Chempublish Journal
Journal Homepage: online-journal.unja.ac.id/chp
Department of Chemistry
Faculty of Science and Technology
Universitas Jambi
ISSN (Online): ISSN 2503-4588
No. 23/E/KPT/2019

Article

Behaviour of SS-316L Hydroxyapatite Coated in Simulated Body Fluids

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Abstract

Hydroxyapatite (HA) is a calcium phosphate mineral that closely resembles the inorganic component of natural bone. The incorporation of polycaprolactone (PCL) into HA enhances its mechanical strength, flexibility, and bioresorbability, producing composites with excellent biocompatibility and bioactivity in simulated body fluid (SBF). This study investigates the bioactivity and degradation behaviour of HA/PCL coatings on SS 316L stainless steel substrates. The relationships among coating thickness, shear strength, crystallinity, and pH variation in SBF were systematically examined. HA/PCL coatings were prepared using the dip-coating method and immersed in SBF at 37 °C for 7, 14, 21, and 28 days. Crystallinity and degradation characteristics were analysed using X-ray diffraction (XRD) and weight loss measurements. The results showed that HA/PCL-coated SS 316L exhibited noticeable weight loss after seven days of immersion due to Ca^{2+} ion release from the composite. Extended immersion led to increased HA crystallinity, indicating continued apatite formation and confirming the coating's bioactive and biocompatible nature. Overall, the HA/PCL composite coating effectively enhances the bioactivity and provides controlled degradation of metallic implants, demonstrating strong potential for orthopaedic and dental biomedical applications.

Keywords: *HA/PCL coating, Hydroxyapatite, Polycaprolactone, Simulated Body Fluid, SS 316L*

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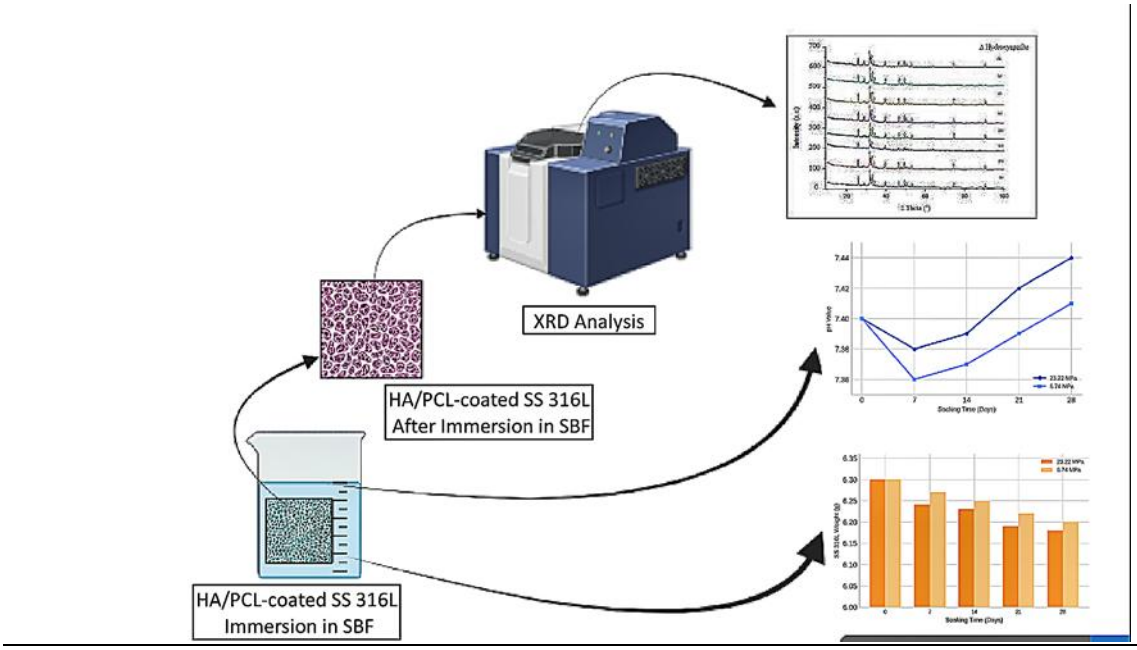
DOI: https://doi.org/10.22437/chp.v9i2.44633
Received May 30th 2025; **Accepted** October 13rd 2025; **Available online** November 25th 2025

Graphical Abstract



Introduction

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a bioceramic material that closely resembles the mineral component of natural bone in both chemical composition and crystal structure. Owing to its excellent biocompatibility, bioactivity, and osteoconductivity, HA has been widely employed as a coating material for metallic implants to enhance osseointegration and bone regeneration [1,2]. When deposited onto metallic substrates such as stainless steel, titanium, or magnesium alloys, HA establishes a strong chemical and mechanical bond at the implant–tissue interface, thereby improving fixation and long-term stability [3].

