Effect of Sericin in [HA/Bioplastic] Composite Composed by 2^k Factorial Design Method on Solidification Time and Tensile Strength

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Abstract

Synthetic hydroxyapatite has been attractive ceramic for a decade as main material of bonegraft. However, in powder form it was difficult to shape into certain geometry due to its brittleness characteristic. For this, composing it with another biomaterial as a matrix in a proper composition can be a challenging. In the present work, bioplastic (B) made of cassava starch was selected as a matrix material and silk sericin (SS) extracted from B.mori cocoon was added. Hydroxyapatite was obtained from bovine hydroxyapatite (H). The composite was prepared by mixing of H/B with ratio of 20/80 and 80/20 and sericin of 8% and 16%w/w was blended to form pasta. It was then casted layer by layer (0.1mm and 0.5mm thick) into mould with size of 6mm diameter and 3mm deep. The composite component was composed by utilizing 2^k Factorial Design Method and selected the highest of diametral tensile strength (DTS). ANOVA was performed to evaluate hypothesis H_0 and H_1 based upon the sources and two regression models of Y=f(X,) that represented solidification time and tensile strength were developed. The result of solidification time model reveals that composite [H80/B20/S16] with 0.1mm layer thickness had the shortest time of 106.36sec and the highest DTS of 3.46MPa. These results were slightly lower than that obtained by experiment i.e. 107sec and 3.88MPa, respectively. Additional sericin twice for this composite gains more significant effect in improving DTS and solidification time compared to that of giving hydroxyapatite.

Keyword-bovine hydroxyapatite, bioplastic, sericin, solidification time, diametral tensile strength

I. INTRODUCTION

Biocomposite based hydroxyapatite (HA) powder material has been intensively investigated for scaffold or bone filler application since HA was bioactive and osteoconductive materials that mimics the human bone, however, pure HA powder was difficult to be shaped due to brittleness characteristic [1-3]. An existing method to shape the pure HA powder was mixing it with distilled water, then casting into a mold and sintering in the furnace for post processing. Applying this method was nearly fit to the requirement, but in term of the shape it was limited only for simple geometry of the mold. In addition, its mechanical strength remains low and even occurs shifting the phase of the HA due to high sintering temperature [4]. In addressing these problems, research has been done not only focusing on improvement of its mechanical property, but also ease of forming and biological property. In mechanical property, improvement was taken place to get a better mechanical strength by forming into composite. Several materials as a matrix of HA composite have been used so far, namely collagen type-I, gelatin, PVA, chitosan, PLA, PLLA, PMMA and bioplastic cassava based material [5]. However, among these matrix materials, collagen-type I biologically was the best one as it was part of the mineralized collagen fibril within the bone tissue, but it was costly. Tensile strength could be achieved using those matrices was low, typically was around 3 MPa [6]. Potential application for this biocomposite with this strength was only for making bonegraft. Another effort to improve this strength was zirconia with a certain portion into the HA composite, although there was affect in the growth of S. Epidermidis colony and 20% concentration zirconia to be an optimum number to inhibit the growth [7].

Another challenge in forming into a certain shape is ease of forming. By adding a matrix within the powder to form a composite, the composite can be easier to be shaped at room temperature into a particular geometry. Several possible methods could be employed such as blue-ray curing [8,9], a gravity casting and an injection moulding, although these still need a further post process to obtain a final product [10]. In case of very complex shape and geometry of hard tissue of human organ such as skull of human head, mould would be no longer applicable. To address this challenge, an additive manufacturing technology called a 3D printing machine might be a solution. However, this technology currently is only available commercially using non-medical materials in the form of powder, filament, sheet and photo-liquid materials of non-medical application. In addition, the

machine works only using the materials were supplied by the compony who manufactures the machine. Beyond these materials, such as in the form of pasta, commercially it is not available yet.

