Characterization of Bacterial Cellulose (Nata De Coco)/Pva Composite for Drug Delivery Application

Ian Paolo I. Azusano, Raymund Angelo C. Memije, Lemmuel L. Tayo

Abstract: ***Numerous innovations in the field of biomedical engineering include the use of synthetic polymers as a drug carrier for drug delivery systems. In this study, polyvinyl alcohol (PVA), with its properties of being a biocompatible, non-toxic, non-carcinogenic polymer, were infused into nata de coco, a bacterial cellulose, by immersing nata de coco slabs into PVA solutions to investigate its capability as a component in drug delivery systems. Different PVA concentrations were prepared to see any effect to the bacterial cellulose. The composites were characterized by analyzing the functional groups present, water uptake capacity and kinetics of methylene blue release. Effect of varying PVA concentration was not seen due to inconsistent amount of pure bacterial cellulose and water on tested samples. Study on the kinetics of methylene blue, modeled as a drug, loaded into the nata de coco/PVA composite was analyzed using Power Law Model. Results showed that the governing diffusion mechanism involved in the release of methylene blue from the composite samples were mainly 'non-Fickian (anomalous). Tests performed, confirmed the capability of PVA to reinforce the bacterial cellulose matrix. Results showed that composites with high amount of PVA in their matrix released smaller amounts of methylene blue. Results showed that composites with high amount of PVA in their matrix released smaller amounts of methylene blue. PVA absorbed less water as the hydroxyl groups of BC were bonded to PVA PVA may have changed the morphological structure of bacterial cellulose affecting the diffusion mechanism of the methylene blue release. Findings of this study, based on nata de coco/PVA composites can be used for future studies of drug delivery systems.***

I. INTRODUCTION

Throughout the years in the field of biomedical engineering, continuous research for innovative methods of treating patients with different diseases have been one of the major concerns. The vital component being studied in this field is the method of drug delivery. In the study of drug delivery knowledge on manipulation of drug release profile, as well as the drug elimination for the increase in efficiency of drugs and safety of the patients is essential. This implies that drug concentration and area of effect can be targeted.

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Ian Paolo I. Azusano, School of Chemical, Biological, and Materials Engineering and Sciences, Mapúa University 658 Murala St., Intramuros, Manila, Philippines.

Raymund Angelo C. Memije, School of Chemical, Biological, and Materials Engineering and Sciences, Mapúa University 658 Murala St., Intramuros, Manila, Philippines.

Lemmuel L. Tayo, School of Chemical, Biological, and Materials Engineering and Sciences, Mapúa University 658 Murala St., Intramuros, Manila, Philippines, ltayo@mapua.edu.ph

Bacterial cellulose is a perfect candidate to administer in this growing field of specialty.

Bacterial cellulose (BC), tantamount to a hydrogel polymer is produced by the acetic bacterium acetobacter xylinum at specific culture conditions. The structural features of BC provides its distinguishable properties, including high water holding capacity, high specific surface area, tensile and wet strength, elasticity, and porosity, which has raised its attention vastly towards biomedical applications. It is also widely utilized in industries producing paper, filter membrane, food and beverages, and pharmaceutical products [1].

Modifications have been incorporated on BC to improve its biomedical compatibility by synthesizing its composites, and further increase its application, such composites are synthesized using bioactive polymers, nanomaterials and solid particles. Currently, various types of polymers have been successfully combined with BC and have improved its properties for biomedical applications. Some of these polymers are chitosan, gelatin, poly(ethylene glycol) (PEG), collagen and polyvinyl alcohol (PVA) [2]. BC/PVA composite has captured the attention of many researchers as PVA can be combined with other polymers or fillers to enhance its properties [3].PVA is a water soluble polymer, has an excellent chemical resistance, optical and physical properties which recommend PVA, and its composites for broad industrial uses [4]. BC/PVA composites were synthesized through freeze-drying of a wet BC pellicle immersed in PVA aqueous solution which can also be used to synthesize BC/PVA composite [5]. Nata de coco which is primarily bacterial cellulose [17,18], a product of microbial cellulose through fermentation of coconut water with the help of A.xylinum will be modified for the first time through incorporation of PVA to improve its capability and properties.

