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Calcium-Enhanced Polydopamine Coating as a Method to Improve Bacterial Viability and Function in Plant Growth-Promoting Rhizobacteria

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Abstract

Microbial inoculants represent a promising alternative to conventional agricultural practices; however, their effectiveness is often limited by environmental stress and reduced bacterial viability following industrial processing and field application. In this study, we investigate calcium-mediated polydopamine (PDA) coating as a strategy to enhance the stability and functionality of plant growth-promoting rhizobacteria (PGPR). PDA is known for its strong adhesive and antioxidant properties,[4,7] making it a suitable candidate for protecting bacterial cells from reactive oxygen species (ROS)-induced damage. Using UV-Vis spectroscopy, transmission electron microscopy (TEM), and bacterial growth assays, we examine how calcium ions influence PDA polymerization and coating efficiency. We demonstrate that calcium ions significantly accelerate dopamine polymerization,[8] but excessive concentrations lead to uncontrolled aggregation. By systematically optimizing dopamine and calcium concentrations, we identify conditions that promote effective bacterial coating while preserving viability. These findings establish a critical balance between polymerization kinetics and surface-controlled deposition, providing a framework for improving PGPR-based agricultural technologies.

Introduction

Plant Growth-Promoting Rhizobacteria (PGPR) play a central role in sustainable agriculture by enhancing nutrient availability, producing phytohormones, and improving plant tolerance to abiotic stress. Despite their potential, their practical application remains limited due to poor survival in competitive and variable soil environments. Introduced bacterial strains must compete with native microbiota, adapt to fluctuating environmental conditions, and respond to plant root exudates, all of which significantly reduce their colonization efficiency and long-term effectiveness.[1–3]

To address these limitations, protective surface engineering strategies have emerged as a promising approach. Among these, polydopamine (PDA) has attracted considerable attention due to its biocompatibility, strong adhesion to diverse surfaces, and redox activity.[4,5] PDA is formed through the oxidative polymerization of dopamine under alkaline conditions and has been shown to protect biological systems from oxidative stress while maintaining functional integrity.[5,7] Previous studies have also shown that PDA-coated bacteria exhibit enhanced antimicrobial properties, which may help mitigate competition with native microbiota and improve survival.[6]

Recent studies suggest that calcium ions (Ca^{2+}) play a critical role in modulating PDA formation. Calcium ions facilitate dopamine oxidation, stabilize intermediate species, and promote uniform deposition onto biological surfaces.[8] Importantly, Ca^{2+} can act as an interfacial mediator, enhancing adhesion without disrupting membrane-bound receptors, which is essential for maintaining bacterial functionality.[9,10]

In this work, we investigate the role of Ca^{2+} in controlling PDA formation and deposition on bacterial cells. Two model PGPR strains, *Pseudomonas defensor* (Gram-negative) and *Bacillus velezensis* (Gram-positive), were selected to evaluate how bacterial structure influences coating behavior. *Bacillus velezensis* is known for producing bioactive secondary metabolites and forming resilient endospores under adverse conditions,[11] whereas *Pseudomonas defensor* is an efficient root colonizer capable of eliciting plant defense responses.[12] The objective is to identify conditions that enable stable PDA coating while preserving bacterial viability, thereby improving the practical applicability of PGPR systems.

Methods

Bacterial cultures of *P. defensor* and *B. velezensis* were grown statically for 24 hours at 27°C in LB medium. Cells were harvested by centrifugation at 2000 g for 5 minutes, washed three times with phosphate-buffered saline (PBS), and resuspended in 0.85% NaCl. The optical density (OD_{600}) was adjusted to approximately 6.0.