In the biological aspect, this has been focused on how to enhance the growth and attachment of fibroblast cell. For this, silk sericin was the choice material to deal with due to a wide range of applications for biomaterials [11-24]. It was hydrophilic, macromelecular glycoprotein synthesized in the middle silk gland of the silkworm, that constitutes 25-30% of the silk cocoon [25]. In this paper, it will only be focused on an investigating the effect of adding sericin with various percentages on solidification time and strength of the composite. Other aspects such as biology and manufacturing were not considered yet. These will be planned for the future work.

II. METHODOLOGY

A. Materials, Preparation and Testing

The main materials have been utilized in this research were bovine hydroxyapatite powder (H), cassava starch powder and sericin (S). H with particle size of 55.62 µm was obtained by calcination of bovine bone at 900^{o}C following the process available in reference [5,6]. Cassava starch was obtained from commercial shop as a bioplastic main material (B) and sericin was a protein extracted from Bombyx mori cocoon. Other additive materials for samples preparation were also used such as citric acid, gliserol and distilled water. This citric acid was used to improve hydrophilic characteristic of the hydroxyapatite particle surface to gain a better homogeneous distribution when it was mixed with bioplastic matrix. Whilst gliserol was employed to improve flexibility characteristic of the matrix; and distilled water was used as a solvent of cassava starch powder, citric acid and gliserol. No further characterization had been carried out for the main materials. Samples of biocomposite were prepared for two purposes i.e. solidification time of 0.1mm and 0.5mm layer thicknesses; and diametral tensile strength. For those purposes, samples were made by mixing of hydroxyapatite and biolastic in a certain ratio, and each of these composition ratio was added with sericin. The shape of samples was a disk with 6 mm diameter and 3 mm thick. Detail of sample preparation was as follow. The samples were made into two steps. The first step was preparation of X_1 by mixing of hydroxyapatite (H) and bioplastic (B) with percentage weight ratios of 20/80 and 80/20. The second step was preparation of X_2 by making solution of sericin of 8 and 16 %w/v. It was then mixed with X_1 to form a paste and casting it into the sample mold. Prior to hydroxyapatite preparation, citric acid solution was firstly made by mixing 10% w/v of citric acid powder in distilled water (H_2O). It was stirred at 350 rpm at room temperature for a few seconds. After citric acid solution was ready, hydroxyapatite powder of 20% w/v was added into the solution and stirred at 1000 rpm, at room temperature for 20 hours to get hydroxyapatite and ready to blended with bioplastic.

In preparation of bioplastic, 20% w/v cassava starch solution and 7.5% w/v gliserol solution was mixed and stirred at 600 rpm, at temperature of 60^{o} C for 10 minutes. This process was carried out by stirring it manually at first stage till the starch blends to become gel, then it was stirred at 350 rpm, at temperature of 25^{o} C for 10 minutes to form a paste. Finally, hydroxyapatite, bioplastic and sericin were blended and casted into disk. The specimen mold with 3 sets of disk cavity of 3 mm depth and diameter of 6 mm was set up in 6 layers with 0.5mm and 30 layers with 0.1mm of each layer thickness. Therefore, 3 samples can be built simultaneously in single process. In making sample shown in Fig.1a, it begins with the first layer by filling the cavity mold of 0.5 mm deep, it was then heated up at 50^{o}C to solidify and solidification time was recorded as fulfilling the first purpose. Next, pushing the cavity base down to 0.5mm deep to allow filling the second layer of pasta on top of the first layer. This process filling-heating-cooling was repeated until the last layer accomplished. After all samples were accomplished, diametral tensile strength (*DTS*) test as illustrated in Fig.1 was then performed using universal testing machine (UTM). Each sample was placed at the specimen holder of the UTS machine with its position as shown in Fig.1b. The sample disk with diameter of 6mm and thickness of 3mm was pressed from top and bottom with force *F* until the sample was deformed shown in Fig.1c.