In this study, characterization of the chemical properties of the nata de coco composite was performed and PVA-reinforced BC was synthesized. Lastly, kinetics of methylene blue, modeled as drug, in the BC-PVA system was studied. The Philippines is known for its good production of nata de coco because of its tropical climate needed for the growth of coconut trees [25]. The use of this tropical dessert could greatly affect the field of biomedical engineering in terms of drug delivery in which the need for production will not be a problem [25]. The cost for the production is relatively cheap and the process is not very difficult [25]. Its application as a BC composite holds great

breakthrough potential that can give new insights to nata de coco as a biocompatible and hemocompatible material. Furthermore, this study will give fundamental understanding of the characteristics in nata de coco. This can pave the way for other studies of drugs incorporated to other microbial cellulose products. This study focuses on the characterization of BC/PVA composite and the kinetics of the release of methylene blue. Nata de coco was used in the synthesis of BC/PVA composite and was purchased directly from a local supplier.

II. METHODOLOGY

Materials

Nata De Coco Slabs Were Purchased From A Local Supplier. These Slabs Were Washed In Running Water Until The Ph Turned Almost Neutral. Poly-Vinyl Alcohol Was Purchased From Sigma-Aldrich With Weight (MW) 31,000–50,000 g/mol, 98-99% hydrolyzed and was used without further purification or treatment. Methylene blue was purchased from Belman Laboratories.

Preparation of Polyvinyl Alcohol Solutions

Three different concentrations of PVA solutions were made: 6%, 8% and 10%, by weight. Amounts of PVA and distilled water necessary to formulate the required concentration of PVA solution were measured. Appropriate amount of distilled water was placed in a beaker and heated in a hotplate while the stirring speed was set to mild setting until the temperature reaches 90°C. Due to the temperature setting used for dissolving the PVA, water loss was compensated by adding water to the resulting solution. PVA was gradually added to distilled water to avoid agglomeration. The stirring speed was set at the highest setting to again help prevent agglomeration. This process was done slowly for two hours on each variant of PVA solution. The solutions were then stored in plastic container inside a refrigerator with a temperature of 20°C. Table 1 shows the distilled water and PVA combinations

Table. 1 Distilled Water and PVA Combinations.

Weight of PVA	Weight of Distilled Water	PVA Concentration (by weight)
6 grams	94 grams	6%
8 grams	92 grams	8%
10 grams	90 grams	10%

Preparation of Methylene Blue

To prepare the 4 mg/L methylene blue concentration, 4 mg methylene blue was dissolved in a litre of distilled water. This served as stock solution for the remaining parts of the experiment.

Preparation of Nata de Coco/PVA

Nata de coco slabs, after purchasing from a local supplier, were washed with running water until the acidity were neutralized. After washing, the slabs were dried for approximately 6-7 hours at 65°C, just enough not to dry out the slabs, to reduce water content. After the slabs were dried, they were subjected to air drying to cool down to room temperature. Upon cooling down, the slabs were immersed to PVA solution enough according to Table 2 to cover the whole surface area for two days.

Table. 2 Concentrations of PVA in Samples

PVA Concentration in Nata de coco	Sample
0% (pure)	SP
6%	S6
8%	S8
10%	S10

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FITR spectroscopy was used to identify chemical features (functional groups) present in the film composites. FTIR spectra of samples SP, S6, S8 and S10 were recorded using Perkin Elmer Spectrum 100 at a room temperature of 16°C. The samples were analyzed directly by placing the samples on the top plate of diamond plate at a range of 4000-600 cm^{-1}.

Water Uptake

To analyze the capability of the film composites to uptake water, the samples were soaked in distilled water for 5 hours. Weights of the samples were recorded for every 1 hour. The water uptake was calculated using the following equation

$$Weight\ Increase(\%) = \frac{w_t}{w_t - w_i} x\ 100\% \quad (1)$$

where w_t is the weight of the sample at certain hour, and w_i is the initial weight of the sample.

Scanning Electron Microscopy (SEM) Analysis

The morphology of nata de coco/PVA composite and pure nata de coco was analyzed using HITACHI TM3000 (can be run at accelerating voltage of of 5 to 15 kV). Images of the surface of the samples were taken at a resolution of 10 µm and with at least magnification of 8000x. The most representative samples were presented in this study. The samples were tested at the Research Center for the Natural and Applied Sciences, Thomas Aquinas Research Complex, University of Santo Tomas.

