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A New Approach to Catalyze Template Polymerization of Aniline Using Electrostatically Multilayered Hematin Assemblies

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ABSTRACT

In this work, we describe a new approach to catalyze the template polymerization of conducting polyaniline (Pani). Electrostatic layer-by-layer (ELBL) self-assembly of a polyelectrolyte and a biomimetic catalyst, hematin has been utilized to construct a nanocomposite film catalyst. Poly(dimethyl diallylammonium chloride) (PDAC) and hematin have been used as polycation and counter anions, respectively. The absorption spectra by UV-VIS-NIR spectroscopy showed that a conductive form Pani was formed, not only as a coating on the surface of the ELBL composites, but was also formed in solution. Furthermore, it was found that the reaction rate was affected by pH and concentration of hematin in the multilayers. The feasibility of controlled desorption of hematin molecules from the LBL assembly was explored and demonstrated by changing pH and hematin concentration. It is believed that hematin sandwiched between positively charged polyelectrolytes in the composite films was slowly released into the solutions and then used to catalyze the template polymerization of aniline with SPS, resulting in a water soluble form of Pani. The polymerization rate of aniline in

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solution was enhanced with decreasing pH of the solutions due to increased desorption of hematin nanoparticles from the multilayers. These ELBL hematin assemblies demonstrated both a way to functionalize surfaces with conductive Pani and a potential method of reusability of the catalyst for improved cost effectiveness.

Key Words: Horseradish peroxidase (HRP); Hematin; Polyaniline; Electrostatic layer-by-layer (ELBL) self-assembly; Multilayer nanocomposites.

INTRODUCTION

Conducting polyaniline (Pani)[1,2] has attracted considerable attention in many applications such as sensors,[3,4] microelectronics,[5] electrochromic displays,[6] electromagnetic shielding,[7] and nanofibers[8] because of its electrical and optical properties as well as good stability. While Pani has promising properties, improvements in cost effectiveness and processability are needed before practical applications can be realized. Many attempts have been made to improve the processability of Pani.[9–11] Compared to chemical preparative routes, enzymatic polymerization has several advantages including environmentally benign reaction conditions and the potential to produce product in high yield.[12,13] Horseradish peroxidase (HRP) is a widely used oxido-reductase. This enzyme polymerizes aromatic compounds such as anilines and phenols. Recently, Samuelson et al. reported enzymatically synthesized conducting Pani using templates such as SPS[11,14–17] and DNA.[18,19] In this approach, a water soluble Pani was formed in the presence of SPS. Conductivity of up to 0.15 S/cm upon acid doping was assessed for these complexes. However, for potential large scale commercialization, the current high cost and low activity of HRP over a wide pH range needs to be improved. Hematin is considered a promising biomimetic alternative to HRP because it is an inexpensive hydroxyferriprotoporphyrine which catalyzes the polymerization of aniline and phenol substrates. Hematin however dissolves only at high pH due to carboxylate salt formation[20] and is not soluble at low pH, which is required for the template polymerization of aniline.[14]

It has been reported that enzymes can be immobilized by using sol–gel[21] and electrostatic self-assembly techniques.[22–25] Among other methods, electrostatic layer-by-layer (ELBL) self-assembly has attracted much attention because it is a simple, controllable, and versatile method for the fabrication of novel surfaces and functional thin films via electrostatic interaction between oppositely charged species. As demonstrated by Decher and Ferguson,[22,23] these techniques have been widely applied to the assembly of many polyelectrolytes,[24] DNA,[25] proteins,[26–28] and nanoparticles[29,30] to fabricate ELBL nanostructured composite films. In the case of protein multilayers where enzymes are immobilized for catalytic or sensing applications, it is important and often difficult to immobilize the enzyme such that the active site is accessible for reactivity. Hematin is an ideal candidate for this approach however because it is a molecular species that is the reactive site of the enzyme, thus orientation is not a factor, and the two carboxyl groups of hematin can be negatively charged for ELBL assembly. In the case of the template polymerization of aniline with SPS in solution, the ELBL immobilized catalyst needs to be desorbed into the solution since the SPS/aniline complex cannot diffuse into the catalytic multilayer assembly. Recently, controlled desorption of a ELBL assembly due to charge compensation was demonstrated by altering the ion concentration of the solution.[31,32]

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Therefore it is expected that the desorption rate of hematin from the ELBL assembly can be controlled in a similar way for the polymerization of aniline and that these hematin ELBL composites can be reused for multiple template polymerization reactions. Thus, with the objective of developing a reusable catalyst to polymerize aniline with template, we have fabricated hematin nanocomposite films using the ELBL self-assembly technique. The two carboxyl groups of hematin can be utilized to construct negatively charged nanoparticles by controlling pH. The negatively charged nanoparticles can be easily deposited with the cationic polyelectrolyte, PDAC. Here we describe a detailed investigation of hematin multilayer formation and the resulting catalytic activity and reusability of these novel hematin nanocomposites for the template polymerization of aniline both on the surface of the hematin multilayers and in solution.

