Modification Of The Surface Properties Of (Gelatin-Tannic Acid)N multilayered Films By Hydrogenophosphate Anions.

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Abstract; *Films deposited by the alternate adsorption of type B gelatin and from tannic acid are shown to undergo partial erosion and some modification of their surface properties (roughness and hydrophilicity) after being put in contact with hydrogenophosphate/dihydrogenophosphate anions at pH = 7.5. Phosphate species are incorporated in the obtained films. It is shown that phosphate species interact with tannic acid in solution at pH = 7.5 in a non yet described manner. It is hence suggested that the partial film erosion is due to the stronger interactions between phosphates and tannic acid than between gelatin and tannic acid.*

I.   Introduction

Surface modification with coatings of well controllable thickness is of major interest for plenty of industrial applications as protection against corrosion, and the design of drug reservoirs in biomaterials science. The most popular method to create coatings of well controllable thickness from the nanometer to several micrometer range is the layer-by-layer (LBL) deposition method initially developed for charged species like polyelectrolytes [1] but extended to species displaying multitopic and complementary interaction sites like hydrogen bond donnors and acceptors [2-4]. Among such species one can distinguish polyphenols which are molecules extracted from plants and displaying a vast repertoire of biological properties, like antioxidant, antiviral and bactericidal ones [5]. Hence interest has been devoted to deposit LBL films containing polyphenols [6-10] whose antimicrobial properties have been established. Of interest is also to know the stability of those films in physiological conditions namely at pH 7.4-7.5 mainly in phosphate containing buffer. Of particular interest is to know if a polyphenol like tannic acid (TA) is released from the film in these conditions. In this article we show that a PEI-(gelatin-TA)$_n$ film, where PEI and n design poly(ethylene imine) as a precursor adhesion layer and the number of deposition cycles respectively, undergoes some partial dissolution in a 50 mM phosphate buffer at pH 7.5 after film deposition from a 50 mM sodium acetate buffer. This partial dissolution, evidenced by means of quartz crystal microbalance with dissipation monitoring (QCM-D) and ellipsometry produces also some important changes in the film roughness (evidenced by means of Atomic Force Microscopy) and wettability (seen by contact angle goniometry). The interactions between TA and phosphate species in these pH conditions (equilibrium between dihydrogeno and hydrogeno phosphates) are investigated in solution and it is shown that TA undergoes some specific chemical modifications in the presence of those species. These interactions may be at the origin of a partial disruption of the gelatin-TA interactions in their LBL films.

II.   Materials And Methods

All solutions were prepared from doubly distilled and de-ionized water (Milli Q-RO+ system) with a resistivity of 18.2 MΩ.cm.

Sodium acetate buffer (50 mM, pH = 5) and sodium phosphate buffer (50 mM, pH = 7.5) were prepared from commercial powders (anhydrous $NaCH_3COO$, Sigma-Aldrich ref. W302406 and NaH_2PO_4, H_2O, Sigma-Aldrich, ref; S-9638, respectively). The pH of the sodium acetate and sodium phosphate buffers was adjusted with concentrated hydrochloric acid (Sigma-Aldrich) with a calibrated Hanna Hi221 pH meter.

Fresh gelatin (Type B from bovine skin Sigma-Aldrich ref. G9382) and TA (Sigma-Aldrich ref. 403040) solutions were prepared before each experiment at a concentration of 1 mg.mL^{-1} in the sodium acetate buffer. PEI (Fluka, ref. P3133) was also dissolved at 1 mg.mL^{-1} in the same buffer and was used as an anchoring layer.

The PEI-(gelatin-TA)$_n$–gelatinfilms were deposited on quartz coated QCM-D crystals (QSX 303, QSense, Göteborg, Sweden)) and on (100) oriented silicon wafers (Siltronix, Archamps, France). Before the deposition experiments the adsorption substrates were cleaned with ethanol and during 30 min with an air plasma (PDC-32G-2, Harrick Plasma). After this cleaning process the substrates were extremely hydrophilic with a water contact angle lower than 10° as checked with an Attension-Theta goniometer (Biolin Scientific).