Despite these advantages, pure HA is inherently brittle and exhibits poor mechanical strength, which can lead to coating delamination or fracture under physiological loading conditions [4]. To overcome these shortcomings, researchers have developed composite coatings that integrate HA with biodegradable polymers such as polycaprolactone (PCL). The incorporation of PCL enhances the coating's flexibility and adhesion to the metallic substrate, while also allowing for gradual degradation and controlled ion release during the healing process [5,6]. Such hybrid HA/PCL coatings combine the mechanical resilience of polymers with the biological functionality of ceramics, making them promising for orthopaedic applications.

The bioactivity of these coatings is typically evaluated *in vitro* using simulated body fluid (SBF), which replicates the ionic composition of human plasma [6]. According to Kokubo and Takadama [7], the ability of a material to induce the formation of a bone-like apatite layer on its surface in SBF strongly correlates with its *in vivo* bone-bonding potential [8]. During immersion, the process of apatite nucleation, crystal growth, and transformation into stable HA phases serves as an indicator of surface reactivity and degradation stability [9].

Recent studies have shown that several parameters, including SBF pH, ion concentration, and coating layer thickness, significantly impact the degradation kinetics

and bioactivity of HA/PCL coatings [10-12]. In particular, coating thickness plays a dual role: a thicker layer generally limits ion diffusion and slows degradation, whereas a porous and thinner layer enhances Ca^{2+} and PO_4^{3-} ion exchange, promoting faster apatite formation [6]. Although these findings highlight the importance of coating design, the precise correlation between HA/PCL coating thickness, structural integrity, and degradation behaviour remains insufficiently understood, especially for stainless steel 316L (SS 316L) substrates that are widely used in orthopaedic implants.

Therefore, this study aims to elucidate the relationship between HA/PCL coating thickness and degradation behaviour in SBF. Specifically, it investigates (i) the correlation between coating thickness, shear strength, and crystallinity; (ii) the influence of immersion time on pH variation and degradation rate; and (iii) the formation and evolution of apatite layers on HA/PCL-coated SS 316L surfaces. The outcomes are expected to provide new insights into the physicochemical interactions governing HA/PCL coatings in physiological environments and to guide the design of next-generation bioactive coatings for durable and functional orthopaedic implants

Materials and Methods

Materials

The AISI 316L stainless steel was selected as the substrate due to its high corrosion resistance, mechanical stability, and widespread use in biomedical implants. Hydroxyapatite (HA) was synthesised using chicken eggshells as a calcium precursor, providing a sustainable and cost-effective source of calcium. Polycaprolactone (PCL), a biodegradable aliphatic polyester known for its flexibility and bioresorbability, was employed as a polymeric binder and matrix material [11]. All chemicals used were of analytical grade and utilised without further purification.

Preparation of Hydroxyapatite and SBF.

Hydroxyapatite powder was synthesized [6], and simulated body fluid (SBF) was prepared by sequentially dissolving reagent-grade salts in distilled water maintained at 36.5 ± 1.5 °C under continuous stirring at 300 rpm. The pH of the solution was adjusted to 7.40 ± 0.01 using hydrochloric acid (HCl) to replicate the ionic conditions of human plasma. The final SBF composition was confirmed to match that reported by Kokubo and Takadama [7], and the solution was stored at 20 °C before use in immersion experiments.

HA/PCL Coating Process

The HA/PCL coatings were applied onto SS 316L substrates using the dip-coating technique [6]. The coating thickness was controlled by adjusting both the immersion duration and withdrawal speed of the substrates. Following coating, the samples were air-dried at room temperature to minimize potential thermal degradation of the polymer matrix. The selection of experimental parameters was guided by the procedures described in Kokubo's Bioactive Glass Ceramics: Properties and Applications (1991) [12]. The primary variables investigated in this study included coating shear strength, layer thickness, and immersion duration in simulated body fluid (SBF). All other experimental conditions-such as solution composition and temperature-were maintained constant throughout the experiments.