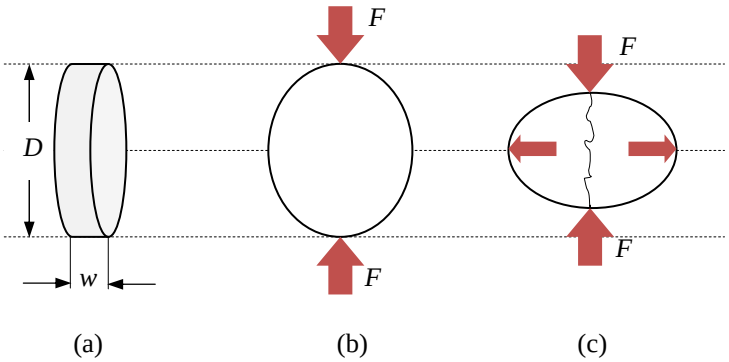

Fig.1. Diametral tensile test: (a). Sample (b) Applied force F and (c) Sample deformed

DTS value was calculated by equation-1 adopted from the reference [5,6].

$$DTS = \frac{2F}{\pi Dw} \quad (1)$$

where F is force (N), D and w are diameter (mm) and thickness (mm) of the disk sample, respectively.

All solidification time and DTS data have obtained in the experiment were processed statistically using analysis of varians (ANOVA) and Minitab 15 to determine coefficient of regression. ANOVA was used to find the most significance factor (X) and interaction between the factors in responding to solidification time and diametral tensile strength (Y).

B. Method of 2^k Factorial Design [26]

In this study, there were two consecutive tasks to be carried out: (1) Design of Experiment (DoE) and (2) ANOVA and regression. In this DoE as shown in Table I, factors, levels and coding the factors were determined. There were 3 factors including ratio of [H/B], sericin content and layer thickness; and 2 levels i.e. low and high. Those factors were coded by X_1, X_2, and X_3, respectively. As a note that X_3 was only used for dealing solidification time experiment purposes since DTS testing did not consider the layer thickness. Here, the layer thickness was fixed as 0.5mm.

TABLE I: DoE: Factors, Levels and Code of Factor

Factors	Level		Code of Factor
	Low (-)	High (+)	
Ratio of [H/B]	20	80	X_1
Sericin content	8%	16%	X_2
Layer Thickness	0.1mm	0.5mm	X_3

In the 2^k Factorial Design, factors of X_1, X_2, and X_3 were developed into a combination of test runs. There were 8 test runs depicting a solidification time experiment shown in Table II and 4 test runs for DTS testing as shown in Table III. In the DTS, it was only factors of X_1 and X_2 to be considered, while X3 was regarded as a fixed-value of 0.5mm layer thick.

TABLE II: Test run for solidification time

Test Run	Code of Factor			Actual Factor			Actual Weight (g)			Sample Code
	X_1	X_2	X_3	X_1 (%w/w)	X_2 (%w/v)	X_3 (mm)	H	B	S	
1	-1	-1	-1	20/80	8	0.1	0.4	1.6	0.16	H20 B80 S8 T01
2	+1	-1	-1	80/20	8	0.1	1.6	0.4	0.16	H80 B20 S8 T01
3	-1	+1	-1	20/80	16	0.1	0.4	1.6	0.32	H20 B80 S16 T01
4	+1	+1	-1	80/20	16	0.1	1.6	0.4	0.32	H80 B20 S16 T01
5	-1	-1	+1	20/80	8	0.5	0.4	1.6	0.16	H20 B80 S8 T05
6	+1	-1	+1	80/20	8	0.5	1.6	0.4	0.16	H80 B20 S8 T05
7	-1	+1	+1	20/80	16	0.5	0.4	1.6	0.32	H20 B80 S16 T05
8	+1	+1	+1	80/20	16	0.5	1.6	0.4	0.32	H80 B20 S16 T05

TABLE III: Test run for Diametral Tensile Strength (DTS)

Test Run	Code of Factor		Actual Factor		Actual Weight (g)		Sample Code
	X_1	X_2	X_1 (%w/w)	X_2 (%w/v)	H	B	
1	-1	-1	20/80	8	0.4	1.6	H20 B80 S8
2	+1	-1	80/20	8	1.6	0.4	H80 B20 S8
3	-1	+1	20/80	16	0.4	1.6	H20 B80 S16
4	+1	+1	80/20	16	1.6	0.4	H80 B20 S16