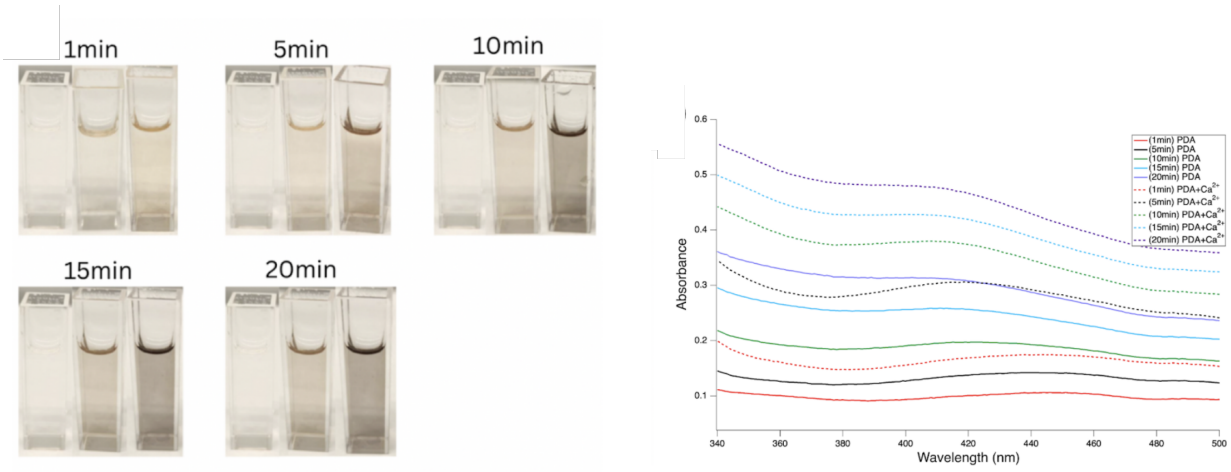
Polydopamine solutions were prepared by dissolving dopamine hydrochloride in 10 mM Tris buffer (pH 8.35). Calcium chloride solutions were prepared separately and combined with dopamine solutions to achieve desired concentrations. UV-Vis spectroscopy was performed at time intervals of 1–20 minutes to monitor polymerization kinetics.

For coating experiments, bacterial suspensions were introduced into reaction mixtures under control (Tris only), PDA-only, and PDA+Ca^{2+} conditions. Samples were incubated for varying durations and collected via centrifugation. Initial centrifugation at high relative centrifugal force (16,639 g) resulted in excessive aggregation; therefore, optimized conditions using 3000 g were employed to preferentially isolate bacterial pellets while minimizing PDA precipitation.

Transmission electron microscopy (TEM) was used to visualize bacterial morphology and aggregation behavior. Samples were prepared on formvar-coated grids, fixed with glutaraldehyde, stained with phosphotungstic acid, and imaged using a Hitachi H7700 microscope. Bacterial viability was assessed using growth curve analysis (OD_{600}) over 24 hours.

Results and Discussion

The role of calcium ions in PDA formation was first evaluated using UV-Vis spectroscopy and direct visual inspection of reaction mixtures. As shown in Figure 1, the presence of Ca^{2+} resulted in a noticeably faster darkening of the dopamine-containing solutions and a corresponding increase in absorbance around 410 nm. Together, the photographic evidence in Figure 1a and the spectral data in Figure 1b indicate that calcium accelerates the oxidative polymerization of dopamine into PDA.[8,13] This behavior is consistent with the proposed role of Ca^{2+} in facilitating catechol oxidation and stabilizing intermediates during PDA formation.[8]



(a) (b)

Figure 1: UV-Vis spectroscopy and visual observations of polydopamine (PDA) formation with and without calcium ions. (a) Representative photographs of PDA solutions acquired at different time points using 2 mg/mL dopamine under various conditions: in deionized water (left), Tris buffer (middle), and Tris buffer supplemented with Ca^{2+} (right). (b) UV-Vis spectra of PDA samples polymerized in the presence or absence of Ca^{2+}.

Although calcium enhanced PDA formation, the initial coating conditions revealed a major limitation of the system. When high concentrations of dopamine and Ca^{2+} were used, centrifugation produced large dark pellets that were visually distinct from bacterial control pellets. Figure 2 shows that pellet size and darkness increased with reaction time, indicating substantial over-aggregation. Rather than promoting uniform surface deposition, these conditions favored bulk PDA self-association. This observation suggested that faster polymerization did not automatically produce better bacterial coatings and that reaction conditions had to be tuned carefully to separate true surface coating from uncontrolled aggregate formation.