EXPERIMENTAL

Materials and Methods

Hematin was purchased from Sigma Chemical Co., St. Louis, MO. Aniline (purity 95%), PDAC (20 wt.% in water, Mw 100,000–20,000), poly(sodium 4-styrenesulfonate) (Mw 1,000,000) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI) and used as received. Buffers of phosphoric acid (0.1 M, pH 1, 2, 3) were used in the polymerization studies. The glass slides used as solid supports for multilayer fabrication were purchased from VWR Scientific. The glass slides (25×75 mm) were hydrophilized with 1% Chemsolv® solution, which was obtained from Mallinckrodt, in deionized water under ultrasonication for 180 min. Multilayer composite films were prepared with PDAC as the polycation and hematin as the counter anion at pH 11. All procedures for LBL self-assembly were similar to that described previously.[33] The PDAC and hematin concentrations were 5 and 3 mM, respectively. The dipping process for PDAC and hematin were performed for 10 and 5 min, respectively followed by washing with deionized water for 1 min. Aniline polymerization was typically carried out at room temperature in a 40 mL, 0.1 M phosphoric acid buffer solution, which contained a 1 : 1 molar ratio of SPS to aniline. Then 0.167 g of (0.81 mmol) SPS was added first to the buffered solution, followed by an addition of 2.1 mL of aniline stock solution (0.036 mL/mL pH 1.4) with constant stirring. A 17 bilayer (one side of the glass) hematin/PDAC composite substrate ($3.5 \times 2.5\ cm^2$) was then immersed into the solution. To initiate aniline polymerization, 10 mL of H_2O_2 (0.25 w/w%) was added dropwise, incrementally, over 10 min. The reaction was maintained for 24 h with constant stirring, and carried out at different pH values (1.0, 2.0, 3.0, 4.0). The rate of polymerization for each hematin multilayer assembly was monitored and recorded by a Perkin-Elmer Lamda-9-UV-VIS spectrophotometer at a concentration (3.3 v/v%) diluted with buffer solutions after 24 h. To compare the polymerization rates on the glass solid supports, a set of samples with different numbers of bilayers of hematin/PDAC composite films were immersed in a pH 3.0 solution. Subsequently, the glass solid support coated with Pani was directly measured by UV-VIS spectroscopy as a function of time. To monitor the reusability of the assembled hematin nanocomposite films for polymerization, composite films of 17 bilayers were chosen and used to polymerize aniline at pH 1.0 for 24 h. This substrate, coated by Pani, was then reused for the polymerization of fresh aniline and SPS at pH 1.0. This test was repeated for

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10 cycles. The UV-VIS-NIR spectra were obtained from a diluted solution (10 v/v%) in a pH 1.0 buffer. To assess the increase of molecular weight and conjugation length of Pani in the SPS complex, the absorption intensity of Pani/SPS solution produced by using the first reused composite films was monitored as a function of time without stirring.

RESULTS AND DISCUSSION

The multilayer hematin nanocomposites were prepared by ELBL sequential deposition of positively charged PDAC and negatively charged hematin at pH 11 (Fig. 1). PDAC was deposited for 10 min as demonstrated in previous results.[34] For hematin deposition, however, a 5 min deposition time was used because the hematin deposition does not change with time. Figure 2 shows that the characteristic absorption peaks of hematin at 395 nm increased linearly and reproducibly with the number of PDAC/hematin deposition cycles.