Based on the coded factors in Table II and III, it was then prepared the samples with the weight composition (in grams) and material code as illustrated in Table IV. Here, samples No.9 to 12 were prepared twice for layer thickness of 0.1mm and 0.5mm. Additional samples were also made as control samples including H, B, BS8, BS16, HS8, HS16, H20B80 and H80B20. There were 20 various ingredients including 12 test runs and 6 control samples. So, if each consists of 3 samples, there would be 60 samples to be prepared in this experiment.

TABLE IV: Weight Composition of Control Samples

No	Sample Code	Actual Weight (g)		
		H	B	S
1	H	2	-	-
2	B	-	2	-
3	B S16	-	2	0.32
4	B S8	-	2	0.16
5	H S16	2	-	0.32
6	H S8	2	-	0.16
7	H80 B20	1.6	0.4	-
8	H20 B80	0.4	1.6	-

In the second task, analysis of varian test was carried out to evaluate the hypothesis of H_0 and H_1 in accordance to the factors of X_1, X_2, and X_3 and the interaction among the factors by comparing lack of fit P_{value} to and lack of fit F to $F_{0.05,df1,df2}$. If $P_{value} < 0.05$ and $F_{value} > 9.55$, the hypothesis H_0 is reject and oppositely the hypothesis H_1 is accepted. Assuming that there was strong bonding between layers in building the whole sample thickness. Response for factors of X_1, X_2, and X_3, would be Y_t representing a solidification time (in sec) that counted of each layer and response for X_1 and X_2 would be Y_d expressing a diametral tensile strength (in MPa). The regression model for solidification time (Y_t) and DTS models (Y_d) were expressed in equation 1 and 2.

$$Y_t = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \epsilon \quad (1)$$

$$Y_d = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \epsilon \quad (2)$$

Processing in Minitab 15, regression coefficient of could be obtained. Finally, Y_t and Y_d calculated by regression model in equation-1 and -2 are compared with that obtained by experiment.

III. Results and Discussion

Measurement results of solidification time (t) and DTS for 8 and 4 test runs were depicted in Table V, VI and VII, respectively. Fig. 2, 3 and 4 were showing another expression of solidification time (t, sec) and DTS (MPa) for varying composite compositions.

TABLE V: Experiment results of 8 test runs for Solidification Time

Sample Code	Test Run	Actual Factors			Solidification time (t;sec)
		X_1 (%wt)	X_2 (%wt)	X_3 (mm)	
B20 H80 S8 T01	1	20	8	0.1	210
B80 H20 S8 T01	2	80	8	0.1	200
B20 H80 S16 T01	3	20	16	0.1	125
B80 H20 S16 T01	4	80	16	0.1	107
B20 H80 S8 T05	5	20	8	0.5	640
B80 H20 S8 T05	6	80	8	0.5	440
B20 H80 S16 T05	7	20	16	0.5	515
B80 H20 S16 T05	8	80	16	0.5	265

Fig. 2 illustrates the comparison of solidification time for two different layer thicknesses of 0.1mm and 0.5mm. The longest solidification time of 640 sec belongs to sample H20B80S8T05 and the shortest solidification time

of 107sec obtained by sample H80B20S16T01. As shown in this figure that increasing concentration of sericin from 8%w/w to 16%w/w gains sorter solidification time about 40.47% to 46.5% for 0.1mm layer thickness and 19.53% to 39.77% for 0.5mm layer thickness. Lowering this solidification time was also affected by making thinner the layer. In this by lowering thickness from 0.5mm to 0.1mm can reduce the solidification time of 54.54% to 67.18% for sericin content of 8%w/w and H/B ratio of 20/80 and 80/20, respectively. Similar trend also occurred for the sample with sericin content of 16%w/w. It was about 75.72% for H/B ratio of 20/80 and 59.62% for H/B ratio of 80/20. Thus, it was obvious that by increasing sericin concentration could speed up its solidification time and this can be improved as well by reducing its layer thickness from 0.5mm to 0.1mm.