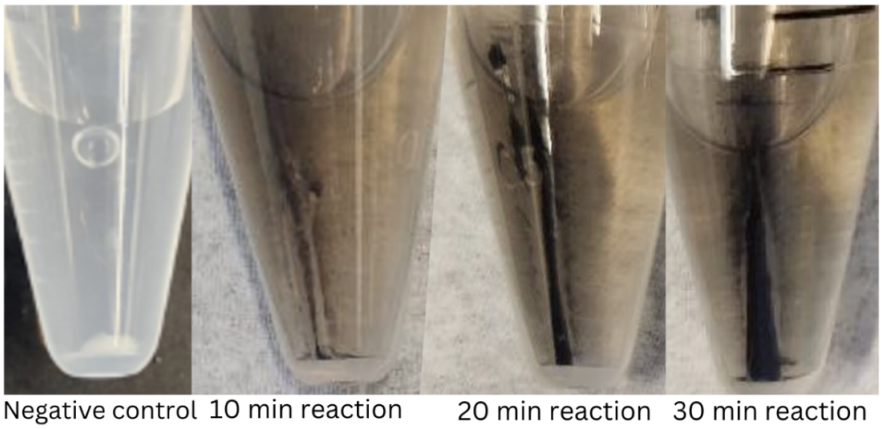


Figure 2: Comparison of pellet formation after centrifugation at 16,639 g for 10 minutes across varying reaction times (10, 20, and 30 minutes) in samples containing 2 mg/mL PDA and 100 mM Ca^{2+}, alongside a negative control (bacteria-only) shown in the left tube. The negative control shows minimal pellet formation, whereas samples containing PDA and calcium ions exhibit increasingly large pellets and pronounced aggregation with longer reaction times.

TEM imaging provided further evidence that aggregation dominated under these initial conditions. As shown in Figure 3, untreated bacterial cells retained the expected rod-like morphology, with *Pseudomonas defensor* and *Bacillus velezensis* displaying dimensions consistent with their known structures. In contrast, samples prepared in the presence of PDA and calcium were dominated by dense amorphous material, with little or no clearly discernible bacterial surface. The TEM data therefore support the conclusion drawn from pellet observations: the early coating conditions generated substantial free PDA aggregates. At the same time, the TEM images also exposed an experimental limitation, namely overstaining and possible sample damage caused during handling, which made it difficult to definitively visualize a thin surface coating. Even so, the images clearly established that aggregation was a central obstacle to coating optimization.

In addition, the structural distinction between Gram-positive and Gram-negative bacteria is relevant when interpreting coating behavior. The thick peptidoglycan wall of Gram-positive bacteria and the outer membrane of Gram-negative bacteria create different interfacial environments for deposition, making *P. defensor* a useful model for coatings intended to resemble eukaryotic cell-surface behavior.[10,14]

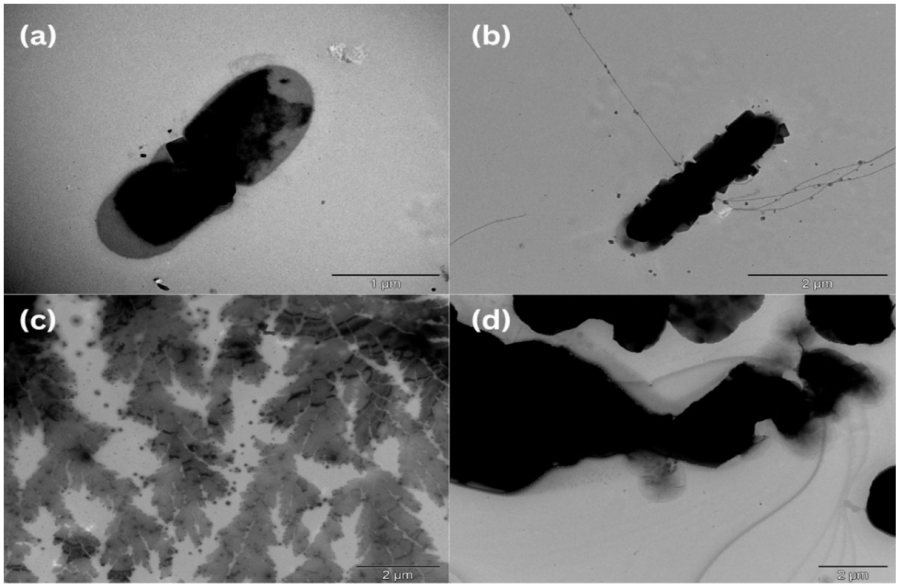


Figure 3: Transmission electron microscopy (TEM) images illustrating bacterial morphology and the effect of PDA and calcium ions on the coating process. (a) Rod-shaped *Pseudomonas defensor* cells with lengths of approximately 2.5–3.0 µm and diameters of 0.7–1.0 µm. (b) Rod-shaped *Bacillus velezensis* cells measuring approximately 2.0–2.5 µm in length and 0.6–0.8 µm in diameter, with visible flagella. (c) PDA aggregates in samples containing *P. defensor*, showing dense amorphous morphology with no clearly discernible bacterial cells. (d) PDA aggregates in samples containing *B. velezensis*, likewise dominated by large irregular PDA formations with limited visible bacterial structure.