The film deposition was first investigated by quartz crystal microbalance with energy dissipation monitoring (QCM-D) [11] at 25°C in order to determine the optimal deposition time of each layer, either PEI, gelatin or TA. The SiO$_2$ coated quartz crystal was first mounted in the flow chamber of the QCM device and equilibrated with flowing sodium acetate buffer at a flow rate of 0.25 mL.min^{-1}. The deposition of the polymer based film was started when the reduced frequency drift of the quartz crystal was lower than 0.1 Hz.min^{-1}. The polymer solutions were injected at a flow rate of 0.25 mL.min^{-1} and the resonance frequency of the SiO$_2$ coated quartz crystal was followed at the third, fifth and seventh overtone of the quartz crystal (this one lying close to 5 MHz). Each polymer or TA injection was continued until the reduced frequency changes were lower than 0.1 Hz.min^{-1}. Each polymer injection was separated from the next injection of TA by buffer rinse during 5 min. After the deposition of a PEI-(gelatin-TA)$_5$-gelatin film, the buffer solution was switched to 50 mM sodium phosphate buffer at pH = 7.5 which injection lasted for 60 min to reach a steady state signal. After that, the buffer flow was switched again to 50 mM sodium acetate buffer at pH = 5. This was mandatory because pH and buffer composition changes can induce changes in the solution density and hence in the recorded QCM-D frequencies and dissipation.

A similar PEI-(gelatin-TA)$_5$-gelatin film was deposited on a freshly cleaned silicon slide, using the optimal adsorption time defined from the QCM-D experiment. The film was then immersed in a 50 mM sodium phosphate buffer solution (pH = 7.5) and was regularly rinsed with distilled water and blown dry under a gentle stream of filtered air. The films were rinsed with distilled water in order to avoid the deposition of salt originating from the buffer. Their thickness was measured at every step, after deposition from sodium acetate buffer and after different immersion times in sodium phosphate buffer by means of single wavelength (λ=632.8 nm) ellipsometry. The used ellipsometer was a PZ2000 device from Horiba-JobinYvonworking at a constant incidence angle of 70°. The measured ellipsometric angles were used to calculate the film thickness assuming a constant refractive index of 1.5 for the polymer based film. The given values correspond to the average ± one standard deviation over 5 measurements taken along the major axis of the rectangular silicon wafers.

The morphology of the films used for the ellipsometry experiments were obtained by means of contact mode Atomic Force Microscopy in the contact mode and in the dry state. The AFM microsope was a Nanoscope IV from Bruker instruments. The used cantilever was an MLCT-C with a nominal spring constant of 0.01 N.m^{-1}. The images were acquired at a scan rate of 1 Hz over surface areas of 20 µm x 20 µm, 10 µm x 10 µm and 5 µm x 5 µm. Some of the films were also needle scratched to get the film thickness by profilometry along line profiles get by AFM in the direction perpendicular to the scratched line.

The contact angles for a 5 µL water droplet was measured on a PEI-(gelatin-TA)$_5$-gelatin film after deposition on a silicon slide from a 50 mM sodium acetate buffer and after 60 min of immersion in a 50 mM sodium phosphate buffer. These experiments were performed at 25°C using anAttension-Theta contact angle goniometer (from Biolin Scientific).

The attenuated total internal reflection Fourier transformed infrared spectra (ATR-FTIR) of the films deposited on a ZnSe total reflection element were measured with a Spectrum Two spectrometer from Perkin Elmer accumulating 16 interferograms between 4000 and 700 cm^{-1} with a spectral resolution of 2 cm^{-1}. After deposition, the films were exposed to a 50 mM sodium phosphate buffer at pH = 7.5 during 60 min before switching back to 50 mM sodium acetate buffer at pH = 5.0. The final spectrum was compared with that obtained immediately after film deposition.

The UV visible spectra of the TA solution at pH = 7.5 with pH adjusted with NaOH or in the presence of 50 mM sodium phosphate buffer (at pH = 7.5) were measured with an mc² spectrophotometer (Safas, Monaco) between 200 and 750 nm using the same buffer as a standard. The spectra were taken 2 days after dissolution of TA in the corresponding solutions. Some digital images of those solutions were also taken after 48 h of aging at pH = 7.5.