Mechanical Properties of Film Composites

To determine the mechanical properties of the samples, tensile strength test was performed. The samples were cut to rectangular strips with dimensions of 40x10 mm before subjecting to Instron 3225 Single Column Universal Testing Machine (at Polymer Laboratory Institute of Chemistry at the University of the Philippines – Diliman). Strain rate of 15 mm/min and grip distance of 20 mm was used.

All measurements were performed for at least two trials on each case, and the average value will be recorded.

Analysis Methylene Blue Release

A solution of methylene blue with a concentration of 4 mg/L was made that served as a stock solution. Samples SP, S6, S8 and S10 were soaked in 40 mL for 2 hours at room temperature. Distilled water, with a pH of 6.6 was used as the medium wherein the samples were placed to let the release of methylene blue took place. It was assumed that the total amount of methylene blue present in the solution was absorbed by the composite and same amount was released as well upon release to the water medium.

The concentration of the released drug in the medium was determined at different time points (every 1 hour) and was quantified using Ultraviolet-visible spectroscopy. Perkin Elmer Lambda 35 was used for the UV-VIS analysis at 668 nm. Calibration curve was made by determining the absorbance of known solutions of methylene blue (0.04 mg/L, 0.05 mg/L, 0.06 mg/L, 0.08 mg/L and 0.1 mg/L). Concentrations of the samples can be determined by using the equation 2 generated from the calibration curve, noted that the y-axis would be the absorbance and the x-axis would be the concentration in mg/L. Concentration of sample (mg/L) = (absorbance ± intercept) / slope. Kinetics of drug release was studied using the Power Law Model [6] modified as

$$\frac{M_t}{M_{inf}} = kt^n \tag{2}$$

where Mt is the cumulative amount of methylene blue released from the samples at time t, and Minf is the cumulative amount of methylene blue released from the samples at infinite time.Cumulative concentration of samples can be calculated using Equation 3.

$$M_t = (C_m x V_m)_t + \sum (C_{ws}\, x\, V_{ws})_{t-1} \tag{3}$$

where C_m is the concentration of the medium where methylene blue is released at present time t, V_m is the volume of the medium where methylene blue is released at present time t, C_{ws} is the concentration of the withdrawn sample at previous time (t-1) and V_{ws} is the volume of the withdrawn sample at previous time (t-1).M_t/M_{inf}, meanwhile, can be calculated using Equation 4

$$\frac{M_i}{M_{inf}} = \frac{cumulative\ release}{C_{MB}\, x\, V_{MB}} \tag{4}$$

where C_{MB} is the concentration of released methylene blue at infinite time which is equal to 4 mg/L, and the V_{MB} is the volume of medium where methylene blue was released. Cumulative release percentage can also be calculated by taking the percentages of M_t/M_{inf}. Plotting ln (M_t/M_{inf}) against ln t was performed to calculate the values of k and n. Finally, the correlation coefficient (r^2) will be determined to report the fitness of data in the model.

III. RESULT AND DISCUSSION

FTIR Analysis

To determine the interaction between the polyvinyl alcohol and nata de coco, the spectrums of the nata de coco/PVA composites were compared with the spectrum of the pure nata de coco (bacterial cellulose) sample. Figure 1 shows the comparison of their spectrums. Based on this figure, the main differences are in the hydroxyl and carbonyl regions. Both the spectrums of samples SP, S6, S8 and S10 showed intensive peaks within the range 3500-3000 cm^{-1}, which signify the presence of OH groups. However, it should be noted that samples S6, S7 and S10 exhibited broader peaks proving the formation of hydrogen bonds. This confirms the intermolecular interaction between hydroxyl groups of bacterial cellulose and PVA.

According to [4], the narrower band exhibited by sample SP shows the dominance of the free hydroxyl groups. Carbonyl groups are strongly exhibited by the samples with PVA compared to the pure bacterial cellulose. Derived from polyvinyl acetate, this is an evidence of incomplete hydrolysis of polymer PVA.

