

Tartrazine Dye and Bovine Serum Albumin: the Influence of pH on Adsorption Process

Jackeline B. Brito, Gean P. S. Aguiar, Júlio C. J. Flores, Josmary R. Silva, Nara C. de Souza*

Grupo de Materiais Nanoestruturados, Universidade Federal de Mato Grosso, Barra do Garças, Mato Grosso, Brazil

Abstract Multilayers from Tartrazine dye (Tart) alternated with bovine serum albumin (BSA) were successfully prepared and a uniform growth has been observed. The influence of two values of Tart solution pH (3 and 7.5) on adsorption process of this molecule on BSA monolayer at physiological pH was investigated using kinetics and isotherms of adsorption, as well as thermostimulated adsorption experiments. All effects found by the changing of the pH were explicated mainly taking into account the ionic attraction (Tart at pH 3) and ionic repulsion (Tart at pH 7.5) that occurs during the adsorption process of Tart onto BSA monolayer.

Keywords Tartrazine Dye, Bovine Serum Albumin, Layer-By-Layer Deposition Method, Adsorption Process, Ph Influence

1. Introduction

Various health problems are associated to the dyes, which are components of a lot of industrial products. Among the dyes, Tartrazine is very popular because is used in food-stuffs, cosmetics, textile, and medicines[1]. It has been also associated to diseases such as hyperactivity[2], asthma[3], and thyroid cancer[4]. Many studies have been carried out concerned to the Tartrazine dye (Tart)[5-8]. In special, the adsorption process of Tart has been investigated because its important role in applications, such as color removal from industrial effluents[9-12].

In this work we prepared Layer-by-Layer (LbL) films in order to investigate the interactions of Tart and proteins. Bovine serum albumin (BSA) was chosen because it is the most abundant protein in blood plasma, and works like a transport protein for several substances (dyes and bioactive molecules)[13,14]. The BSA has been focus of several studies for their properties, such as pH-dependent binding[15] and interactions with phospholipids[16]. Besides that, BSA and human serum albumin, HSA, have a similarity of approximately 80% of their primary sequences[17]. This means that BSA and HSA are homologous proteins that should have very similar biological functions. Thus, as the Tartrazine is associated with various health problems, an investigation about the adsorption process of the dye and albumin can be used as an approach to analyze the biological process. Although there are several works on Tart and also

BSA, we are not aware of any report about the preparation of multilayer systems and study of pH influence on adsorption process of Tart and BSA using the layer-by-layer (LbL) method. In this paper, we have not only studied the multilayer growth of Tart alternated with BSA, but also investigated the effect of pH on adsorption process of Tart on BSA monolayer focusing on the possible interactions associated to this process.

2. Materials and Methods

Tartrazine and BSA (fraction V, purity 96–100%) from Acros Organics were used as received. The experimental film fabrication procedures are essentially the same as those described by Decher[18]. To build the multilayers, Tartrazine was alternated with 0.5 g/L BSA diluted in ultrapure water. The pH of the BSA solution was adjusted to 7.5 (close to physiological pH) by adding appropriate amounts of ammonium hydroxide. The final BSA solution was completely clear. The rinsing solution was ultrapure water with pH adjusted to 7.5. The Tartrazine solution was prepared to concentration of 0.5 g/L by dissolution in ultrapure water. The pH of the Tartrazine and the rinsing solutions were adjusted to 7.5 (anionic solution) and 3 (cationic solution) by adding an ammonium hydroxide solution or HCl respectively. The films were adsorbed on BK7 optical glass and the cleaned procedures are essentially the same described by previous works[1,19]. The adsorption of Tartrazine, which is proportional to absorbance, was monitored by measuring the UV–Vis absorption spectra with a double-beam Thermo Scientific spectrophotometer model Genesys 10.

* Corresponding author:

ncsouza@ufmt.br (Nara C. de Souza)

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3. Results and Discussion

We have employed the layer-by-layer (LbL) method to growth multilayers from BSA alternated with Tart (BSA/Tart). Fig. 1 shows the absorbance at 425 nm versus the bilayer number, and an inset with the spectra of Tart solution and BSA/Tart (pH7.5/pH7.5) films. A linear increase in the absorbance was observed, suggesting that the same amount of material was adsorbed in each deposition cycle. The spectra (inset in Fig. 1) are essentially the same as those found in the literature for the Tartrazine[1,20]. The maximum absorption wavelength λ_{max} of Tart molecules appears at 408 nm in aqueous solution (symbol line) and 425 nm in LbL films. These results reveal a λ_{max} shift ($\Delta\lambda = 17$ nm) to red for the BSA/Tart bilayer in relation to the Tartrazine aqueous solution[1], indicating that the dye molecules join in J-aggregates with in-line transition dipoles[21]. The capacity of forming multilayers of the BSA and Tart reveals clearly that there are interactions between these two kinds of molecules, which permit the assembly of films[1].