III. Results And Discussion

The build-up of the PEI-(gelatin-TA)$_n$-gelatin film was followed *in-situ* and in real time by means of quartz crystal balance with dissipation monitoring (QCM-D). It appears that the deposition of each layer, either PEI (an anchoring layer), gelatin or TA is achieved in less than 5 min according to the criterion defined in the Materials and Methods section (Figure 1). This deposition duration of 5 min as well as 5 min of buffer rinse between the deposition of two layers was maintained constant in all forthcoming experiments. After the formation of a PEI-(gelatin-TA)$_5$-gelatin deposit from a 50 mM sodium acetate buffer at pH =5.0, the buffer was switched to 50 mM sodium phosphate buffer at pH = 7.50. A sudden frequency decrease and an increase in

energy dissipation was observed with the obtention of a minimum and a maximum in the resonance frequency (Figure 1A) and dissipation (Figure 1B) after a few minutes of contact with the sodium phosphate buffer. Later on during the contact with the flowing sodium phosphate buffer the frequency increased and the dissipation decreased again to reach constant values after about 60 min. A subsequent switch to 50 mM sodium acetate buffer at pH = 5.0 did not restore the resonance frequencies and the energy dissipation values obtained after the initial deposition step. This experiment shows clearly that the PEI-(gelatin-TA)$_5$-gelatin multilayer depositundergoes some irreversible changes when the buffer is switched from 50 mM sodium acetate (pH = 5.0) to 50 mM sodium phosphate (pH = 7.5). In particular the resonance frequency changes suggest that the film undergoes partial erosion in the presence of sodium phosphate. This effect may be due either to a pH change or to a specific effect of hydrogeno/dihydrogenophosphate anions. Indeed when the film deposition is done in the presence of 50 mM sodium acetate buffer and subsequently exposed to a 50 mMNaCl solution with pH adjusted to 7.5 during 60 min before switching back to 50 mM sodium acetate buffer at pH = 5.0, less than 20 % of reduced frequency changes are observed (data not shown) in comparison to more than 60% variation in the presence of sodium phosphate buffer. This shows clearly that the hydrogeno/dihydrogenophosphate anions plays a specific role in the erosion of the PEI-(gelatin-TA)$_5$-gelatin films. The film erosion induced by hydrogeno/dihydrogenophosphate anions was further confirmed by means of single wavelength ellipsometry (Figure 2). After 60 min of contact with the sodium phosphate buffer, the film thickness does not change any more but has decreased to about 40% of its original value. Note that the thickness of the dried (but nevertheless keeping some hydration water) film is of (115 ± 5) nm after its deposition. Let us compare this value with that estimated from the QCM-D experiment where the reduced frequency change at the 3rd overtone of the quartz crystal is equal to -920 Hz with respect to the pristine silica coated quartz crystal. It appears that the reduced frequency change is independent of the overtone number (±10%, Figure 1) justifying the use of the Sauerbrey equation to estimate the surface coverage Γ in adsorbed molecules [11]:

$$\Gamma = -17.7 \, \frac{\Delta f_v}{v} \quad (1)$$

Where C=17.7 ng.cm^{-2}.Hz^{-1} is the sensitivity constant of the used quartz crystal provided by the furnisher. The obtained surface coverage of the PEI-(gelatin-TA)$_5$-gelatin film is hence 1.63 x 10^4 ng.cm^{-2}. This surface coverage is related to the average film density, ρ, and its thickness in the wet state, d_w, according to:

