The Effect of Variation of Collagen-Hydroxyapatite Synthesis Parameters on Compressive Strength using Taguchi Method

Wibawa, T[1*], Fitri, F[1], Putro, G.M, Setiawan, A[2], Prijoto[3]

[1] Department of Industrial Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta, Yogyakarta, 55283, Indonesia
[2] Department of Metallurgical Engineering, Faculty of Mineral Technology, Universitas Pembangunan Nasional Veteran Yogyakarta, Yogyakarta, 55283, Indonesia
[3] Department of Geophysical Engineering, Faculty of Technology Mineral, Universitas Pembangunan Nasional Veteran Yogyakarta, Yogyakarta, 55283, Indonesia

*Corresponding author: tri.wibawa@upnyk.ac.id

Abstract

Bone is a vital human organ that has many functions. One of the functions is as a buffer for the human body. In 2015, the rate of bone damage due to osteoporosis in Indonesia reached 19.7%. Meanwhile, in 2018 fracture sufferers in Indonesia reached 5.5% of the entire population of Indonesia. From the case above, the problem that arises is the increase in demand for a bone graft that exceeds capacity. Synthetic bone graft is the solution to this problem. Collagen-Hydroxyapatite is the main component in the manufacture of bone grafts. The results of the bone graft were tested for compressive strength, then tested for SEM-EDX-Mapping. The compressive strength test value obtained is processed using the Taguchi method to find how much influence the selected factors have. The compressive strength of the trabecular bone is the target value for this synthetic bone graft. Based on the research, the best compressive strength value in sample 1 is 0.176 MPa. While, the most influential factor on the compressive strength value is the ratio of collagen and hydroxyapatite, which has a percent contribution value of 6.8504%. The results of the SEM-EDX test showed that there were three components with the largest percentage, namely 35.27% carbon, 33.89% phosphorus oxide, and 24.03% calcium oxide.

Keywords: Bone graft, hydroxyapatite, collagen, compressive strength, taguchi method

1. Introduction

The bone is a vital human organ that serves as the attachment site for muscles. So, there is movement between the joints bones to each other. Bones also have other functions as a buffer for the human body, protecting organs from impact, storing minerals and energy, and producing blood cells. Therefore, this vital function of bones encourages humans to maintain bone health. The bone disease often goes unnoticed. A bone disease characterized by rheumatic pain. It must be treated quickly to avoid the occurrence of an epidemiological transition towards chronic disease. Based on data from the Hospital Information System (in the Indonesian Ministry of Health, 2015), there are 200 fractures out of 100,000 cases due to osteoporosis. In addition, data from the Indonesian Ministry of Health (Santhi, 2020), fractures/broken bones in Indonesia reach 5.5% of the entire population. The solution is a bone graft to replace and regenerate damaged bone. Based on data from the hospital dr. Soetomo (Taufik et al, 2017), freeze-dried bone allograft production showed an increase from year to year. In 2001 and 2006, the hospital produced 148 and 199 bone grafts. The availability of autograft and allograft is insufficient to supply the increasing demand for bone grafts (Ichsan, 2012). Thus, a synthetic bone graft is an alternative for bone healing. Synthetic bone graft has biocompatible properties. In addition, bone graft also provides advantages in the process of osteoconduction, osteoinduction, and osteogenesis. Collagen and hydroxyapatite are the main ingredients for synthetic bone grafts. The manufacturing process uses the freeze-drying method, which begins with the freezing process and continues with the drying process. Then, testing compressive strength and SEM-EDX for the bone graft.

There are two purposes of this research. First, determine the optimal level parameters, so the bone graft characteristics are similar to real human bone. Second, analyzing bone graft through compressive strength testing and SEM-EDX, and finding the most influential factors on compressive strength using the Taguchi method. Based on previous research, Ichsan, M.Z. et al (2013), bone graft from collagen-hydroxyapatite synthesis has a compressive strength value of 842 KPa (0.8 MPa). This study used process parameters, namely the comparison of the composition of collagen-HA, freezing time, and the composition of collagen, CH_3COOH, and $Na_2HPO_4.H_2O$. The results of the compressive strength of each experiment based on these design parameters will be processed using the Taguchi method. The compressive strength of bone graft compared with the compressive strength of human bone in the trabecular bone, which was 0.5-50 MPa (Grimm, 2004).

2. Methods

The research was carried out at Material Physics Laboratory Airlangga University, Institute of Tropical Disease Airlangga University, and the UPT Integrated Laboratory Diponegoro University. Data processing is described using the Taguchi method. This research uses the quality characteristic, namely Larger The Better, by choosing the highest value. The SNR formula for the Larger The Better quality characteristic is:

$$M.S.D. = \frac{1}{N}\sum_{i=1}^{n}(\frac{1}{y_i})^2 \qquad (1)$$

$$\eta = -10.Log(M.S.D)$$

y_i as data for each experimental number. N is the number of replications for each experimental number.

Experimental factors consist of controlled and uncontrolled factors. The controlled factors showed in Table 1. While, the uncontrolled factors are the temperature of the laboratory room used for the experiment. Based on the obtained orthogonal array, the experiment was made four times with different parameters, and the experimental layout used was L_4.