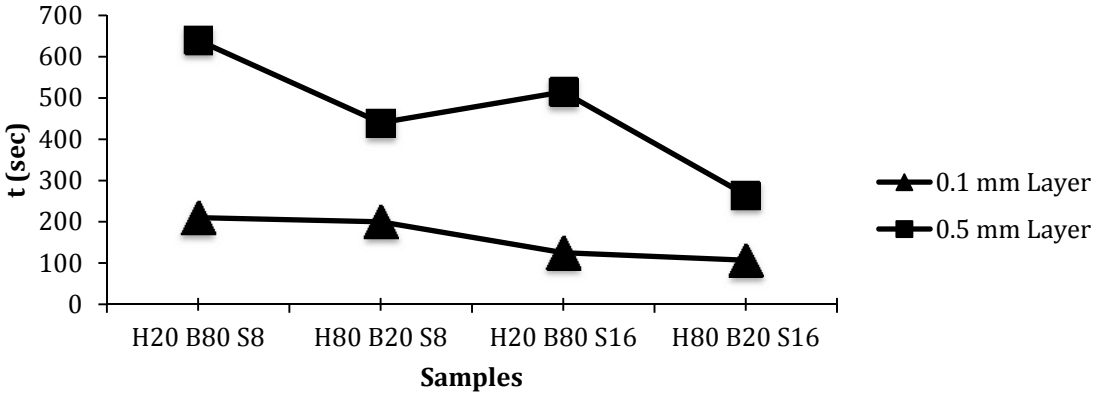

Fig.2. Solidification time (t) of biocomposite for various layer thicknesses and composition.

In DTS experiment as shown in Table VI appeared that among those samples which composed by three components of bioplastic, hydroxyapatite and sericin, sample B20H80S8 has the lowest DTS of 2.58 MPa and sample B80H20S16 has the highest DTS value of 3.88 MPa. Diametral tensile strength (DTS) as depicted in Table VI and VII, and it was plotted in Fig.3 that DTS of hydroxyapatite alone was lower than that of bioplastic (B) and the composites (BS8, BS16, HS8, HS16, H20B80, H80B20, H20B80S8 and H80B20S16). It was clear that due to hydroxyapatite is bioceramic which has a brittle characteristic, there was no strong bonding between the particle. However, when the hydroxyapatite (H) was added with bioplastic (B) or sericin (S) or both (BS), its DTS was better compared to that of the individual material (hydroxyapatite/H or bioplastic/B). This was in agreement with the rule of mixture in the composite theory. The higher content of sericin, it would get better DTS value. When sericin was added to hydroxyapatite or bioplastic to become HS and BS respectively, the DTS of these materials increase, even though the DTS of BS was better than that of HS. Adding sericin of 16%w/w to the hydroxyapatite improved its DTS better than that of 8%w/w. Similar trend of increasing DTS was also happen when adding sericin to the bioplastic. Again, 16%w/w sericin to the bioplastic was better than that of 8%w/w. Following the phenomenon of the previous material (BS and HS) explained in the Fig.3, experiment was continuous to other composition of HB (H20B80 and H80B20) and HBS (H20B80S8, H20B80S16 and H80B20S8 and H80B20S16). Higher content of hydroxyapatite or sericin within the bioplastic leads to have higher DTS value. The composite with composition ratio of [H/B]=80/20 gains higher DTS value than the composite with ratio of 20/80. Similarly, composite of [H/B] with 16%w/w sericin content shows better DTS value than that with 8%w/w sericin content. Increasing content of sericin by twice of the previous one (8%w/w) with the same content of hydroxyapatite can improve its DTS value about 31.44% to 32.63%. Surprisingly, sericin which is counted as a protein, gives better DTS improvement rather than hydroxyapatite. By adding hydroxyapatite to the [H/B] composite with the same sericin content, its DTS was only improved by 1.29% to 3.0%. Improvement would be more significant to the composite when hydroxyapatite content within the composite was 4 times of bioplastic and sericin of 16%w/w hydroxyapatite. This better improvement probably due to sericin as a protein plays as a cross-linker to the bioplastic as a composite matrix, causing strong bonding to the hydroxyapatite particle.