$$\Gamma = \rho.d_w \quad\quad (2)$$

Assuming a film density of 1200 kg.m^{-3} (a reasonable value lying between that of pure water, 1000 kg.m^{-3}, and that of gelatin, ie about 1400 kg.m^{-3}), one obtaines a hydrated film thickness of (135 ± 5) nm. This value is in close agreement but slightly higher than the value obtained by ellipsometry, namely 115 nm. This is not surprising owing to the fact that QCM-D measures the whole mass of the film, the total amount of hydration included, whereas the thickness calculated from the ellipsometry data is obtained on a partially deshydrated film [12].
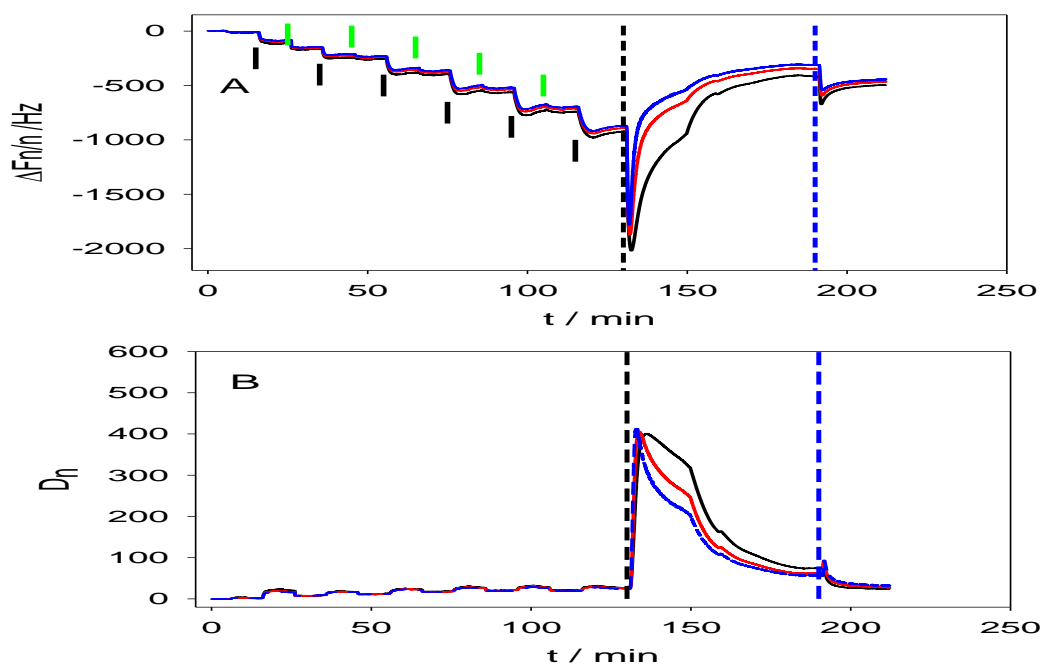
Figure 1: A: Variation of the reduced frequency changes of the silica coated quartz crystal at the third (——), the fifth (——) and the seventh (——) overtone during the deposition of a PEI-(gelatin-TA)$_5$-gelatin film in the

presence of 50 mM sodium acetate buffer (pH = 5.0) before switching to 50 mM sodium phosphate buffer at pH = 7.50 (long dashed black vertical line) and again to 50 mM sodium acetate buffer (long dashed blue vertical line). During the film deposition, the injections of gelatin are labelled with a black vertical line and the injections of TA with a green vertical line.

B: A: Variation of the energy dissipation of the silica coated quartz crystal at the third (——), the fifth (——) and the seventh (——) overtone during the deposition of a PEI-(gelatin-TA)$_5$-gelatin film in the presence of 50 mM sodium acetate buffer (pH = 5.0) before switching to 50 mM sodium phosphate buffer at pH = 7.50 (long dashed black vertical line) and again to 50 mM sodium acetate buffer (long dashed blue vertical line)
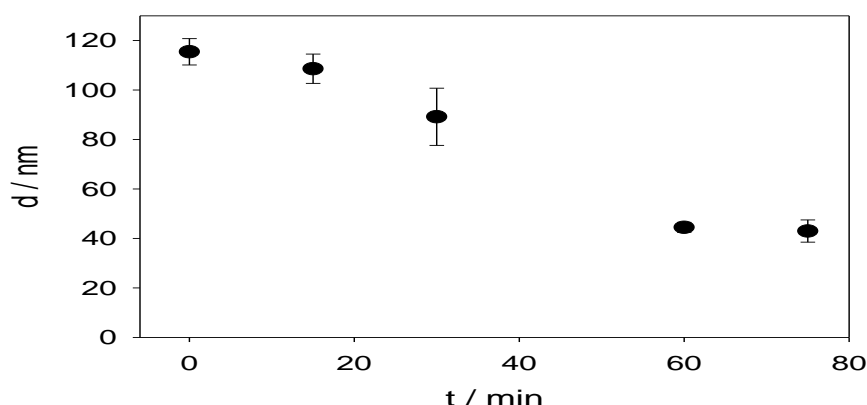