HA/PCL Soaking Testing of HA/PCL in SBF.

The degradation and bioactivity of the HA/PCL-coated samples were assessed following ISO/FDIS 23317 guidelines and the

procedure established by Kokubo and Takadama [7]. The coated samples were immersed in 80 mL of SBF at 37 °C for 7, 14, 21, and 28 days to simulate physiological conditions [8]. During the immersion period, the pH of the SBF solution was periodically monitored using a calibrated pH meter to detect ionic exchanges and potential degradation processes. A schematic flowchart summarising the SBF synthesis and immersion testing procedure is presented in Figure 1.

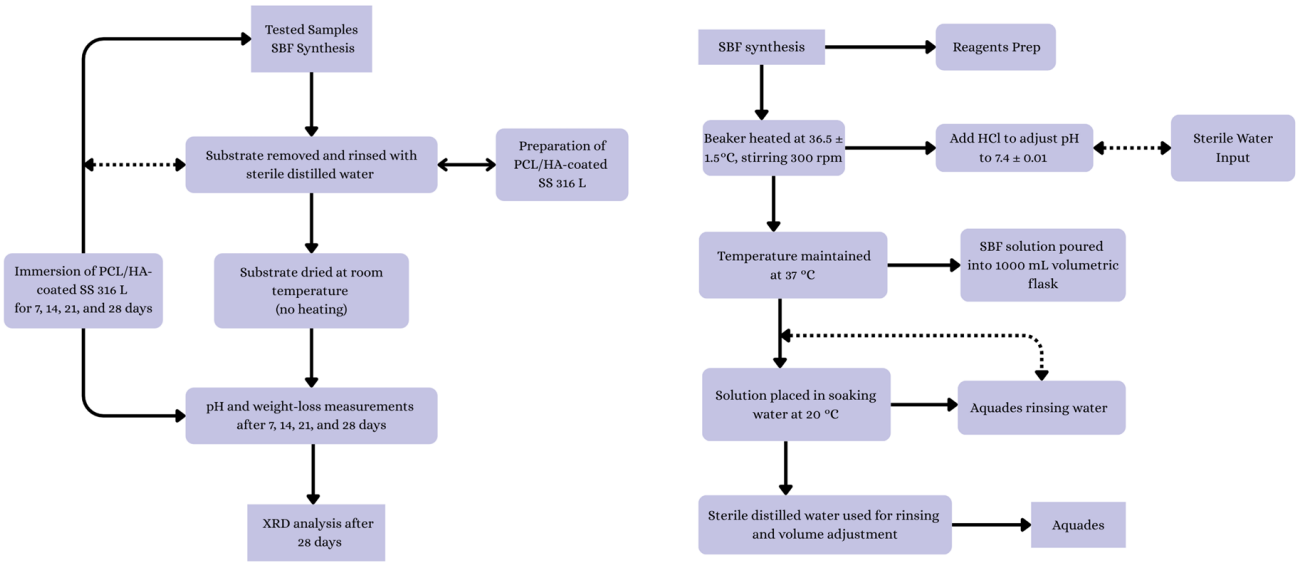


Figure 1. Flow diagram of the HA/PCL-coated SS 316L immersion process and simulated body fluid (SBF) synthesis steps.

XRD and Weight Loss Analysis

After each immersion interval, the samples were removed from SBF, rinsed with distilled water, and dried at room temperature. X-ray diffraction (XRD) was performed to identify crystalline phases and evaluate changes in the degree of crystallinity of the HA/PCL coatings. The weight loss of each sample was recorded before and after immersion to determine the degradation rate according to equation 1.

$$\text{Weight Loss } (\%) = \frac{\text{Wo} - \text{Wt}}{\text{Wo}} \ 100\% \qquad (1)$$

Where Wo and W_t represent the sample weights before and after immersion, respectively. All measurements were repeated three times to ensure reproducibility, and the mean values were used for analysis.

After immersion in SBF for 7, 14, 21, and 28 days, the samples were dried and analysed using X-ray diffraction (XRD) to identify the phase structure and crystallinity of the formed HA layer. The pH of the SBF solution was measured periodically using a calibrated pH meter to monitor ion exchange and potential degradation of the coating [13].

Result and Discussion

X-Ray Diffraction (XRD) Analysis

The XRD analysis was performed to evaluate biocompatibility and apatite formation on HA/PCL-coated SS 316L samples after 28 days of immersion in SBF (Figure 2).