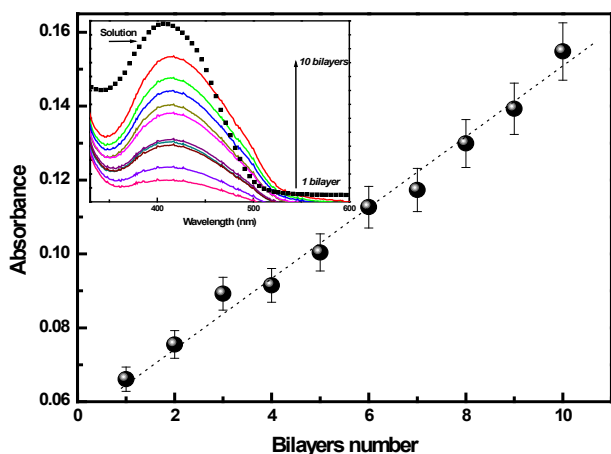


Figure 1. Absorbance at 425 nm versus bilayer number for BSA/Tart LbL films on BK7 glass. The experiment was carried out at pH 7.5 for both Tart and BSA. Each experimental point is the average of measurements of three films fabricated under identical conditions. The inset shows the UV-Vis spectra of the Tart solution and also for LbL multilayers

Solution pH is one of the parameters that can influence on the interaction of Tartrazine and BSA during the adsorption process[22]. Fig. 2 shows the adsorption kinetics curves of BSA/Tart bilayers at different pH values. The curves were obtained by depositing Tart at pH 3 or 7.5 on BSA at pH 7.5 using an immersion time of 3 min for Tart solution. We can observe that Tart at pH 3 presents the maximum absorbance larger than at pH 7.5. Moreover, the absorbance at pH 7.5 achieves the saturation at ca. 2 s, and at pH 3 at ca. 120 s. We can consider that the Tart molecules at pH 7.5 are repelled from away the BSA surface, so the saturation of Tart molecules on BSA monolayer surface should occur quickly, because only some Tart molecules close to BSA surface can adsorb by other interactions different of that ionic one. That is, the others interactions can

overcome the ionic repulsion close to BSA monolayer surface[23]. On the other hand, in the case of Tart molecules in pH 3 the ionic forces dominate the adsorption process.

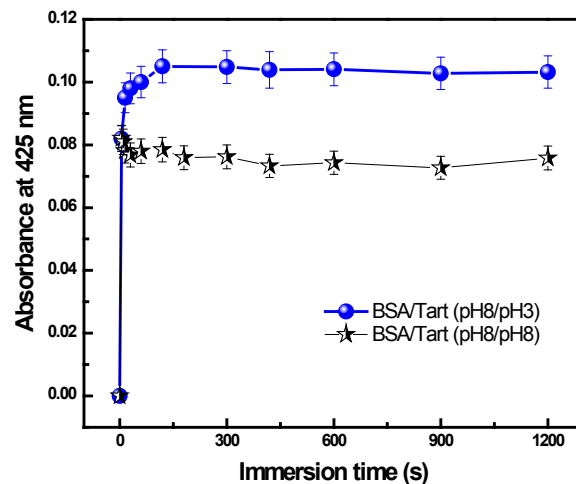


Figure 2. Absorbance at 425 nm versus immersion time for BSA/Tart LbL film at different pH values. The solid line is a guide for the eye

We have performed adsorption isotherm experiments by depositing Tart at pH 3 (Fig. 3) and pH 7.5 (Fig. 4) onto BSA monolayer with immersion time of 10 minutes for BSA and 3 min for Tart solution. As shown in the Fig. 3, we have observed three different regimes for the absorbance. For small concentrations, the absorbance increase fast and linearly, after that achieve a constant value, and thus increases again. The saturation absorbance suggests that a whole layer was achieved and, as a result, the monolayer surface tends to repel the solute because all active sites for adsorption already were filled. The growth after saturation can be indicates the formation of a second monolayer[24].

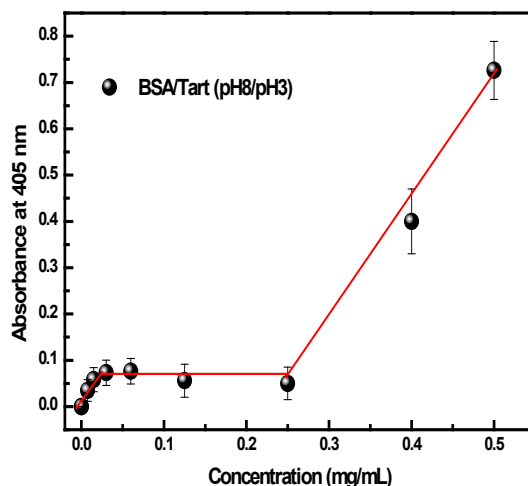


Figure 3. Adsorption isotherm for Tart (pH 3) adsorbed on BSA monolayer (pH 8) with immersion time of 3 min. The solid line is a guide for the eye