To reduce aggregation and recover conditions more favorable for true bacterial coating, both centrifugation force and reagent concentrations were optimized. High-speed centrifugation was replaced with 3000 g in order to reduce co-precipitation of free PDA. After this procedural adjustment, dopamine and calcium concentrations were systematically lowered. The results of this screening are summarized in Figure 4. Distinct dark pellet formation was observed most clearly at 0.5 mg/mL PDA, indicating that this concentration was sufficient to support coating-related deposition, whereas lower dopamine concentrations produced much weaker responses. This result suggested the existence of a practical threshold concentration for detectable coating under the experimental conditions used here.

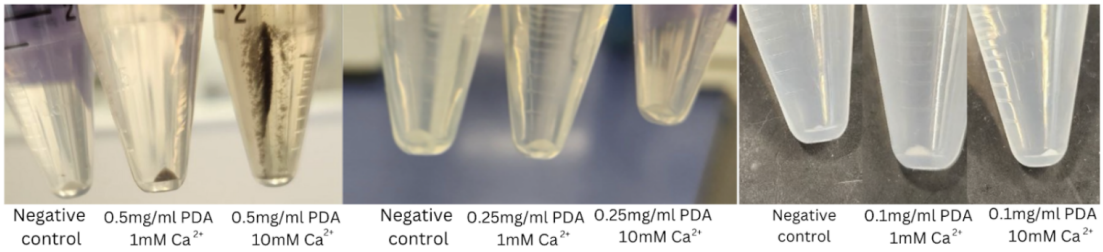
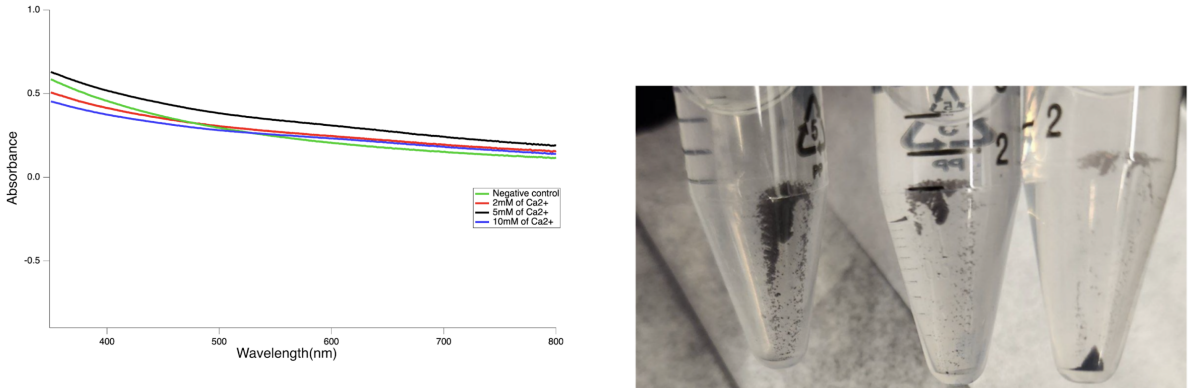


Figure 4: Centrifugation results illustrating the effect of varying dopamine (PDA) concentrations (0.1, 0.25, and 0.5 mg/mL) and calcium ion concentrations (1 and 10 mM Ca^{2+}) on pellet formation. Negative controls are included for comparison. Distinct dark pellet formation was observed most clearly at 0.5 mg/mL PDA, supporting this concentration as the most promising for subsequent coating optimization.

Having identified 0.5 mg/mL dopamine as the most promising concentration, the calcium dependence of the coating process was examined in greater detail. Figure 5 shows that calcium concentration had a non-monotonic effect on coating quality. At 2 mM Ca^{2+}, pellet formation and spectral broadening were relatively weak, suggesting limited PDA retention on the bacterial surface. At 10 mM Ca^{2+}, aggregation again became prominent, indicating that excessive calcium drove the system toward bulk polymer formation rather than controlled deposition. In contrast, 5 mM Ca^{2+} produced the most favorable combination of pellet morphology and post-wash UV-Vis behavior. The broader absorbance profile and compact dark pellet at this condition are consistent with more effective PDA association while minimizing bacterial loss and excessive aggregation.[13] These observations identify 0.5 mg/mL dopamine with 5 mM Ca^{2+} as the optimal condition among those tested.