To determine the pH effect on the catalytic activity, the aniline polymerization was performed with pHs ranging from 1.0 to 4.3. It was observed that HRP does show catalytic activity for aniline polymerization at pH 4.3 but the hematin ELBL assemblies showed relatively no activity at this same pH. Therefore, the pHs of solutions were adjusted to enhance the catalytic activity of the hematin films. As shown in Fig. 3, at a pH of 3 the absorption peaks of the Pani/SPS complex were negligible and a pale yellow color was observed after 1 day. However, at pH 1 and 2 the solution showed a dark green color with absorption bands at 650–750 nm and 300–400 nm, which are assigned to the known polaron transitions of Pani.[35] After 1 day, the absorption intensity of Pani at pH 1 was about 10 times higher than that observed at pH 2.0. Even though preferential alignment of aniline monomers with SPS at low pH was clearly established,[11,14,15] this cannot explain why the polymerization rate depends on pH when the acidity is already low enough for their reactions. To understand this difference in behavior with pH we assumed that the hematin molecules would exhibit different desorption behavior at different pHs. To corroborate this hypothesis, hematin/PDAC ELBL films of 16 bilayers were immersed

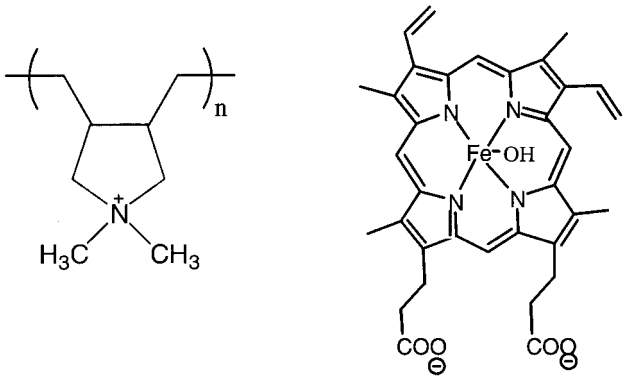


Figure 1. Schematic diagram of (a) PDAC and (b) hematin.

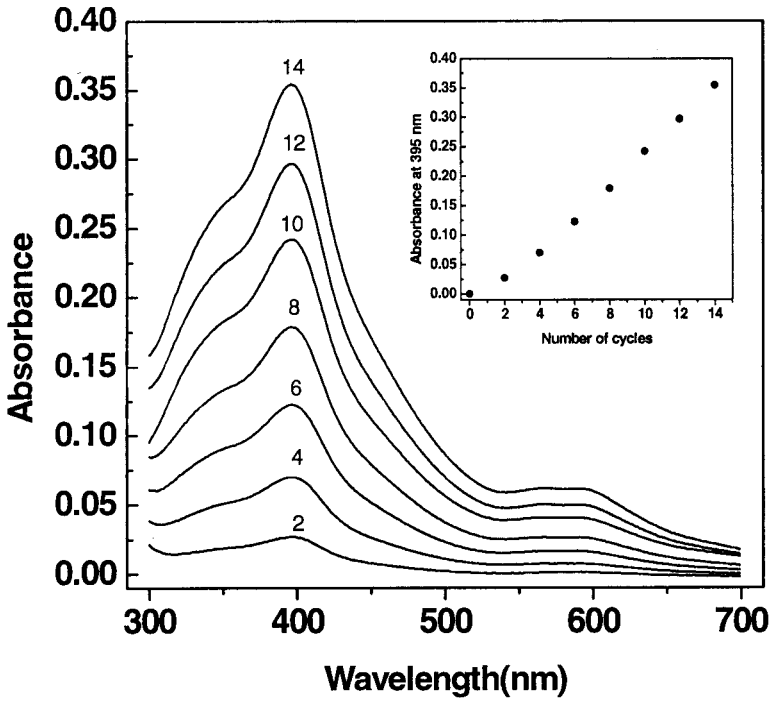
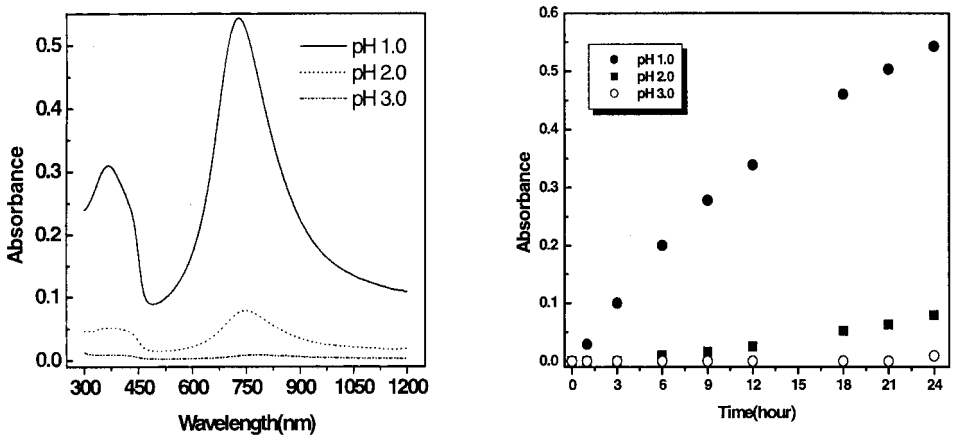