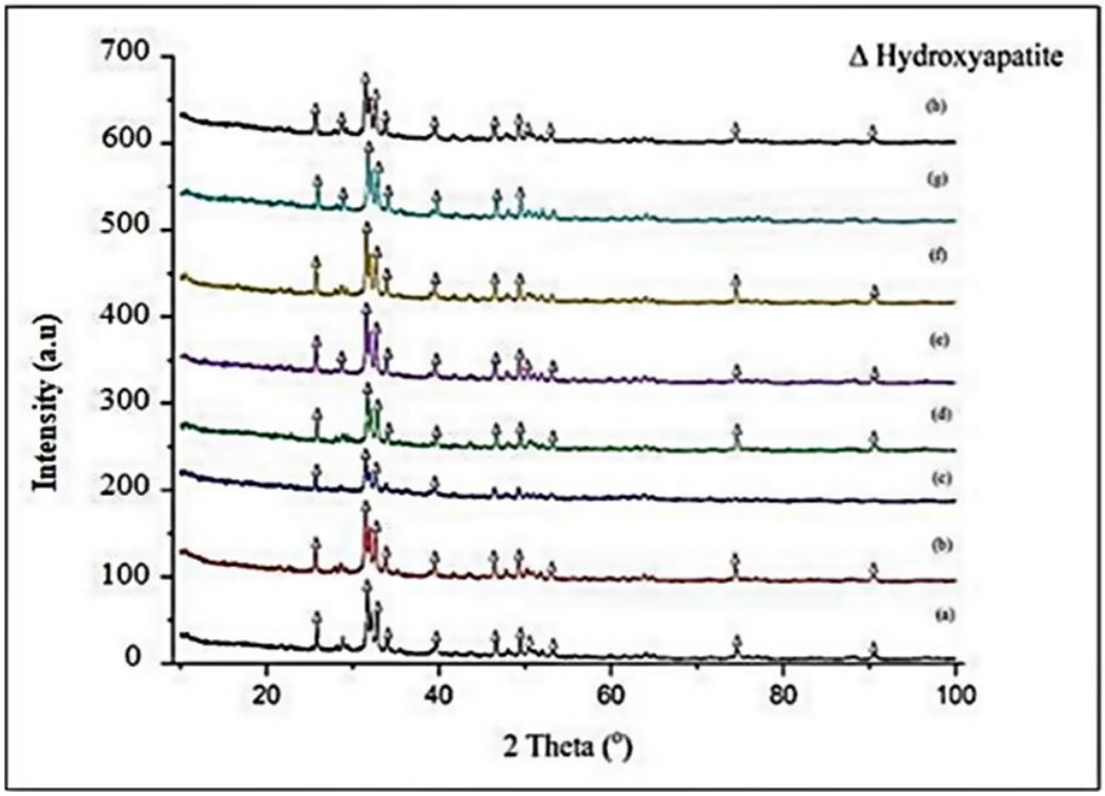


Figure 2. XRD patterns of HA/PCL-coated SS 316L specimens after immersion in SBF, corresponding to shear strength values of (a) 1.45 MPa, (b) 1.95 MPa, (c) 1.99 MPa, (d) 2.22 MPa, (e) 6.74 MPa, (f) 23.22 MPa, (g) 24.17 MPa, and (h) 25.79 MPa.

The highest peak intensity appeared in sample (f) at 750.98 a.u. with a shear strength of 23.22 MPa, while the lowest was in sample (c) at 362.79 a.u. with 1.99 MPa. The peaks corresponded to ICDD card No. 01-072-1243 for hydroxyapatite, confirming the formation of a biocompatible HA phase with a well-ordered crystal structure. Polycaprolactone (PCL) peaks were not detected, as PCL primarily acts as an adhesive polymer [14]. During immersion, an ion-exchange process occurred between the coating and the SBF medium, in which Ca^{2+} ions were released from the coating into the solution, increasing the local calcium concentration [15]. These Ca^{2+} ions, derived from $Ca(OH)_2$, reacted with PO_4^{3-} to form apatite, while OH^- ions from hydroxyapatite promoted further apatite bonding throughout the immersion period. Sharma et al. (2019) also reported that Ca^{2+} ion release facilitated apatite nucleation and crystal growth in PCL/HA composites [16]. In the present study, the crystallinity increased up to 86.8%, which falls within the typical range for hydroxyapatite coatings (60–90%) [14]. The increase in crystallinity correlated positively with adhesion strength, indicating improved coating stability and biocompatibility.

