

Methylene Blue

A Trendy Photosensitizer in Medicine and in Solar-Energy Conversion Systems

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Abstract: The photosensitizer methylene blue (MB) has been investigated as a deoxyribonucleic acid (DNA) intercalant with the main objective of understanding the chemical/physical processes, which occur when adequate wavelength light is impinging on DNA intercalated with MB. This understanding is crucial for the creation of dynamic phototherapy procedures and for the development of new dye sensitized solar cells. During this work we developed and optimized all the conditions to efficiently produce [MB/DNA] multi-layered films by self-assembly. Our study revealed that pH strongly influences the growth of our multilayer films. Our UV studies revealed that the UV radiation causes damage of DNA through opening of aromatic ring and by breaking the DNA phosphate groups. The FT-IR studies on cast films with [MB/DNA] revealed that the denaturation ratio decreases as the irradiation increases, meaning that MB is an intercalant of DNA chain. This study paves the way to develop new dye sensitized solar cells which employ inexpensive materials and take advantage of the intercalation process in order to adjust the intermolecular arrangement of the donor/acceptor molecules to improve the device performance.

1 INTRODUCTION

Radiation exposure carries with it the risk of diseases once it can damage crucial biomolecules such as deoxyribonucleic acid (DNA), compromise the immunological and the nervous systems and contribute to the development of cancer. (Teoule, 1987).

Several studies reported that ionizing radiation as UV, X-rays, β and γ particles have enough energy to excite or ionize the biomolecules. Interestingly, low-energy species (secondary electrons) also induce chemical and physical modifications in DNA such as change of nucleobases, deletion of molecular groups (phosphate and sugar) and both single and double DNA strand formation. (Li et al., 2003) In healthy physiological conditions, living systems have several enzymatic DNA repair systems, which efficiently remove this damage from DNA. If this damage is not repaired in a cell, serious genetic changes such as mutations occur, thus leading to cancer.

Photodynamic therapy destroys the target malignant cells using a photosensitizer, a light sensitive dye, which in the presence of oxygen, activates and forms reactive oxygen species to induce the cellular destruction. (Martinez and Chacon-

Garcia, 2005, Mansuri-Torshiza et al., 2001).

In 1992, Decher et al., introduced the layer-by-layer technique: a revolutionary adsorption technique consisting in the production of thin films by immersing the film alternately in solutions of oppositely charged materials with rinse steps in between to remove any material unbound to the surface. (Decher and Schmitt, 1992) LbL technique requires small amounts of material offering a fine control over the materials structure and is a cost-effective, reproducible, robust and user-friendly technique.

Lbl technique have been used in different areas which integrate the health, electronics and environment in order to develop smart nanostructured devices, drug delivery systems, sensors and solar cells.

In this paper, MB/DNA films were prepared using the LbL technique and was assessed the influence of several factors such as the pH, drying process and immersion time in the loading of MB molecules. The results showed that the pH value of MB solution has a significant effect on the adsorption of the dye into the film.

This study paves the way to develop new dye

sensitized solar cells (Wang et al., 2010) which employ inexpensive materials and take advantage of the intercalation process in order to adjust the intermolecular arrangement of the donor/acceptor molecules to improve the performance of the device.

2 EXPERIMENTAL DETAILS

The LbL films were prepared from MB (MW= 373.90 g/mol) and DNA sodium salt from calf thymus, obtained from Aldrich. The MB was dissolved in pure water supplied by a Milli-Q system from Millipore (resistivity of 18 M Ω cm) to a concentration of 10 mM and pH 7. The DNA aqueous solutions were 0.5 mg/mL concentration. The MB/DNA LbL films were adsorbed onto quartz supports that had been hydrophilized in a Piranha solution for 30 min. Deposition comprised the following steps: (i) immersion of the support in MB solution for 5 s; (ii) washing the support plus MB layer with pure water; (iii) immersion of the support plus MB layer into the DNA solution for 60 s; and (iv) washing the substrate MB/DNA bilayer with pure water. The number of deposited bilayers is equal to the number of repetitions of steps (i)–(iv).

Film growth was monitored by measuring the UV visible using a Shimadzu UV-2101PC spectrophotometer. The damage caused by UV exposure was characterized using a FTIR spectrophotometer Thermo Scientific Nicolet-model 530 (Waltham, MA, USA).

3 RESULTS AND DISCUSSION

3.1 [MB/DNA] Film Growth

The LbL technique is based on physical adsorption processes resulting, mostly, from electrostatic interactions but also the existence of van der Waals forces, hydrogen bonding and hydrophobic forces. (Oliveira Jr et al., 2001, Oliveira Jr et al., 2002).