TABLE VI: Experiment results of 4 test runs for DTS

Sample Code	Test Run	Actual Factors		DTS (MPa)
		X_1 (%w)	X_2 (%w.v)	
B20 H80 S8	1	20	8	2.58
B80 H20 S8	2	80	8	2.66
B20 H80	3	20	16	3.83
B80 H20	4	80	16	3.88

TABLE VII: Experiment results of DTS for Control Samples

Control Sample Code	DTS (MPa)
H	0.81
B	1.13
B S8	1.38
B S16	2.03
H S8	1.14
H S16	1.26
H20 B80	1.35
H80 B20	1.82

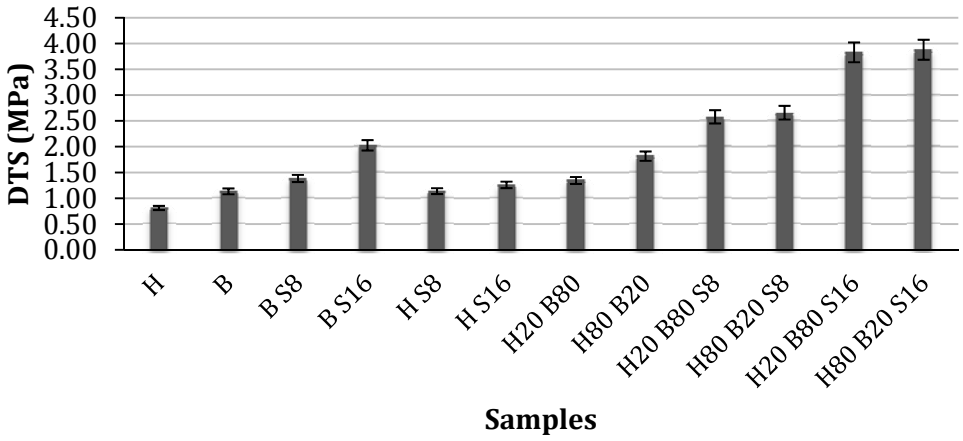

Fig.3. DTS of samples: H, B, BS, HS, HB and HBS composition

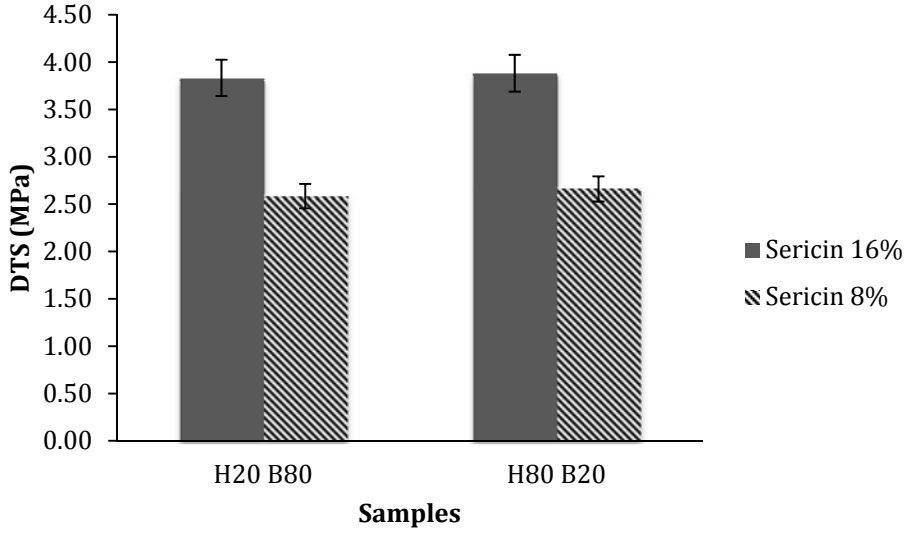

Fig.4. Effect of sericin content on DTS of various [H/B] composition samples

Table VIII and IX show results of ANOVA for Solidification Time (Y_A) and DTS Testing (Y_B), respectively. It depicted status of hypothesis Ho of each factor and its interaction between the factors on the basis of DOF, Fo and P_{value}. It was found that all individual factor (X_1, X_2, X_3), interaction factor of X_1*X_3 and X_2*X_3 show that hypothesis H_0 were rejected as indicated by $F_{value} > 9.55$ and $P_{value} < 0.05$. Only X_1*X_2 that hypothesis was