Fig. 4 shows the adsorption isotherm for Tart at pH 7.5. It is noted a different behavior of this isotherm in comparison to that showed in Fig. 3. We can observe only an increase of absorbance and, after that, a saturation. This can be attributed

to ionic repulsion between Tart and BSA molecules when Tart is at pH 7.5 and BSA monolayer at pH 7.5. In this situation, there is a competition between the attractive interactions and the ionic repulsion. Even the concentration is increased, it does not contribute to increase the Tart adsorbed molecule amount, and the saturation is achieved at lower value than that found for Tart at pH 3.

In order to determine the activation energy associated to the adsorption process of Tart on BSA monolayer for the different pH values, we have performed thermostimulated adsorption experiments. Fig. 5 shows the Arrhenius plot from absorbance at 425 nm as a function of temperature for Tart and BSA solutions.

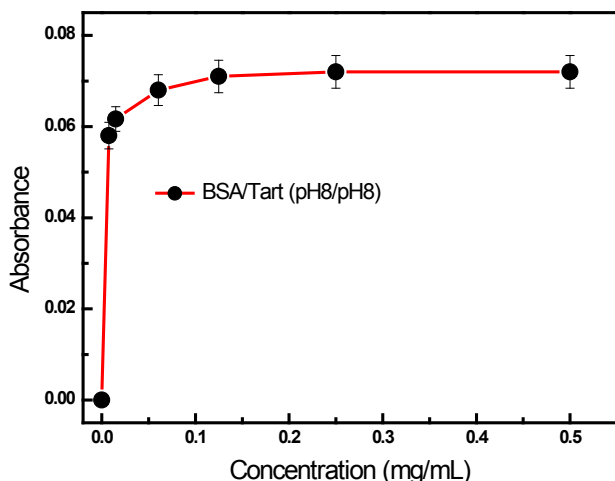


Figure 4. Adsorption isotherm for Tart (pH 8) adsorbed on BSA monolayer (pH 8) with immersion time of 3 min. The solid line is a guide for the eye

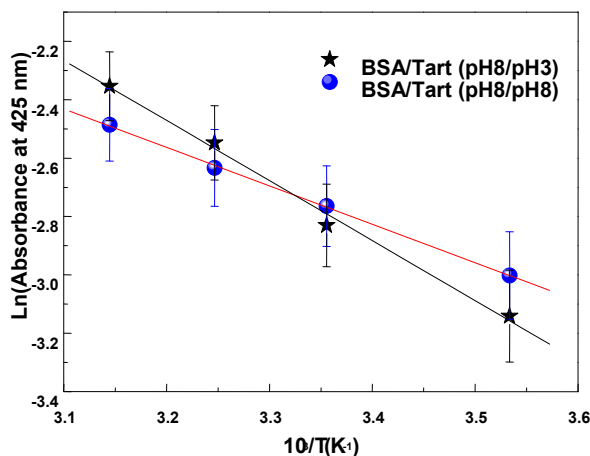


Figure 5. Arrhenius plot for adsorption process of Tart (pH 8 and 3) adsorbed onto BSA monolayer (pH 8)

The activation energies were found to be about 2.6 kcal/mol for BSA/Tart (pH7.5/pH7.5) and 4.1 kcal/mol for BSA/Tart (pH7.5/pH3). The higher activation energy observed for the Tart at pH 3 in comparison with the Tart at pH 7.5 can be explained taking into account that at the first one there is an ionic attraction contribution that increases the interaction

magnitude between the Tart and BSA molecules. However, when the pH of Tart is 7.5, the ionic repulsion dominated the process and the adsorption of Tart can be governed by hydrogen or van der Waals interactions. The values of activation energy are consistent with those found for the Eosin and BSA system[15].

4. Conclusions

We have successfully prepared multilayer films from Tart and BSA and shown that the Tart interacts depends on the conditions of the solution pH used in the preparation of films. The adsorption process of Tart at pH 7.5 has not only adsorption rate faster, but also maximum adsorbed amount and activation energy lower than at pH 3. All this results was attributed mainly to ionic attraction associated to Tart at pH 3 and ionic repulsion associated to Tart at pH 7.5 when it is adsorbed on the BSA at pH 7.5. The possibility of forming multilayer films based on dyes and proteins can be used in investigations of biological sensors, whereas the knowledge of influence of pH on adsorption process of Tart on BSA monolayer may be useful to studies of interactions associated to health problems.

ACKNOWLEDGEMENTS

This work was supported by CNPq and CAPES.

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