Multilayers were deposited on quartz substrates using the alternate dipping method into dilute aqueous solutions of MB (pH=4) for 5 sec and DNA (pH=6.8) for 60 sec. Contrary to what was expected, the growth of the film is reduced, since the absorbance values not suffered meaningful changes as the increasing of the number of bilayers, since in according to the literature, the amount adsorbed is proportional to the number of bilayers.

Absorption bands at 260 nm (characteristic of

DNA and MB) and at 600 nm e 670 nm (characteristic of MB) are clear evidences that some molecules were adsorbed in the surface of substrates, possible reflecting the non-ionic physical interactions. The question we asked ourselves was: can the pH influence the multilayers formation?

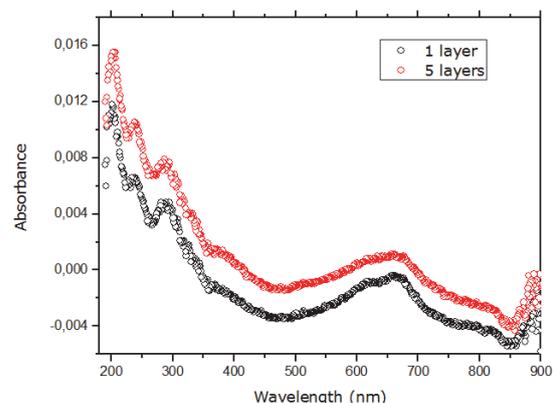


Figure 1: Absorption spectra of [MB/DNA]1 (black line) and [MB/DNA]5 (red line) films with AM at pH=4.

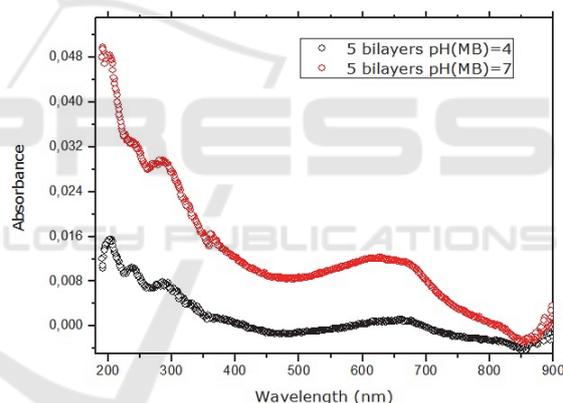


Figure 2: Absorbance spectra of [MB/DNA]5, a pH (MB) 4 (black line) and 7 (red line).

3.2 PH Affects MB Ionization and, Consequently, the Growth of LbL Films [MB/DNA]

Multilayers were deposited on quartz substrates using dilute aqueous solutions of MB with a pH=7 instead of a pH=4. Absorbance spectra of LbL films [MB/DNA] with the same number of bilayers, at different pH, are depicted in the Figure 2, showed that the pH dramatically influences the growth of the films. This can easily be justified by the increase of the degree of ionization of the molecules. In fact, at pH 7, the MB molecules are electrically charged which contribute to the formation of the films due to the ionic forces. (Impert et al., 2003) The absorption

spectra of the LbL films, with the MB at pH = 7 shows the characteristic absorption bands, (mentioned before) of MB and DNA in the visible and ultraviolet.

Given that the assembly conditions were optimized, the quartz substrates were coated with 20 bilayers of MB/DNA. The absorption band at 618 nm and 676 nm correspond to the dimeric and monomeric form of MB, respectively. (Spencer and Sutter, 1979) In Figure 3, we can also see an absorption band at 285 nm (characteristic of DNA and of MB) and other at 292 nm which is characteristic of MB and, according to literature, this assignment correspond to $\pi \rightarrow \pi^*$ transitions. It should be referred also that, for these LbL films, the absorbance in the 600 to 700 nm range is lower than the absorbance in the 280 nm and the absorbance has a nonzero value at 200 nm. As the spectra of MB solutions the absorbance is higher in the 600 to 700 nm than the absorbance at 280 nm and the absorbance decreases to zero for 200 nm, see figure 3b), one can conclude that both DNA and MB molecules are incorporated in the films. These results proved that both MB forms have the ability to link to the DNA, allowing the formation of LbL films.