accepted with F_{value}=1495.101 and P_{value}=0.072. This means that there was any interaction between factor of X_1 (percentage ratio of hydroxyapatite and bioplastic) and X_2 (sericin content) in the solidification time. Since in the DTS factor X_3 was not considered, thus accepted interaction between X_1 and X_2 was also applicable for H_0 of DTS. Fig. 4 and 5 taken from Minitab 15 show interaction plot depicting the interaction between factors in solidification time and main effects plot of contributing factors in DTS, since the interaction between factors in DTS testing was insignificant.

TABLE VIII: ANOVA of Solidification Time

Source of Variation	DF	F_{value}	P_{value}	Status
X_1	1	230.462	0.000	Reject H_0
X_2	1	230.462	0.000	Reject H_0
X_3	1	1495.101	0.000	Reject H_0
X_1*X_2	**1**	**1495.101**	**0.072**	**Accept H_0**
X_1*X_3	1	3.427	0.000	Reject H_0
X_2*X_3	1	179.092	0.000	Reject H_0
Interaction	**1**	**14.910**	**0.194**	**Accept H_0**
Error	40			
Total	47			

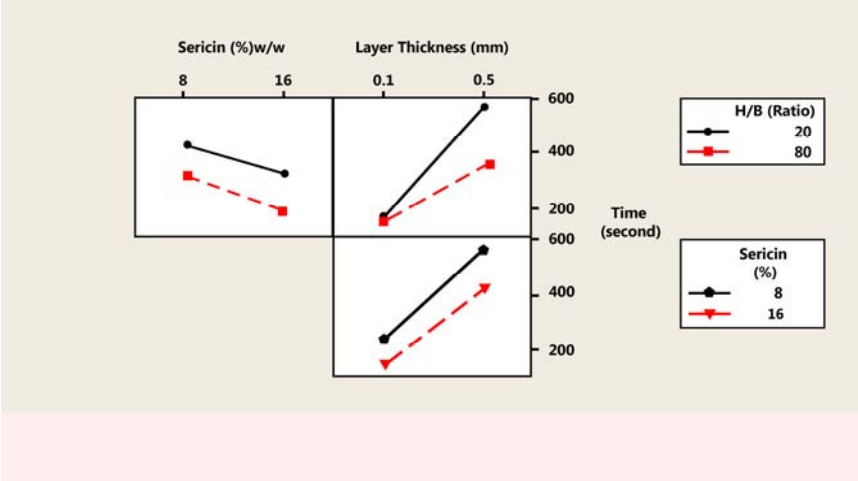

Fig.5. Interaction between factors and its solidification time.

TABLE IX: ANOVA of DTS

Source of Variation	DF	F_{value}	P_{value}	Status
X_1	1	40.33	0	Reject H_0
X_2	1	16963.04	0	Reject H_0
Interaction	**1**	**2.62**	**0.144**	**Accept H_0**
Error	8			
Total	11			