(a) (b)

Figure 5: Optimization of calcium concentration for PDA coating. (a) UV-Vis absorbance spectra for samples treated with 0 mM (negative control), 2 mM, 5 mM, and 10 mM Ca^{2+}. The 5 mM condition shows the broadest spectrum, consistent with optimal PDA coating and reduced bacterial loss. The 10 mM condition reflects excessive PDA aggregation, whereas the 2 mM condition indicates weaker coating efficiency. (b) Pellets formed after washing bacteria coated with PDA in the presence of varying Ca^{2+} concentrations. The 5 mM Ca^{2+} sample exhibits a compact dark pellet indicative of effective coating, whereas 2 mM shows weaker attachment and 10 mM shows excessive aggregation.

The final question was whether the optimized coating conditions preserved bacterial viability. Growth curve analysis showed that bacteria treated under the optimal condition retained the capacity for robust growth after washing. As shown in Figure 6, the growth profile of the 5 mM Ca^{2+} sample closely tracked that of the positive control, whereas the negative control showed no growth. This result is particularly important because it demonstrates that the optimized coating strategy does not merely reduce aggregation; it also maintains biological function. The combined evidence from Figures 4–6 therefore supports the conclusion that effective coating requires balancing two competing processes: sufficient calcium to accelerate surface-associated PDA formation, but not so much that bulk aggregation dominates.

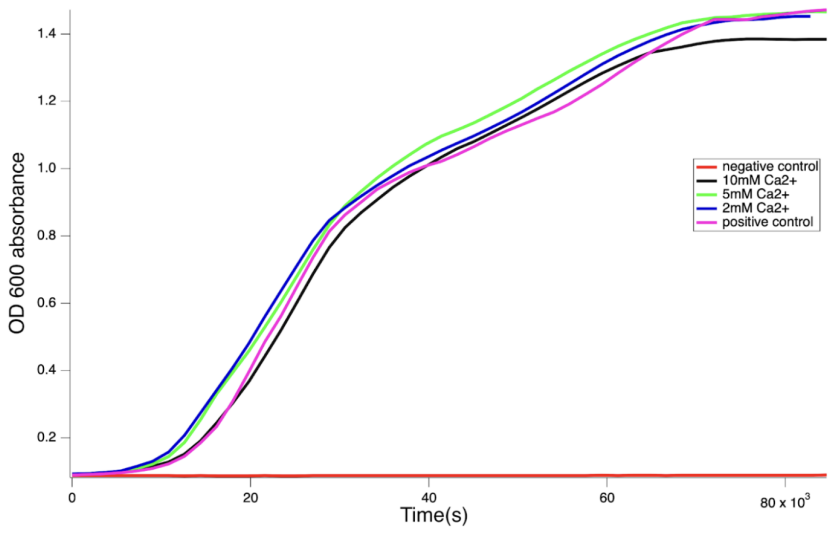


Figure 6: Growth curves of *Pseudomonas defensor*, monitored by OD_{600} over time under different coating conditions: 2 mM Ca^{2+}, 5 mM Ca^{2+}, 10 mM Ca^{2+}, negative control, and positive control. The 5 mM Ca^{2+} condition most closely mirrors the positive control, indicating that bacteria remained viable after coating and washing, whereas the negative control showed no growth.

Taken together, these results reveal a key trade-off governing PDA coating: increasing calcium concentration enhances polymerization kinetics but also promotes aggregation, while insufficient calcium results in weak coating. Effective surface engineering therefore requires precise control of reaction conditions so that polymerization remains sufficiently rapid to support deposition, yet sufficiently constrained to prevent the formation of large free aggregates.

Conclusion

This study demonstrates that calcium ions play a dual role in polydopamine-based bacterial coating, simultaneously enhancing polymerization and promoting aggregation. By systematically optimizing reaction conditions, we identified an operating regime in which effective PDA deposition could be achieved while maintaining bacterial viability. Among the conditions tested, 0.5 mg/mL dopamine and 5 mM Ca^{2+} provided the most favorable balance between coating efficiency and aggregate suppression. These findings provide a mechanistic basis for understanding calcium-mediated PDA formation on bacterial systems and establish design principles for improving PGPR stability in agricultural applications.