Figure 2: Variation of the thickness of a PEI-(gelatin-TA)$_5$-gelatin film build-from a 50 mM sodium acetate buffer (pH = 5.0) after exposure at time t= 0 to a 50 mM sodium phosphate buffer (pH = 7.5). The film thickness was measured by ellipsometry and the given values correspond to the average of 5 measurements ($\pm$ 1 standard deviation) along different positions along the major axis of the silicon substrate.

The surface topography of the PEI-(gelatin-TA)$_5$-gelatin film is also markedly modified after 60 min of contact with the 50 mM sodium phosphate buffer: most of the large aggregates present on the deposited film disappear after contact with the new buffer at pH =7.5 (Figure 3).

A   B

PEI-(gelatin-TA)$_5$-gelatin   PEI-(gelatin-TA)$_5$-gelatin + 50 mM phosphate buffer
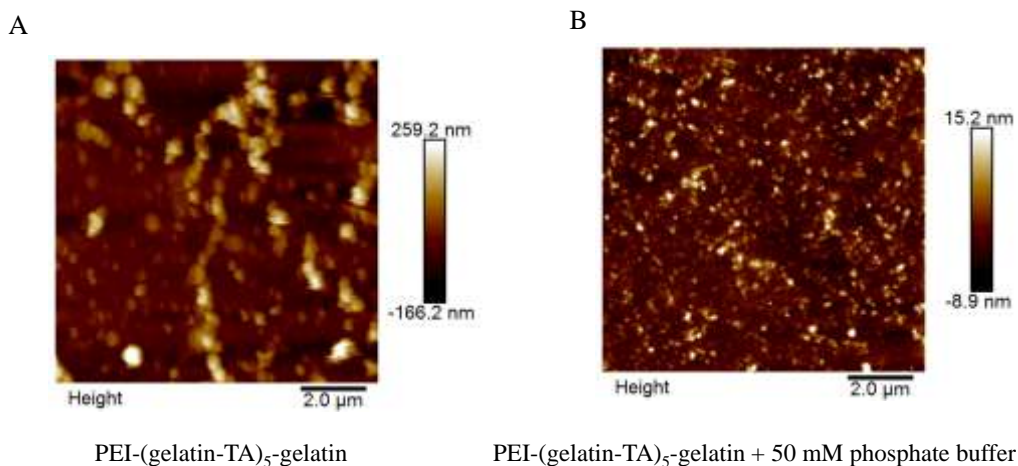
Figure 3: Surface topography over a 10 µm x 10 µm surface area, as determined by contact mode AFM, of a PEI-(gelatin-TA)$_5$-gelatin film before (A) and after (B) 60 min of contact with a 50 mM sodium phosphate buffer at pH = 7.5.