All significant peaks were both shown by pure BC and BC/nata de coco composite, suggesting that mechanism of forming the network present in pure BC was the same mechanism involved in forming the network between BC and PVA. However, Figure 1 shows that sample S6 has the greatest transmittance among the three composites, suggesting that it may have the greatest amount of PVA absorbed. Sample S6 has only 6% PVA concentration compared to other composites, S8 and S10, which has 8% PVA and 10% PVA, respectively. This may be attributed to the inconsistent amount of water present in the samples, which has the plasticizing effect, the water bonds with hydrogen of bacterial cellulose instead of that PVA [7]. As mentioned earlier, the particles of PVA have the tendency to agglomerate at higher concentration - this could be the reason why sample S6 showed the highest transmittance. Particles of PVA at 6% concentration may had penetrated easily the matrix of BC because of small particle size while the particles of PVA at 8% and 10% concentration found some difficulties penetrating because of larger particle size. This possibility of limiting the PVA particles to penetrate the BC because of agglomeration at higher concentration is important since it the amount of PVA present in the matrix affects the properties of the composite.

Chiciudean [7], discussed in his study, after fabricating a bacterial cellulose-epoxy resin composite, that delamination occurs in the internal sheets and not on the interface between the resin and bacterial sheet. Therefore, according to him, the polymer must be able to penetrate between the nano cellulose fibers, thus it should be soluble in water and alcohol. This is the penetration mechanism of PVA as it interacted with bacterial cellulose. The PVA will crosslink with the intact BC membrane creating a network, where the PVA fibers will fill the porous BC matrix [8].

One important factor that must be accounted is the inconsistency of the bacterial cellulose purchased from the local supplier. The amount of water content is critical as it will affect the results of how the PVA will behave and bond with hydrogens present in the PVA. The slabs were also inconsistent when it comes to physical dimensions. These factors are important especially to the investigation on the

effect of varying concentration.

Picheth[9] discussed that increasing the PVA concentration results to hydrogels with higher crystallinity and added stability upon swelling. This increases the mechanical property of the composite such as the tensile strength and tear resistance. However, the following results, especially on tensile test of the conducted research, did not reveal these features and resulted to the inability of the researchers to see the effect on varying PVA concentration. One explanation could be the particle size of the PVA particles, PVA tends to agglomerate and form bigger particles at a higher concentration leading to inability to penetrate the porous membrane. This phenomenon is also observed at higher concentrations with other components similar to PVA which is polyethylene glycol.

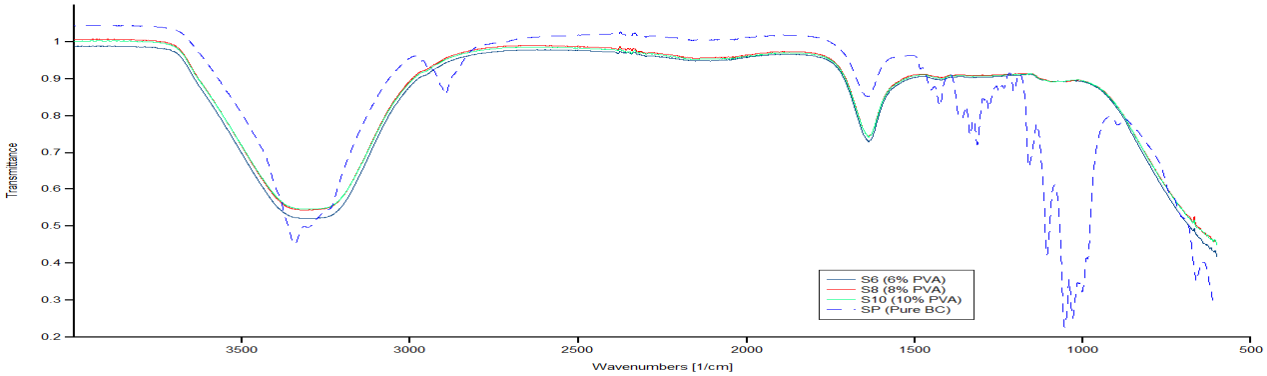

Fig. 1 Comparison of FTIR spectra of nata de coco/PVA samples

Water Uptake Test

Figure 2 shows the behavior of the samples as they were subjected to water uptake test. From the results of the FTIR analysis, the researchers have inferred that sample S6 (6% PVA) has the highest amount of PVA present in itself or has the highest amount of PVA absorbed when it was immersed into PVA solution. This was supported by the results shown in the water uptake test. It was observed that the sample S6 has the lowest amount of absorbed water as time passed by when quantified by using the increase in its mass. According to Peresin [10], the interactions occurred between the PVA and BC, in terms of hydrogen bonding and crosslinking, inhibits the hydroxyl groups to bind with water molecules. The decrease in free volume and chain flexibility when PVA was added restricts the composite to increase its mass by absorbing water.

