sensitivity for this heavy metal ion. The stripping currents for Pb recorded for different concentrations of Pb in the accumulation solution are shown in figure 10. The dependence is linear till about 10^{-7} M Pb(II). The signal for Pb(II) is visible at 10^{-8} M, but the detection limit could be situated even under this concentration. The work is in progress to evaluate the detection limit taking into account all the other conditions and parameters that could diminish this limit.
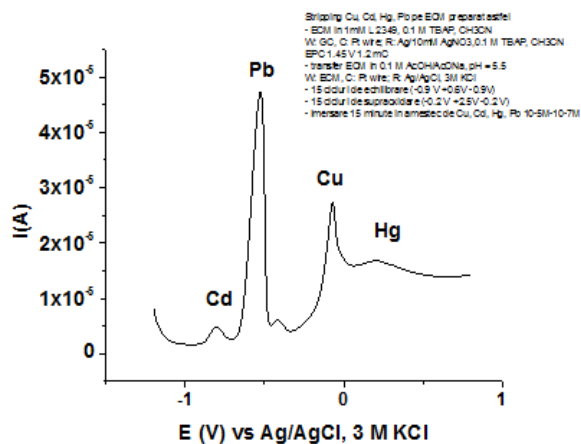
Fig. 9. Stripping curve for the modified electrode with poly**L** after immersion in a solution containing the mixture of all cations, each of them in concentration of 10^{-5} M

Fig. 10. Dependence of Pb stripping currents on Pb(II) concentration using poly**L** modified electrodes

The poly**L** modified electrodes have proved to be complexing electrodes, leading to very good results for the recognition of lead.

Conclusions

We successfully prepared polymer modified glassy carbon electrodes based on (5-[(azulen-1-yl) methylene]-2-thioxothiazolidin-4-one monomer (**L**) and examined by scanning electron microscopy and atomic force microscopy in order to investigate the morphological and mechanical properties of the deposited poly**L** films. Also heavy metal ions recognition experiments werw carried out.

SEM studies showed a relatively uniform arrangement of the polymer surface and the presence of some clusters which are disturbing sometimes the planarity.

Regarding the morphological and mechanical properties of the deposited polymer layers onto the glassy carbon electrode, the topography of AFM images exhibited the presence of columnar shape features. AFM results also revealed a dependence of surface roughness on the electropolymerization parameters (potential/charge). The extra treatments applied for some of the prepared samples, i.e. equilibration of the modified electrodes and overoxidation of the obtained films, are leading to a significant change in the adhesive properties of the layers. The immersion of the modified electrodes in Pb^{2+} containing aqueous soluti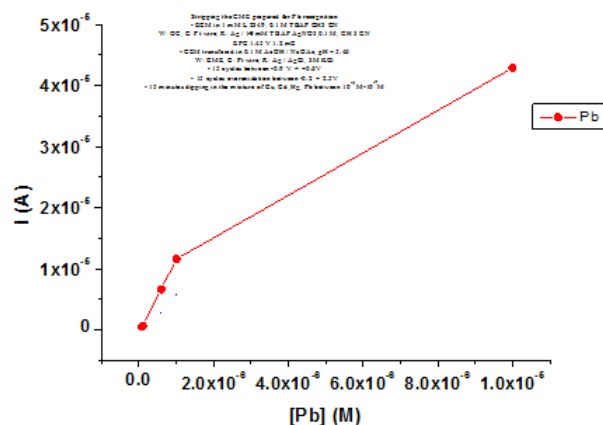on translates into a decrease of the mean adhesion force value, probably due to the Pb contribution of Pb species present in the films.

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