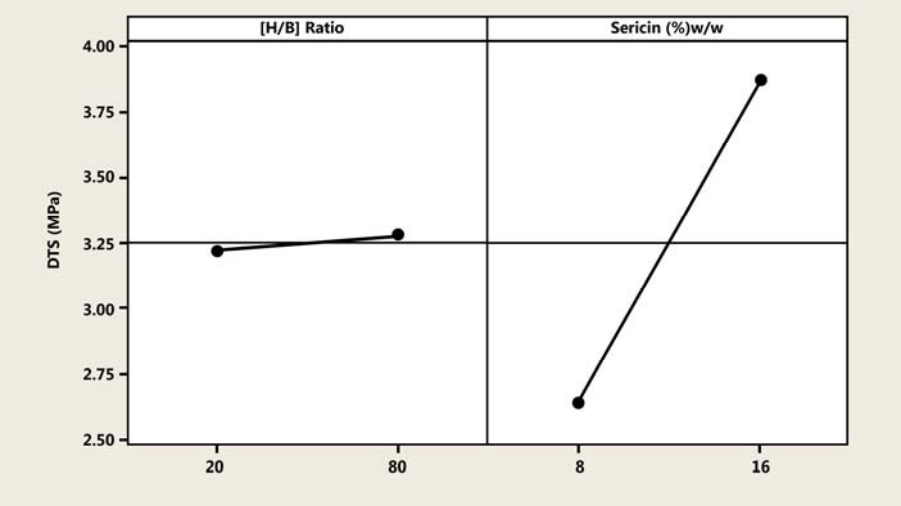

Fig. 6. Interaction between factors and its diametral tensile strength.

Once the significant factors are identified (X_1=80/20; X_2=16%, X_3=0.5mm), using Minitab 15's Factorial Design analysis tool, regression coefficient for Y_t and Y_d could be determined. By putting the regression coefficient into equation1 and -2, these could be written in equation-3 and -4. In these, Y_t and Y_d noted as solidification time of *t* and diametral tensile strength of *DTS*. Both were described in equation-3 and -4.

$$Y_t = 312.71 - 59.79X_1 - 59.79X_2 + 152.29X_3 - 52.71X_1X_2 \quad (3)$$

$$Y_d = 3.24 + 0.03X_1 + 0.62X_2 \quad (4)$$

By inserting the value of X_1=80/20, X_2=16% and X_3=0.5mm into equation-3 and -4, the prediction of solidification time, Y_t=106.39sec with confidence level of 97.31% and Y_d=3.46MPa with confidence level of 99.89%. These prediction results obtained by equation-3 and -4 slightly lower than that confirmed by experiment of *t*=107sec (0.57%) and *DTS*=3.88MPa (10.82%), respectively. In term of tensile strength of [H/B/S] composite, it was found that sericin could improve diametral tensile strength better than that of hydroxyapatite. However, as hydroxyapatite and sericin have different potential benefit for biomaterial that both of them can be complement and give contribution in another way. Thus, these materials can be contained into one single composite material. Hydroxyapatite has bioactive and bioinductive characteristic as found in the reference [1-9], whilst sericin is a protein [11-19,25] that could enhance the growth and attachment of fibroblast cell in live bone as suggested by Sukjai *et.al* [19] and Bhumiratna *et.al.* [24]. These valuable finding in the present work of solidification time (t) and diametral tensile strength would be very important and useful information in the development of the new additive manufacturing process of biomaterial as part of this research.

IV. CONCLUSIONS

Investigation has been done to find the shortest solidification time and the highest diametral tensile strength of the biocomposite. There have been two composition ratios of hydroxyapatite to bioplastic [H/B]=20/80 and 80/20 with varying sericin content of 8-16%w and layer thickness of 0.1-0.5mm to obtain the highest DTS and the lowest solidification time. Method of 2^k Factorial Design was used to develop the composite composition and ANOVA was applied to evaluate it statistically. the highest diametral tensile strength was achieved by the sample with composition ratio of [H/B]=80/20 which contained sericin of 16%w/w. Applying this ratio, the sample with layer thickness of 0.5 mm and sericin content of 16%w, its diametral tensile strength calculated by regression model Y_t=3.46 MPa. It was 10.82% slightly lower than that of experiment result of 3.88MPa. While, for solidification time Y_d=106.39 sec was 0.57% lower than that obtained by experiment of 107sec. All these information could open the new way in developing an additive manufacturing method for biomaterials based-product.

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