Future work should focus on directly visualizing the PDA coating using improved TEM staining protocols, quantifying coating thickness, and evaluating the performance of coated

bacteria in plant systems. Additional studies comparing Gram-negative and Gram-positive bacterial surfaces in greater detail would also clarify how cell-envelope architecture influences coating behavior and long-term functionality.

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Supporting Information for Calcium-Enhanced Polydopamine Coating as a Method to Improve Bacterial Viability and Function in Plant Growth-Promoting Rhizobacteria

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Characterization

The oxidative chemical synthesis of polydopamine (PDA) involves a complex series of multi-step processes, including redox reactions, cyclization, polymerization, and cleavage reactions, all of which contribute to the resulting composition of PDA films (Scheme 1). Among the key steps, the rate-limiting reaction is the one-electron oxidation of deprotonated catechol by molecular oxygen, leading to the formation of dopamine-semiquinone and superoxide radicals.

Calcium ions (Ca^{2+}) have been shown to significantly accelerate this process. Studies indicate that Ca^{2+} promotes the deprotonation of catechol groups, thereby facilitating their oxidation and enhancing oxygen consumption during PDA formation. Moreover, Ca^{2+} ions associate with and stabilize semiquinone radicals, further accelerating the polymerization process and influencing the final composition of PDA films.

This Ca^{2+}-mediated acceleration underscores its role in tuning oxidative pathways and improving the efficiency of PDA synthesis, making it a promising approach for surface engineering applications.[1]

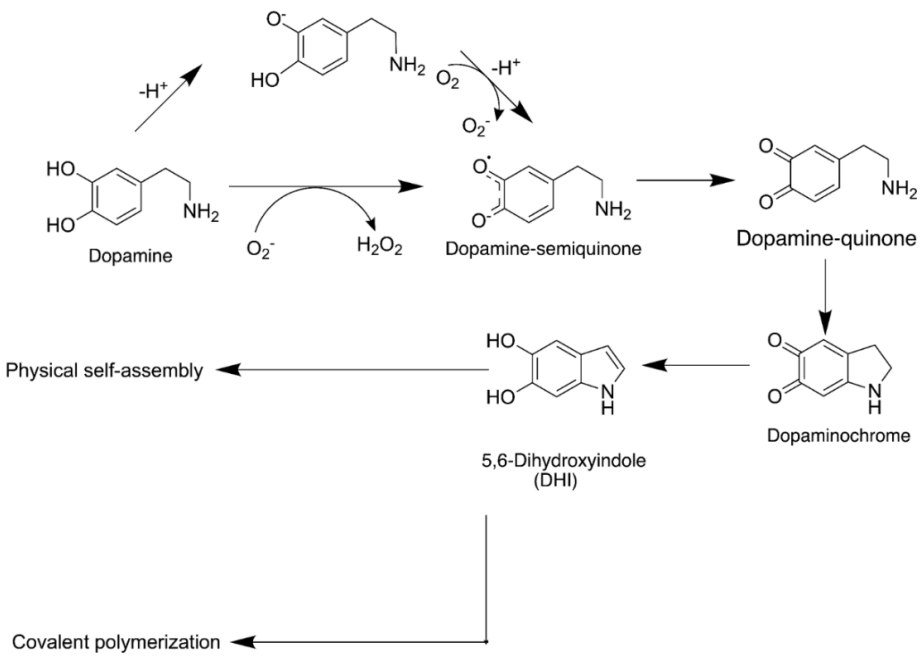

Figure 7: Mechanistic pathway for the oxidative synthesis of polydopamine (PDA) from dopamine under alkaline conditions.[1,2]