Meanwhile, sample SP has the greatest amount of water absorbed. Since there is no PVA present in its matrix, the hydroxyl groups present in bacterial cellulose are free to bind with water molecules. Sample S8 and S10 follow sample SP, respectively. Some of the free hydroxyl groups not bonded to PVA participated in interaction with water molecules which explain why these two samples have greater amount of water absorbed compared to sample S6. This also suggests that these two samples have lesser amount of PVA present in their composites

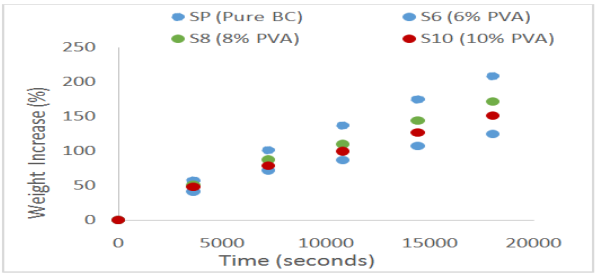

Fig. 2 Water absorption of samples SP, S6 S8 and S10

Tensile Test

The results from tensile tests of samples SP, S6, S8 and S10 did not exhibit high elastic behavior compared to the results presented by Castro et al. [11]. This may be attributed to the plasticizing effect of water, which was still present in the samples. Since there are water present in the composites, the water molecules tend to bond with the hydroxyl groups of bacterial cellulose, reducing the capability of PVA to crosslink and strengthen the fiber network. As such, this decreases the mechanical property of the composites [7].

Nevertheless, sample S6, as expected, exhibited exceptional mechanical strength since it had the highest amount of PVA absorbed. Based on Figure 3 the ultimate stress point is way beyond that of pure bacterial cellulose. The results of sample S8 and S10, however came out different with what was expected, considering the fact these two samples were impregnated with PVA. One possible explanation for this was that the amount of water present in these two samples may be greater than the amount of water present in sample SP. Another factor could be the anisotropic property of natural polymer composites. Contrary to isotropic, anisotropy that pertained to the property of material that varies with orientation, such as ultimate strength [12,13]. This also explains the large standard deviation shown by samples SP and S10. This means that the mechanical property of the samples varies, as it was stretched in a particular orientation. This suggested that, if the material was tested in another orientation, the results could have been different.

Considering the results of tensile test and water uptake test, it might be said that sample S10 has greater amount of PVA present than that of sample S8. Table 3 shows the tensile strengths and young's modulus of the samples