Compared to the absorption peak of aqueous MB (at 664 nm), the visible maximum peak at 623 nm of [MB/DNA]₂₀ film is blue-shifted by 41 nm. According to Chao et al., phenothiazine dyes such as MB, suffered a blue-shift absorption band because they form H-aggregates via π - π stacking. (Gao et al., 2008)

The absorbance at the peak (at 285 nm, 618 nm and 676 nm) was used to monitor the buildup of multilayers, as illustrated in Figure 4. One can see that the data follow straight lines, indicating that the films increase linearly with the number of bilayers, meaning that each deposited bilayer contributes an equal amount of deposited polymer.

The amount of MB adsorbed per bilayer was calculated by taking the intensity of the 618 nm and 667 nm peak in the UV-Vis spectra for [MB/DNA]₂₀. From the absorbance intensity using the Beer-Lambert law, we concluded that the MB adsorbed layer per unit area was $96 \pm 5 \mu\text{g}/\text{m}^2$.

During this work, we assessed the influence of the drying process as well the immersion time on the morphology of the LbL films. The drying process using a nitrogen stream (data not shown) seems to be an important factor during the preparation of LbL films since it promotes conformational changes of the molecules through modifications of their arrangement in space, which allows a greater interaction between charges and facilitates the bonding between the materials.

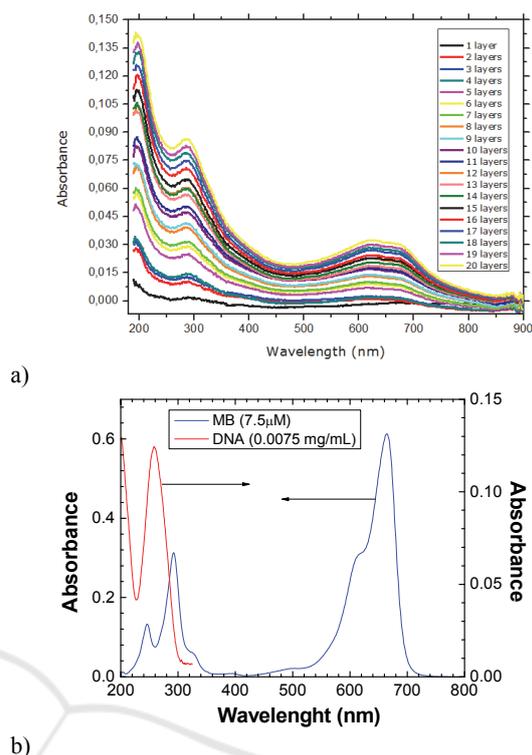


Figure 3: Absorption spectra of: a) [MB/DNA]₂₀ LbL film. b) aqueous solutions of MB and DNA.

The kinetic study of DNA immersion time (data not shown), showed that immersion in DNA solution for 5 sec is enough to obtain a thin [MB/DNA] film with all characteristic absorption bands of each material. The immersion for long times can lead to a desorption phenomena.

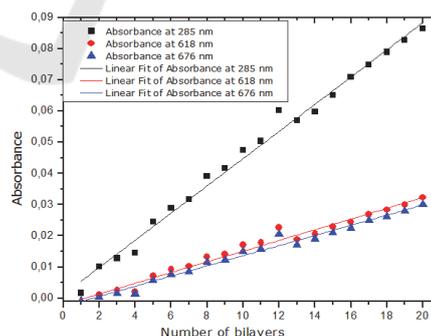


Figure 4: Maximum absorbance versus number of bilayers of MB/DNA self-assembled films adsorbed on quartz substrates.

3.3 FTIR Characterization of Films

The damage caused by UV radiation on DNA in the presence of the intercalator MB, are characterized by fourier transform infrared spectroscopy (FTIR). The FT-IR results obtained to $[MB/DNA]_{100}$ film were inconclusive since the adsorbed amount of MB and DNA was not sufficient to obtain a spectra with adequate resolution.

Cast films were prepared with mixture of DNA and MB, and were irradiated for different time intervals.

Several changes in the IR spectra of DNA and mixture MB/DNA have seen observed after irradiation, as illustrated in Figure 5a) and b). In order to better analyse the infrared spectra changes, spectra baselines were removed and the peaks which did not change as a result of exposure to UV radiation were identified. According to Gomes et al., the FT-IR band detected near 1018 cm^{-1} is very stable since it does not suffer any change when exposed to a radiation with 140 nm . (Gomes et al., 2009) This peak is due to