The decrease in film thickness after one hour of contact with the 50 mM sodium phosphate buffer is further confirmed by means of thickness measurement by AFM (Figure 4A). This technique also allows to evidence a considerable decrease in the film roughness (Figure 4B).
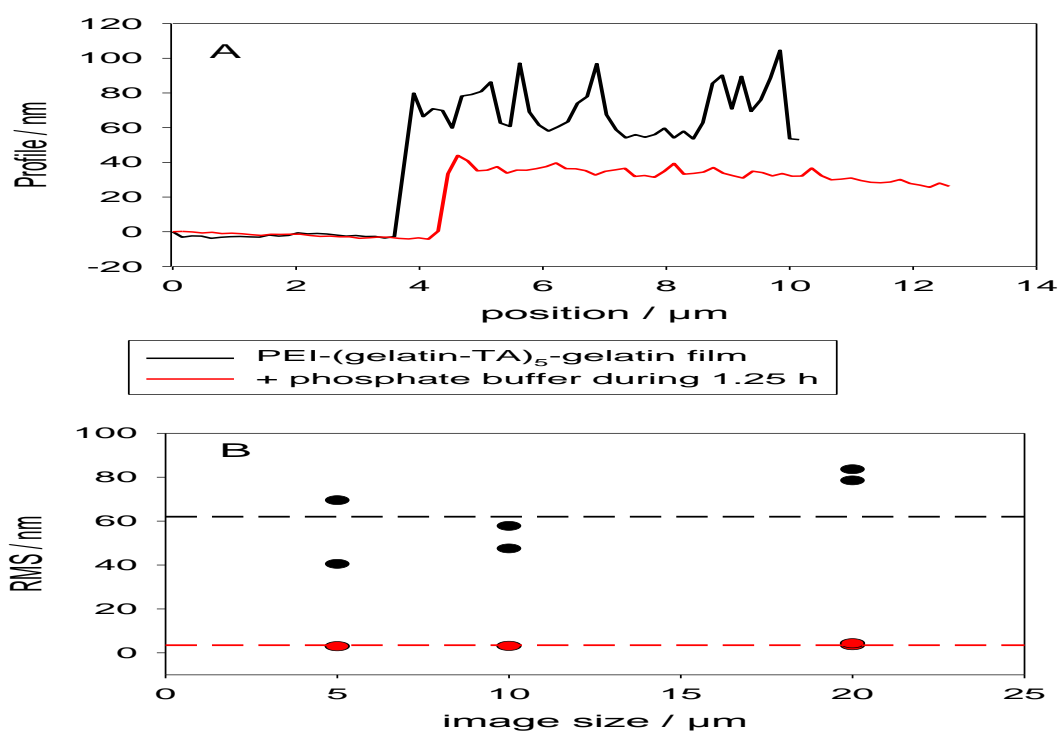
Figure 4: A: Line profiles over s needle scratched PEI-(gelatin-TA)$_5$-gelatin film before (——) and after exposure to a 50 mM sodium phosphate buffer (pH = 7.5, ——) during 60 min.
B: Evolution of the root mean squared film roughness (RMS) as a function of the image size obtained by AFM in the contact mode for PEI-(gelatin-TA)$_5$-gelatin films after their deposition from 50 mM sodium acetate buffer (●) and after 60 min. of immersion in a 50 mM sodium phosphate buffer at pH = 7.5 (●). The horizontal dashed lines indicate the average RMS values respectively of 62 and 3.5 nm for the as deposited film and for the film treated with sodium phosphate buffer.

This decrease in the film roughness may have a marked influence on the wettability of the film according to Wenzel's law [13]:
$$cos\theta_{apparent} = r.cos\theta_{real} \qquad (3)$$
Where $\theta_{apparent}$, θ_{real} and r are the apparent contact angle (on the rough surface) , the real contact angle (on an ideally flat surface) and the film roughness respectively. The apparent water contact angle on the as prepared film was equal to $(25.6 \pm 0.4)°$ and increased to $(45.8 \pm 5.9)°$ after 75 min of exposure to the 50 mM sodium phosphate buffer which is in qualitative agreement with a decrease in the average film roughness evidenced in Figure 3B. However the ratio between the apparent $cos\theta_{apparent}$ values is only equal to 1.3 between the non and phosphate treated films, much smaller than the expected value of about 18 based on the AFM determined surface roughness values. This suggests strongly that the decrease in film wettability after treatment with the sodium phosphate buffer is not only attributed to the decrease in film roughness but also to a change in the film composition.

This compositional change is most probably related to the incorporation of hydrogeno/dihydrogenophosphate anions in the films as suggested by means of ATR-IR spectroscopy where the appearance of an intense band at around 1000-1050 cm^{-1} is evidenced after 60 min of contact between the PEI-(gelatin-TA)$_5$-gelatin film and the 50 mM phosphate buffer and subsequent rinse with the 50 mM sodium acetate buffer to eliminate the excess in non bound phosphate species (data not shown).