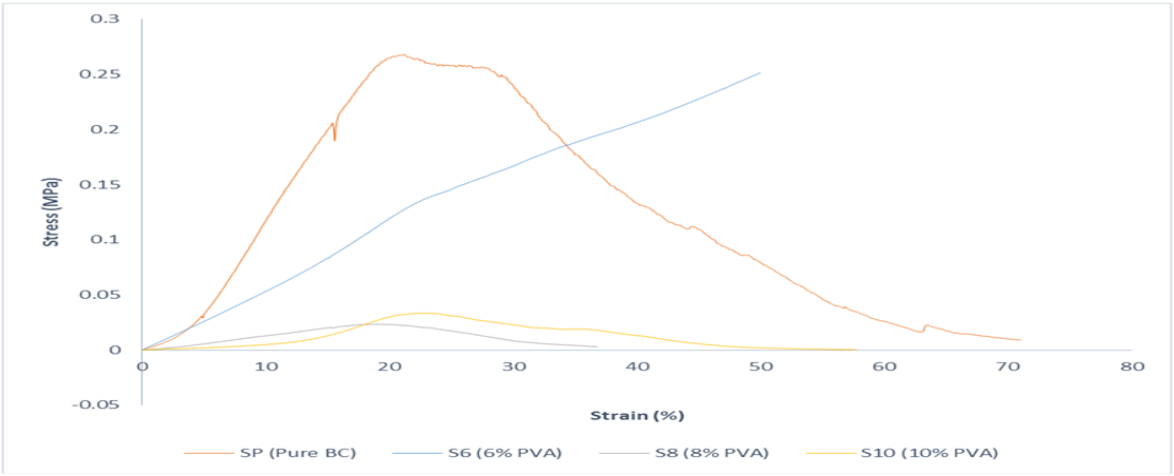

Fig. 3 Stress-strain behavior of tensile test result on BC and BC/PVA composites

Table 3 Tensile Strengths and Young's Modulus of Samples

Sample	Tensile Strength (MPa)	Young's Modulus (MPa)
SP	0.00033 ± 0.00051	1.94749 ± 0.85567
S6	0.00299 ± 0.00014	0.31311 ± 0.20082
S8	0.00112 ± 0.00114	0.20151 ± 0.02396
S10	0.00026 ± 0.00002	0.35191 ± 0.08018

Morphological Analysis

Scanning electron microscopy images on the surfaces of samples SP (Pure BC), S6 (6% PVA), S8 (8% PVA) and S10 (10% PVA) are shown in Figure 4. Corresponding images for each sample is a result of the impregnation method used to form composites. All of the samples show random 'holes' with sample S6 the most visible distribution. Also, crosslinking is not clearly seen as the sample is only capable of being magnified of up to 8000 times.

Nevertheless, formation of these holes already suggest the network present in the samples. As discussed by Perotti et al. [14] and Yang et al .[15], the network present in the bacterial cellulose itself is a fibrilar organization wherein each fibril of this organization comprised of about a thousand net-like structure with large holes. Therefore, the holes presented in Figure 3.3 could denote that these were the gaps between the networks already presented and interacted with PVA.

Since the presence of large holes denote the presence of network of fibrils between the bacterial cellulose and PVA, this can be attributed to the mechanical property of the sample. The greater the number of random 'holes' present in the SEM image of the sample may infer that the organizational network formed between bacterial cellulose, and PVA is more complexed, thus increasing the mechanical property of the substance.

Sample S6 showed greater amount of randomly distributed holes, and may therefore suggest that this sample is more porous compared to samples S8 and S10. Random distribution of the 'holes' in the samples show that fibrils present in the structure are also random in orientation. As described by Chiciudean [7], it is a 3D interwoven network of nanofibrils. It may not be clearly seen the orientation of fibrils, and how they are connected to one another but the presence of random 'holes' already reveals this morphological feature of the samples.