Apatite Formation Within Simulated Boy Fluids.

The formation of bone-like apatite on the coating surface followed a two-stage mechanism: nucleation and crystal growth. In the early stage, the hydroxyl (OH^-) functional groups on the coating surface served as nucleation sites, attracting Ca^{2+} and PO_4^{3-} ions from the SBF. As immersion continued, these nuclei grew into a dense

and continuous apatite layer [16]. Hydroxyl (OH^-) groups at the surface attracted Ca^{2+} and PO_4^{3-} ions. Their bonding formed apatite, which thickened as immersion proceeded. The progressive thickening of the apatite layer indicates enhanced bioactivity of the HA/PCL-coated SS 316L. This process is consistent with the general model of apatite formation proposed by Kokubo and Takadama [7] and further elaborated by Dridi et al. (2021), who described the transformation from amorphous calcium phosphate (ACP) to crystalline hydroxyapatite with increasing immersion time [17]. Therefore, the observed evolution of crystallinity and surface mineralisation behaviour confirms the coating's ability to support in vitro apatite formation, a key indicator of bioactivity and osteointegration potential [16].

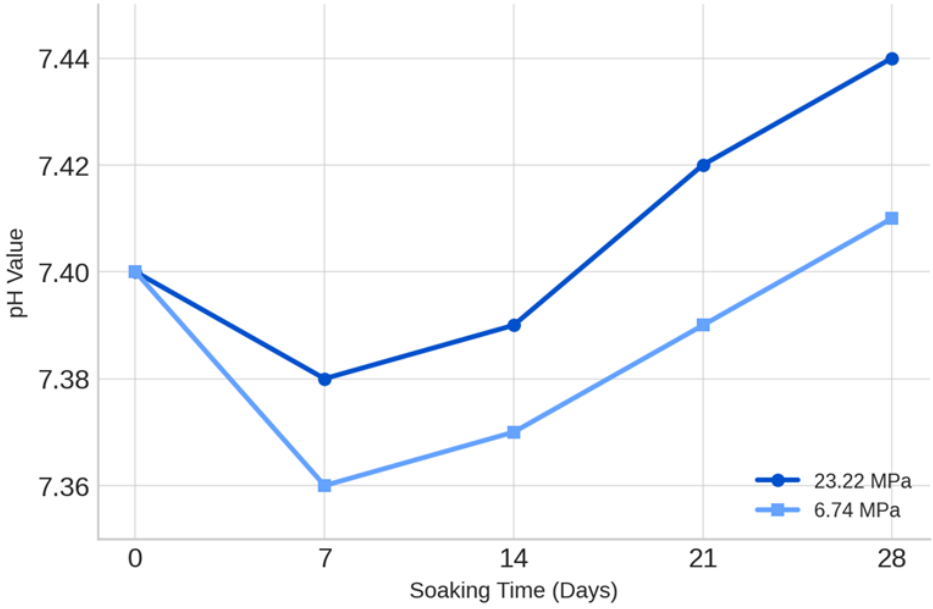


Figure 3. XRD values of HA/PCL-coated samples with different shear strength values: (a) 1.45 MPa, (b) 1.95 MPa, (c) 1.99 MPa, (d) 2.22 MPa, (e) 6.74 MPa, (f) 23.22 MPa, (g) 24.17 MPa, and (h) 25.79 MPa, after immersion in simulated body fluid (SBF).

The Effect of pH Variation During Immersion.

The pH of the SBF solution serves as an indirect indicator of the ionic exchange between the coating and the surrounding medium. As shown in Figure 3, for coating thicknesses of 123.6 µm and 102.6 µm, a slight decrease in pH was observed during the first seven days of immersion, attributed to the release of Ca^{2+} and PO_4^{3-} ions into the solution. After approximately 14 days, the pH stabilized, indicating a dynamic equilibrium between ion release and apatite precipitation. This behavior suggests that the degradation and reprecipitation processes occur simultaneously, promoting the formation of a stable apatite layer on the coating surface. The initial decrease in pH reflects partial dissolution of the HA/PCL composite, while the subsequent stabilization demonstrates the coating's chemical balance and capacity to maintain physiological pH—an essential factor for biocompatibility [16,17].