Spectra

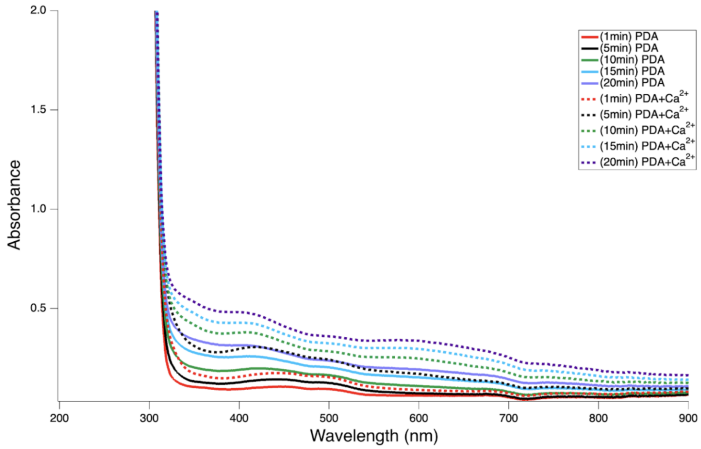

Figure 8: UV-Vis spectra of PDA formation in Tris buffer with and without calcium ions (Ca^{2+}) over time. Solid lines represent PDA formation in Tris buffer at 1, 5, 10, 15, and 20-minute intervals, while dashed lines show PDA formation in Tris buffer supplemented with Ca^{2+} under the same time intervals. The presence of calcium ions accelerates dopamine polymerization, as indicated by higher absorbance and spectral shifts in the PDA+Ca^{2+} samples compared to PDA-only samples.

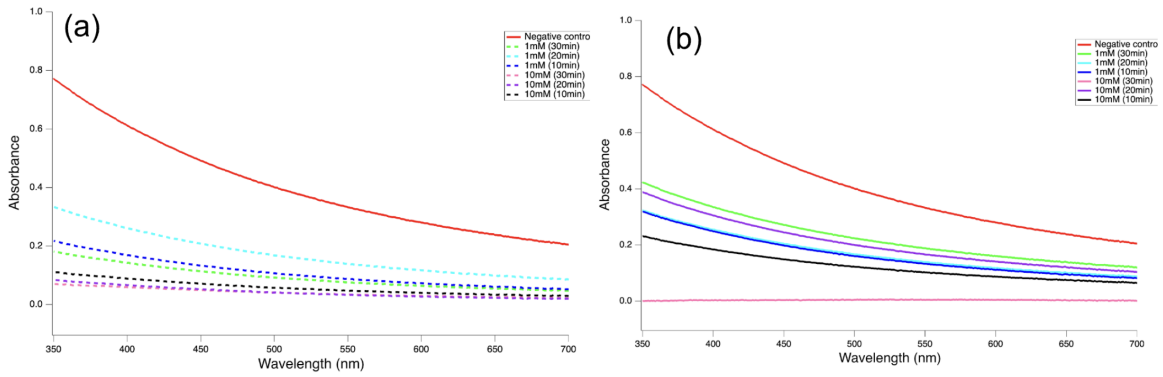

Figure 9: UV-Vis spectra of (a) two washes (dashed lines) and (b) one wash (solid lines) on 0.25 mg/mL PDA-coated samples treated with 10 mM and 1 mM calcium ions after 10, 20, and 30 minutes. The absence of a peak around 410 nm indicates minimal PDA coating. Tighter spectra compared to the positive control suggest bacterial loss during washing, particularly after two washes.

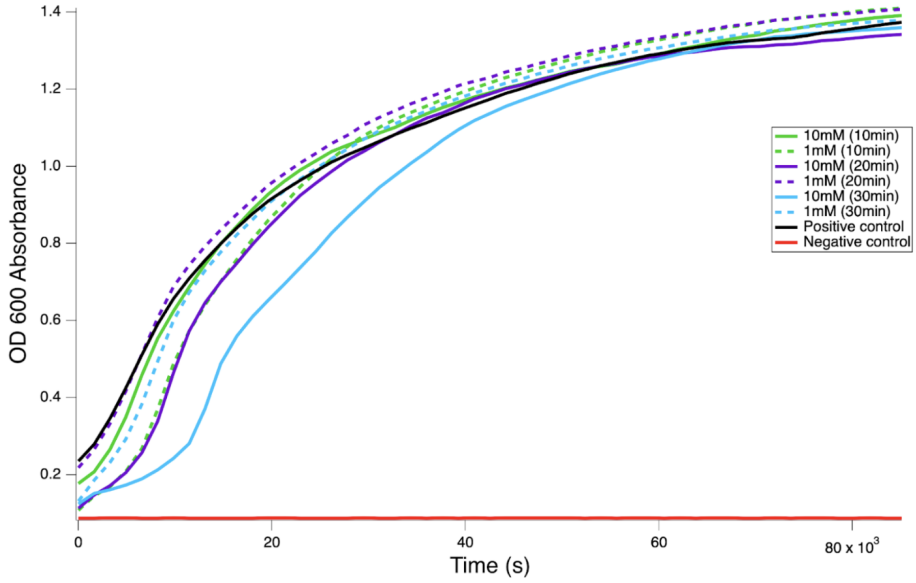

Figure 10: Growth curves of bacterial cultures treated with 0.1 mg/mL PDA under two calcium ion concentrations (1 mM and 10 mM) and three reaction times (10, 20, 30 minutes). Solid lines represent 10 mM Ca^{2+}, dashed lines represent 1 mM Ca^{2+}. Positive (black) and negative (red) controls are included. No significant differences in bacterial growth were observed.