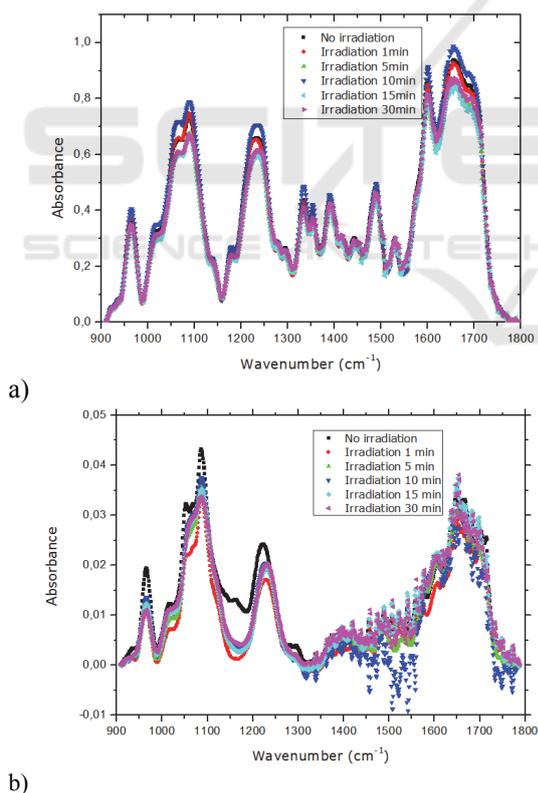


Figure 5: Infrared absorbance spectra of: a) DNA cast sample before and after irradiation with 254 nm UV light; b) a mixture of MB/DNA cast sample before and after irradiation with 254 nm UV light.

furanose vibrations and was used to normalize the obtained data, dividing the other peaks areas by the area of this peak, avoiding the possibility that the small changes due to the measurement of the infrared spectra in different regions of the sample are affecting the observed peak areas decrease or increase.

The vibrations associated with C-O stretching of nucleic acid sugar (1067 cm^{-1}) and PO_2^- stretching (1089 cm^{-1}) decreased with the irradiation time. According to Paulo et al.,¹³⁻¹⁵ UV radiation causes damage of DNA through opening of aromatic ring and by breaking the DNA phosphate groups.

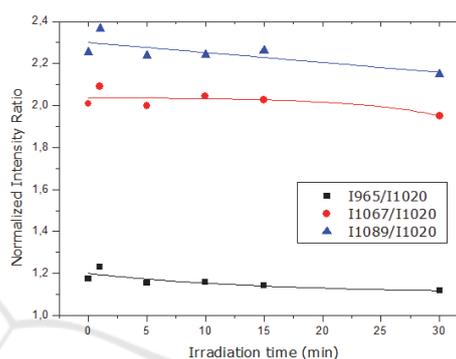


Figure 6: Normalized infrared intensity ratios at 965 cm^{-1} (CC stretch of backbone), 1067 cm^{-1} (CO stretch of the furanose backbone), 1089 cm^{-1} (Symmetric PO_2^- stretching of the backbone) relative to peak area at 1020 cm^{-1} of a DNA cast sample irradiated for different periods of time with 254 nm UV light. The solid lines are guidelines.

As illustrated in Figure 6, as the irradiation time increases the ratio of the peaks 1089 cm^{-1} and 1067 cm^{-1} decrease, meaning that radiation preferentially affects the PO_2^- groups than sugars.

One of the goals of this work was understand if the UV radiation leads to a DNA denaturation in the MB/DNA film. The infrared intensity ratio at 1690 cm^{-1} ($\text{C}=\text{O}_2$ strength of thymine single stranded or double stranded and $\text{C}6=\text{O}_6$ stretching of guanines), was normalized relative to peak at 1652 cm^{-1} ($\text{C}=\text{O}_2$ strength of cytosine single stranded or double stranded and $\text{C}4=\text{O}_4$ strength of thymine single stranded or double stranded) of a DNA cast sample irradiated for different periods of time with 254 nm UV light. As the irradiation time increases the ratio $1690/1652\text{ cm}^{-1}$ decreases, meaning that the presence of MB induces an intercalation process which forces the opening of the DNA chain.

4 CONCLUSIONS

This work showed an efficient protocol to produce [MB/DNA] multi-layered films by self-assembly. The pH value should be 7, since at this pH the MB molecules are electrically charged.

An MB adsorbed layer per unit area of $96 \pm 5 \mu\text{g}/\text{m}^2$ was achieved if the film is dried with nitrogen at the end of each bilayer and immersed for 60 sec in the DNA solution.

The UV studies revealed that the UV radiation causes damage of DNA through opening of aromatic ring and by breaking the DNA phosphate groups. Our results also showed that UV radiation preferentially affects the PO_2^- groups than sugars.

The FT-IR studies on cast films with [MB/DNA] revealed that the denaturation ratio decreases as the irradiation increases, meaning that MB is an intercalant of DNA chain.

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