Figure 2. UV-VIS absorption spectra of PDAC/hematin multilayer films as a function of the number of bilayers. Furthermore, a linear relationship between absorbance and number of dipping cycles at λ_{max} (395 nm) is reported.

into different pH solutions (pH 1.0, 2.0, 3.0). The UV-VIS absorption spectra of just the hematin on each slide were obtained, without template (SPS), monomer (Aniline), and H_2O_2 to avoid any interference in the assessment (Fig. 4). The remaining contents (%) of hematin were calculated by the absorbance ratio of initial to after desorption of hematin as a function of dipping time. The absorbance of hematin slowly decreased with time and a relatively large difference in absorption with time was observed at pH 1. These results show that the release rate of hematin depends on pH, i.e., ionic strength. In other words, the hematin carboxylate groups were compensated with acidic proton at low pH. As suggested in other reports,[31,32] the charges in the ELBL assemblies are compensated by electrostatic interaction of ions. Consequently, hematin desorption is considered to be relatively enhanced at lower pH. To further prove this point, one bilayer of PDAC/hematin followed by 10 bilayers of PDAC/SPS, to make a final assembly of glass//(PDAC/hematin)$_1$(PDAC/SPS)$_{10}$ was fabricated. It was found that this composite film could catalyze aniline polymerization, indicating that hematin molecules sandwiched between the polyelectrolyte layers could diffuse and be released due to charge compensation. As expected, it is difficult for positively charged aniline at low pH to diffuse into the multilayered hematin composite films because the aniline monomers associate in solution to form a charged complex with the negatively charged SPS template. This is not the classical mechanism of immobilized catalysts, where low molecular substrates such as



(a) (b)

Figure 3. (a) UV-VIS-NIR spectra of SPS/Pani catalyzed by hematin ELBL assemblies after reaction for 24 h and (b) absorbance at λ_{max} as a function of reaction time.

glucose diffuse to the immobilized enzymes in multilayers, yielding products that are subsequently released.[26] In these reactions, conducting Pani/SPS complexes were observed both on the hematin film surface and in solution at pH 1.0 and 2.0. However, at pH 3.0 the Pani/SPS complex was observed only on the film surface and not in solution.[33] The absorption peak intensity of the Pani/SPS coating on the ELBL hematin slide increased with a decrease in pH as was observed in solution. Hematin multilayer assemblies of 8, 12, 14 bilayers were prepared and tested at pH 3 to see the effect of the

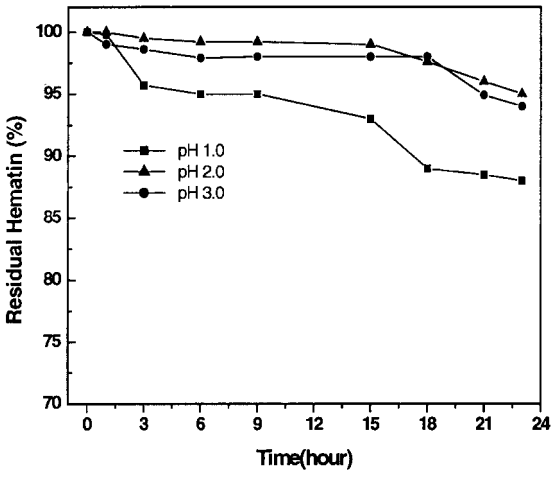


Figure 4. UV-VIS spectra of a 16 bilayer ELBL hematin assembly on a glass support as a function of dipping time.

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catalyst concentration deposited on the glass. As seen in Fig. 5, the polymerization rate on the glass increased with the number of hematin layers. Figure 5 shows that the absorption intensity saturated with reaction time and suggests that the in situ formed Pani was completely deposited on the film surface after 12 h as demonstrated in the case of a polyelectrolyte deposition.[34]