Those hydrogeno/dihydrogenophosphate anionsremain in the film owing to favourable interactions with TA as evidenced by means of UV-visible spectroscopy (Figure 5A) and digital pictures of the TA containing solutions (Figure 5B).
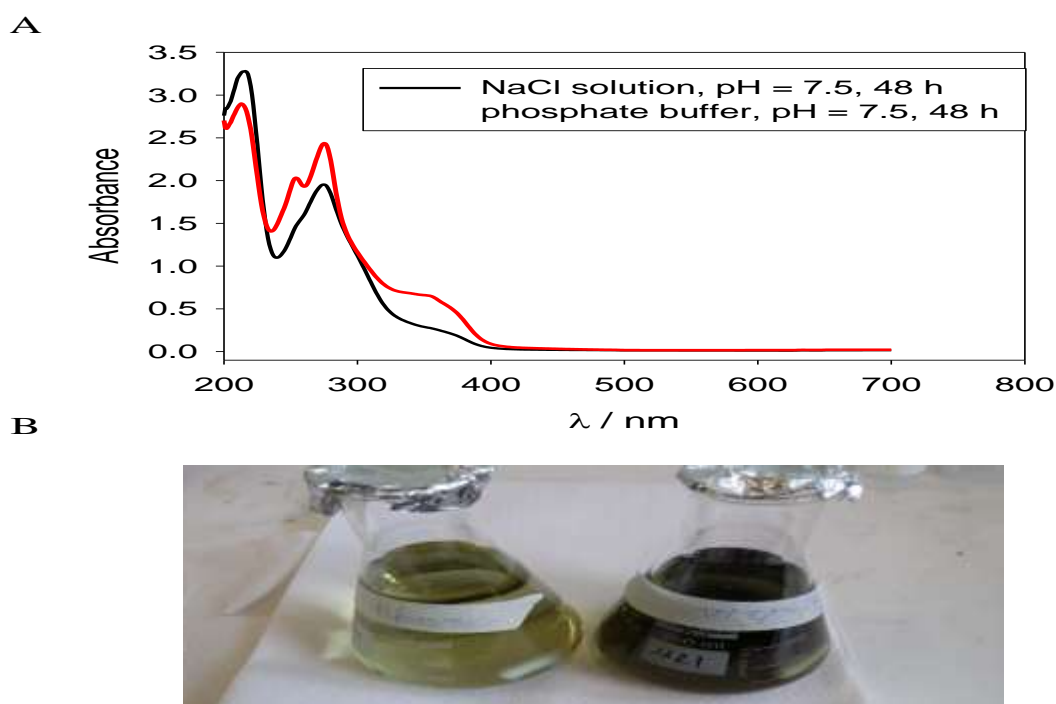
Figure 5: A: UV-visible spectra of a TA solution at 0.1 mg.mL^{-1} in the presence of 50 mMNaCl with pH adjusted to 7.5 with NaOH (——) and of a TA solution at 0.1 mg.mL^{-1} in the presence of 50 mM sodium phosphate buffer at pH =7.50 (——). Both solutions were aged 48 h between their preparation and the spectral characterization.

B: Photographs of the 0.1 mg.mL^{-1} TA solutions prepared in 50 mMNaCl at pH 7.5 (left) and in 50 mM sodium phosphate buffer at pH = 7.5 (right) after 48 h of storage at the ambient atmosphere.
At pH =7.5, TA undergoes some oxidation, triggered by dissolved O2, but it is apparent that the oxidation process is modified in the presence of phosphate species (Figure 5)
These data strongly suggest that when hydrogeno/dihydrogenophosphate anions diffuse in the gelatin and TA containing films, they displace some gelatin /TA bonds, mostly hydrogen bonds, which are replaced by TA-phosphate preferential interactions with some concomitant release of gelatin and gelatin/TA complexes. These released species from the film induce some important reduction in film thickness (Figure 1 and 2) as well as other properties like its roughness (Figure 4) and wettability.

IV. Conclusions

We showed herein that the exposure of PEI-(gelatin-TA)$_n$-gelatin films to 50 mM sodium phosphate buffer (pH = 7.5) is an easy way to modify the film roughness and wettability for water. This investigation underpins some interesting interactions between polyphenols like TA and hydrogeno/dihydrogenophosphate species at pH = 7.5. The formation of phosphate esters may be postulated and needs to be confirmed in forthcoming investiagtions by means of NMR spectroscopy.

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