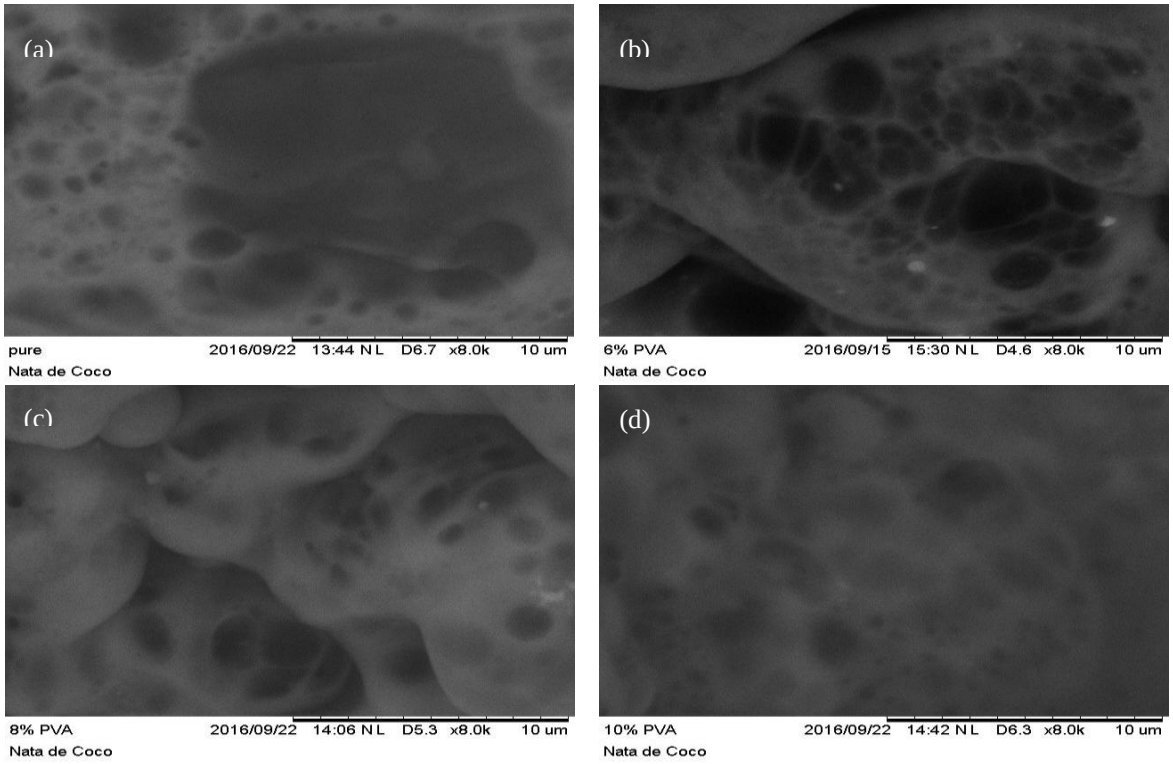

Fig. 4 SEM images of the surface of the samples (a) SP (Pure BC) (b) S6 (6% PVA) (c) S8 (8% PVA) and (d) S10 (10% PVA)

Test Kinetics of methylene blue release

Figure 5 shows the calibration curve for determination of methylene blue concentrations. The results were presented in terms of percentage of M_t/M_{inf} and is shown in Figure 6 and Figure 7. It was assumed that the amount of methylene blue released from the samples at infinite time would be the 4 mg/L, and 5 hours would be enough to attain that concentration, the same with the concentration of absorbed methylene blue of the samples. When PVA was introduced to the bacterial cellulose matrix, morphological changes occurred due to crosslinking between the hydroxyl groups of BC and PVA [10]. Furthermore, the diffusion mechanism of methylene blue was affected as it released from the composites. It was observed that sample S8 had the highest amount of released methylene blue at a duration of 5 hours. As explained earlier, this sample was not much affected by the addition of PVA, as exhibited by the results of tensile test and water uptake test. The little effect of adding PVA may not cause significant morphological changes that helped faster diffusion of methylene. On the other hand, sample S6 has the least amount of methylene blue released for 5 hours. This result can be attributed to the significant changes of PVA to the structure of BC matrix affecting the diffusion mechanism

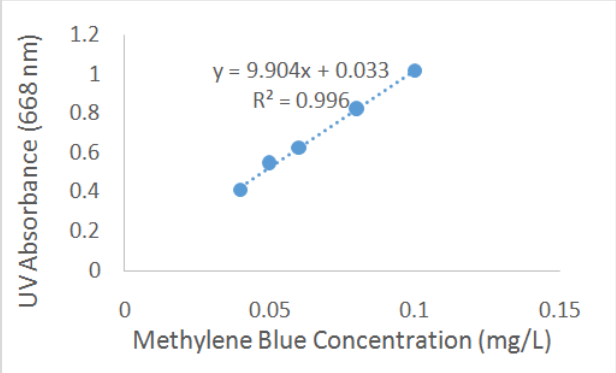

Fig. 5 Calibration curve for determination of methylene blue concentrations

As discussed by Ghanbar et al. [19], there are three different classes of diffusion that can be identified based on the relative rates of diffusion and polymer relaxation. Polymer relaxation is basically the time the polymer requires to go back to equilibrium after it was disturbed upon application of stress [20]. Thus as shown in Figure 6, The first case, where n=0.5, suggests a Fickian diffusion mechanism in which the rate of relaxation is greater than the rate of diffusion. The second case indicates that diffusion process is much faster than the relaxation process, n = 1. The third case is the non-Fickian (anomalous) diffusion mechanism, which states that the rates of diffusion and relaxation are comparable with one another [21]. Fickian

diffusion is defined as the solute transport process in which the polymer relaxation time (t_r) is much greater than the characteristic solvent diffusion time (t_d). When tr ≈ td, the macroscopic drug release becomes anomalous which is known as non-Fickian There are also cases in which the values n are less than 0.5 [22,23] . This kind of diffusion mechanism is called 'Less Fickian' – the penetration rate of penetrant is much below the relaxation rate. Also, Munday and Cox [24], discussed the occurrence of n values greater than 1, regarded as Super Case II kinetics. In this case, the polymer will release at later stages.