The Weight Loss and Degradation

The degradation behaviour of the HA/PCL coatings was further evaluated through weight loss measurements after immersion in SBF for 7, 14, 21, and 28 days. As

presented in Figure 4, significant weight loss was recorded after seven days, with the thicker coating (123.6 µm). It was exhibited a weight loss of 0.71%, while the thinner coating (102.6 µm) showed 0.43%. The degradation rate gradually decreased with prolonged immersion, reaching 0.43% and 0.32%, respectively, after 28 days. This trend indicates that degradation slows as a stable apatite layer forms on the surface, reducing further ion exchange between the coating and the SBF. The morphology suggests that the porosity of the PCL matrix influences the degradation rate by modulating the diffusion of ions. Limited ion exchange results in slower weight reduction, confirming that layer thickness and coating density are critical parameters controlling the effective degradation rate [6,18]. These results align with observations by Mehdizade et al., who reported that thicker bioceramic layers in Mg–HA composites exhibited lower degradation rates and enhanced mechanical stability [19]

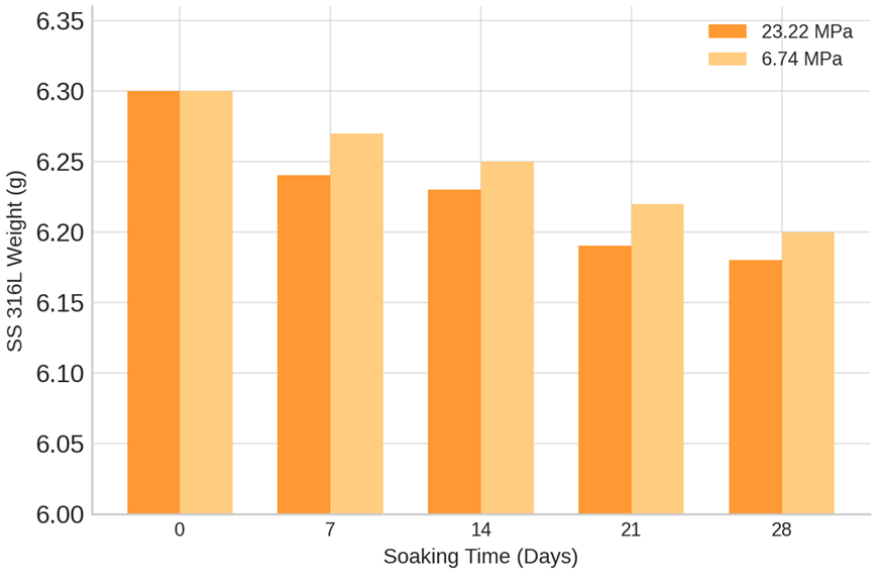


Figure 4. Weight variation of HA/PCL-coated SS 316L samples with 123.6 mM and 102.6 mM ion concentrations after immersion in SBF

Conclusions

This study confirms that the structural and mechanical characteristics of HA/PCL coatings on SS 316L significantly influence their degradation behavior and bioactivity in simulated body fluid (SBF). The coating shear strength (6.74–23.22 MPa) and thickness (102.6–123.6 µm) were found to have a direct influence on pH stability and ionic exchange, leading to a controlled release of Ca2+ ions, as evidenced by the gradual weight loss over the 28 days. The reduction in degradation rate indicates the formation of a protective apatite layer that contributes to long-term coating stability. A notable increase in hydroxyapatite crystallinity, reaching 86.8% confirms enhanced apatite nucleation and surface bioactivity.

Acknowledgement

This research is supported by the University of Riau, Chemical Engineering Department. We extend our sincere appreciation to the University of Riau, Chemical Engineering Department for providing facilities, resources, and technical assistance essential to the completion of this study. We would like to express our special thanks are also extended to Muhammad Ghazy Fernandes

for preparing and providing the coating samples used in this study. This research would not have been successfully completed without the collective effort of all involved.

Author Contributions

Conceptualization, YMS and YDNBB.; Methodology, YMS and YDNBB; Software, YMS and YDNBB.; Validation: AF and SH.; Formal Analysis, YMS and YDNBB.; Investigation, YMS and YDNBB.; Resources, YMS and YDNBB.; Data Curation, YMS and YDNBB.; Writing – Original Draft Preparation, YMS and YDNBB, ; Writing – Review & Editing, YMS and YDNBB, AF, SH; Visualization: YMS and YDNBB.; Supervision, AF and SH; Project Administration, AF.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Standards

This article does not contain any studies involving human or animal subjects.

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