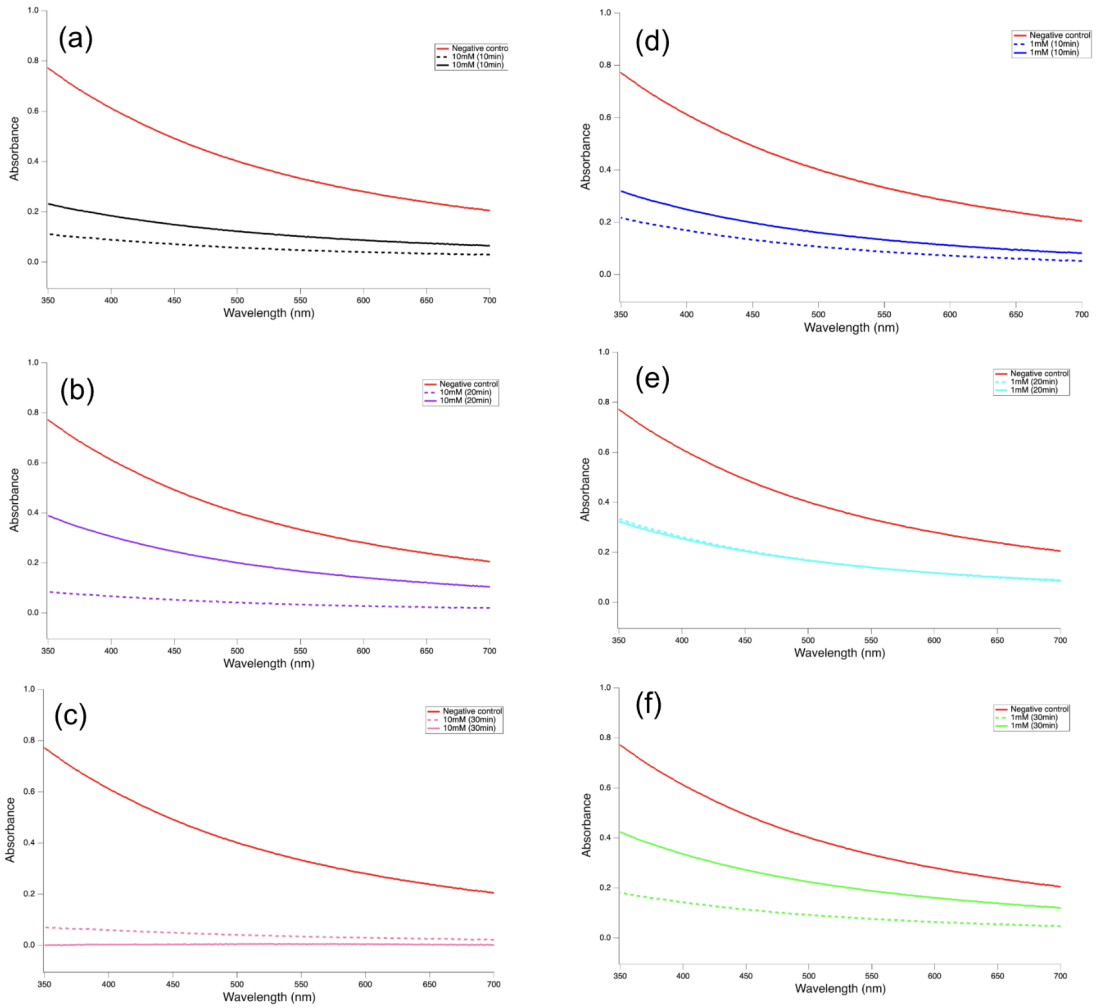

Figure 11: UV-Vis absorption spectra comparing the effects of first and second washes of 0.25 mg/mL PDA at varying Ca^{2+} concentrations (1 mM and 10 mM) and reaction times (10, 20, 30 minutes). Dashed lines represent second wash; solid lines represent first wash. Reduced absorbance and absence of a peak near 410 nm indicate minimal PDA coating and progressive disassembly during washing.[3]

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