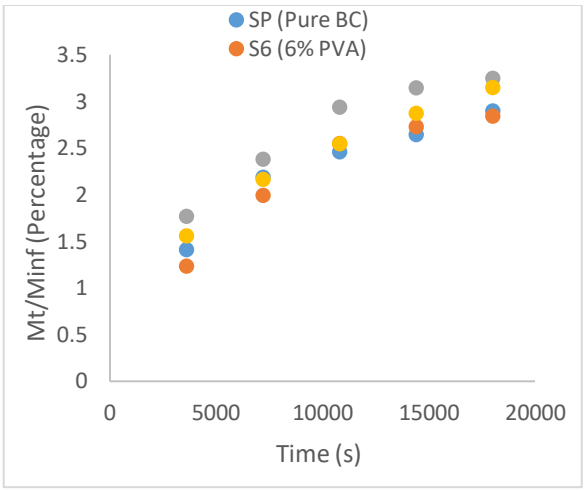

Figure. 6 Percentage release of methylene blue from nata de coco and nata de coco/PVA composites

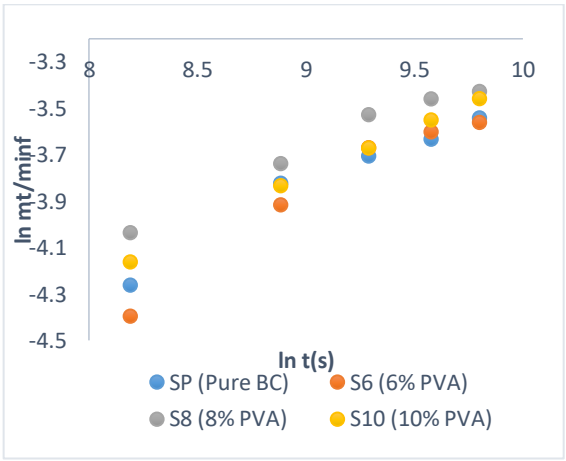

Fig. 7 Variation of ln(M_t/M_{inf}) versus ln Time (s) for samples SP, S6, S8 and S10.

Table 4 shows the values of calculated diffusion exponent as well as the model parameter k. Good correlation was observed between the experimental data and Power Law Model as the least correlation coefficient is 0.9593. Samples SP, S8 and S10 show 'Less Fickian's behavior with their diffusion with their values of diffusion exponent less than 0.5. This explains why S8 has the highest amount of release methylene blue. The rate of methylene blue penetrating the composite was very slow, thus when released, the release rate would be very fast. The amount of water present could helped the diffusion mechanism. Sample S6 meanwhile showed non-Fickian diffusion mechanism with a diffusion exponent of 0.5327. A possible explanation is the fact that the interaction between BC and PVA is great, changing the network of the composite matrix, thus affecting the diffusion of methylene blue and releasing at much lower rate.

Table. 4 Power Law Model Calculated Parameters

Sample	Diffusion exponent n	Model constant k	Correlation coefficient R2
SP	0.4344	-7.7659	0.9609
S6	0.5327	-8.7004	0.9593
S8	0.3943	-7.2438	0.9779
S10	0.4356	-7.7184	0.9986

IV. CONCLUSION

Reinforcing nata de coco, a bacterial cellulose, with polyvinyl alcohol was successfully done by immersing nata de coco into polyvinyl alcohol solutions. Experimental tests performed, confirmed the capability of PVA to reinforce the bacterial cellulose matrix. Water uptake test revealed that composite with high amount of PVA absorbed less water as the hydroxyl groups of BC were bonded to PVA, preventing interaction with water. Kinetics on the release of methylene blue from nata de coco/PVA composites was also investigated. Results showed that composites with high amount of PVA in their matrix released smaller amounts of methylene blue. Addition of PVA have changed the network structure of the composite matrix affecting the diffusion mechanism of methylene blue as it was released. It was observed that the samples exhibited 'less Fickian' behavior and non-Fickian (anomalous) behavior as indicated by their diffusion exponents. Findings suggested that polymer composites such as nata de coco/PVA could be modified and exploited as vital components in the field of drug delivery. More studies are needed to further expand the knowledge on drug deliveries using polymer composites as well as increasing the amount of drug release.

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