To assess reusability of the ELBL hematin catalyst assemblies 14 bilayer PDAC/hematin composites, with a Pani coating from polymerization at pH 3.0 were used. The polymerization was performed at pH 1.0 to enhance desorption of the hematin from the assembly. While the hematin multilayers showed a potential to be reusable, the intensity of the Pani/SPS complex in the solution dramatically decreased with each reaction.[33] In fact, the multilayers covered showed a significant decrease of absorption intensity in the solution after just 2 reactions. This may suggest that the amount of released hematin decreases with each reaction. In an attempt to understand and improve the reusability of the multilayered hematin nanocomposites for template polymerization of aniline, 17 bilayer PDAC/hematin assemblies with a Pani coating from a pH 1.0 reaction were prepared. It was found that in this case the rate of polymerization, as indicated by absorbance of Pani in solution, did not significantly change up to 7 cycles (Fig. 6). This may be explained by a more controlled release of hematin nanoparticles due in part by a thicker Pani film that was formed from the pH 1.0 reaction and a higher amount of hematin. It is possible that the Pani coatings may be helping to control the release of hematin from the layers. From these findings, it may be concluded that the reusability of the ELBL hematin multilayer assemblies for the template polymerization of aniline, depends on the

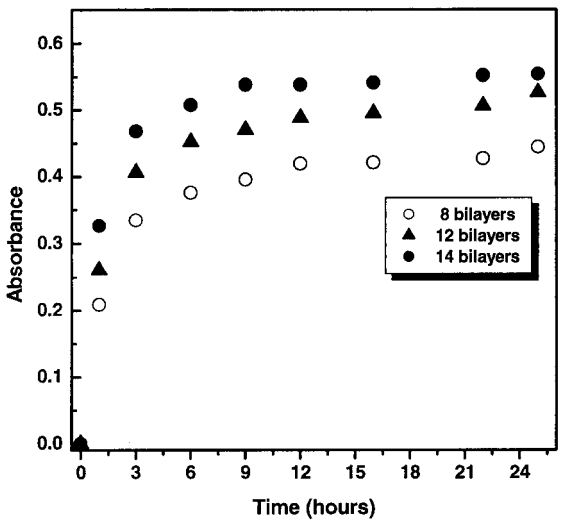


Figure 5. The maximum absorption intensity of the SPS/Pani complex on the glass solid supports with varying number of layers as a function of reaction time.

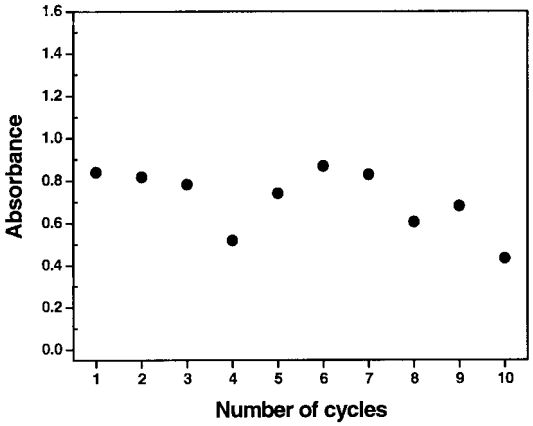


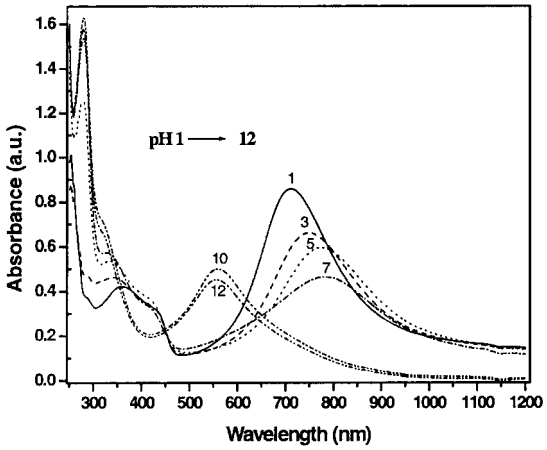
Figure 6. Maximum absorption intensity of the SPS/Pani complex in a series of SPS/Pani solutions at pH 1.0. For each cycle, fresh aniline/SPS solutions were prepared and polymerized with the same multilayered hematin nanocomposite.

pH, amount of hematin (number of bilayers), and the Pani film thickness on the surface of the multilayers.

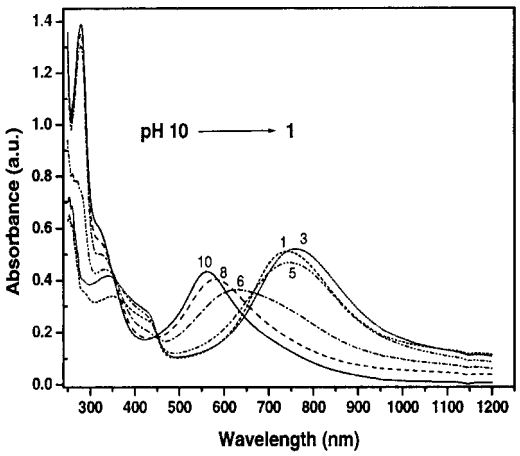
To assess the redox behavior of the Pani/SPS complex formed from this approach, the absorption spectra of a solution prepared at pH 1.0 was studied with varying pH. Figure 7(a) shows the shift in absorption spectra of the complex with increasing pH from 1.0 to 12. At pH 1.0, the Pani is in the doped state as revealed by the presence of the polaron band transition at 365 and 700 nm.[35] As the pH of the complex is increased, the polaron bands at 700 nm gradually disappear, and a strong absorption due to exciton transition of the quinoid rings at 560 nm begins to emerge. At pH 12, the exciton band of the Pani/SPS complex increased, indicating that the Pani has been fully dedoped to the emeraldine base form. The dedoped Pani showed redox reversibility upon titration with 0.1 N HCl [Fig. 7(b)]. The Pani/SPS complex that formed on the surface of the multilayer assemblies prepared at pH 3.0, showed a similar shift in absorption with increasing pH from 3.0 to 11.[33] These results show that multilayered hematin nanocomposites can catalyze aniline polymerization with template both in the solution and on the substrate. The release of hematin from the ELBL composite films was confirmed by both an absorption increase of the Pani/SPS complex in solution and an increase of λ_{max} of Pani. The absorption spectra of the Pani/SPS complex were monitored by UV-VIS-NIR spectroscopy. Figure 8 shows that the absorbance of the complex at λ_{max} increased linearly with time and λ_{max}, which supports that the conjugation length and molecular weight also increased as a function of time.

CONCLUSION

We have demonstrated a new approach to catalyze the template polymerization of aniline. In contrast to the known immobilization methods of enzymes into multilayer composites, here the catalyst desorption, (i.e., hematin) is required for the polymerization



(a)



(b)

Figure 7. UV-VIS-NIR spectra of the Pani/SPS complex during (a) dedoping and (b) doping in solution.

of aniline with a template such as SPS. This is mainly due to inaccessibility of aniline monomers, which electrostatically interact with SPS, to diffuse into the hematin ELBL layers. Therefore, the polymerization rate strongly depends on the degree of hematin desorption which can be controlled through. It was found that the reaction rate was affected by ionic strength of media, amount of hematin, and the type of Pani that is grown

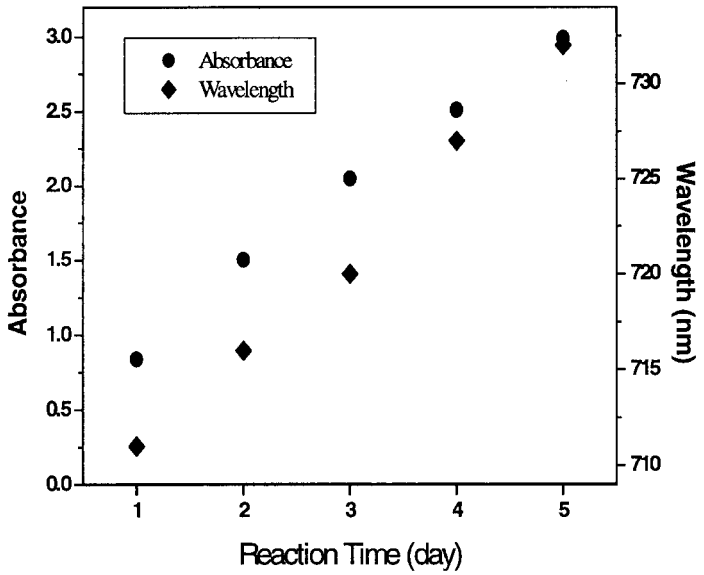


Figure 8. Maximum absorption intensity and λ_{max} of the SPS/Pani complex in solution as a function of reaction time.

on the surface of the films. It is believed that with further optimization, these hematin ELBL nanocomposite films may find wide application as catalysts for the synthesis of various Pani and polyphenols at extremely low or high pH where many enzymes cannot function. In addition, this approach offers a new way to functionalize materials with conductive Pani coatings by simply electrostatically layering hematin onto the surface. Use of these assemblies as potential biosensing devices is also envisioned. Controlling the desorption of hematin from the ELBL assembly and optimizing the polymerizations are subjects of ongoing work.

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This paper is dedicated to the memory of Dr. Sukant Tripathy.

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