Table 1. The controlled factors

Code	Factors	Level 1	Level 2
A	Ratio of collagen : HA	1:2	1:4
B	Freezing time	3 hours	5 hours
C	Ratio of collagen : CH_3COOH : $Na_2HPO_4.H_2O$	1:2:2	1:1:1

Making bone graft based on previous research. A bone graft consists of a mixture of collagen and hydroxyapatite. Next, dissolve the collagen in acetic acid, and add $Na_2HPO_4.H_2O$ in the solution (ratio w/v/w 1:1:1). Then, neutralize the mixture with 1 M NaOH. Meanwhile, the hydroxyapatite was dissolved in 0.6 M phosphoric acid (ratio w/v 1:4) and stirred. The solution was neutralized by NH_3 dropwise with a glass pipette. Neutrality (pH) was measured using litmus paper. Then, mix the neutral hydroxyapatite and collagen solutions using a magnetic stirrer. The mixture was deposited overnight, then molded using a straw. Then, freeze the sample with a freezing temperature of -80°C and a predetermined time based on the experimental layout. Put the composite in a freeze-dryer for 12 hours. Then, test the sample through mechanical testing. Mechanical testing carried out is the compressive strength test

3. Results and Discussion

3.1. The Result of Bone Graft

Hydroxyapatite and collagen used were obtained from Genelinx International Inc and Nitta Gelatin India Limited. Bone graft made using the freeze-drying method. The level factor was selected using references from previous studies and original data regarding human bones. Based on the composition of healthy bones, there are 20-25% collagen and 60-66% hydroxyapatite (Ficai et al, 2011). It is the basis for choosing the factor value in the ratio of collagen and hydroxyapatite. Freezing time affects the crystals formed in the sample. The crystals encourage the hydroxyapatite to form strong bonds so that the compressive strength test results are high. However, there is a limit to the period used to freeze the sample. Because at high temperatures, there will be changes in the properties of the test object if it is not in the range. It is the basis for choosing the factor value in the freezing time. The graph of the period for freezing the sample showed in Figure 2. The last factor is the ratio of collagen: CH_3COOH: $Na_2HPO_4.H_2O$ affects the stability of the deposition of chemical elements (Jebahi, et al., 2013).

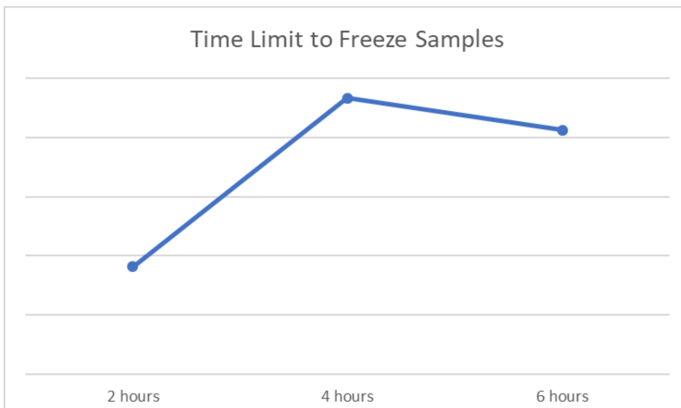

Figure 2. Freezing time range
(Saraswati et al, 2015)

The results of the bone graft were four experimental types with three replications each. The resulting bone graft showed in Figure 3.

Figure 3. Bone graft

3.2. The Result of the Taguchi Method

Mechanical testing of the bone graft using compressive strength test. The results of the compressive strength test were processed using the Taguchi method. Calculations use the mean and SNR values. It showed in Table 2 and Table 3. And, the results of the response table for the mean value and SNR showed in Table 4 and Table 5.

Table 2. The result of calculating the mean

No.	Repeat to-			*Mean*
	1	**2**	**3**	($\bar{y}$)
1	0.176	0.014	0.037	0.0757
2	0.019	0.017	0.030	0.0220
3	0.014	0.029	0.017	0.0200
4	0.008	0.008	0.010	0.0087

Table 3. The result of calculating the SNR

No.	Repeat to-			SNR
	1	**2**	**3**	
1	0.176	0.014	0.037	-32.9113
2	0.019	0.017	0.030	-33.8866
3	0.014	0.029	0.017	-35.1194
4	0.008	0.008	0.010	-41.3830

Table 4. The response table of mean value

Level	A	B	C
1	0.0488	0.0478	0.0422
2	0.0143	0.0153	0.0210
Delta	0.0345	0.0325	0.0212
Rank	1	2	3

Table 5. The response table of SNR value

Level	A	B	C
1	-33.40	-34.02	-37.15
2	-38.25	-37.63	-34.50
Delta	4.85	3.62	2.64
Rank	1	2	3

Based on the results of the response table, the mean value has an optimum point in the combination of $A_1B_1C_1$ process parameters. And, the SNR value has an optimum point in the combination of $A_1B_1C_2$ process parameters. The calculation of ANOVA for the mean values is shown in Table 6.

Table 6. ANOVA calculation for the mean value

Source	v	SS'	p%	SS	MS	F Ratio
A	1	0.0016	68.504	0.0036	0.0036	18.322
B	1	0.0012	51.524	0.0032	0.0032	16.259
C	1	-0.0006	-25.547	0.0013	0.0013	0.6897
Se	8	0.0214	905.519	0.0156	0.0019	10.000
SS_t	11	0.0237	1.000.000	0.0237		
Sm	1			0.0120		
ST	12			0.0356		

Based on the ANOVA calculation, the value of the F-table ($F_{0.05;1;8}$) is 5.3177. Factor A is the factor with the most contribution value. Factor A has a contribution value of 6.8504%. However, based on the value of the F-table and F-ratio, factor A is insignificant to the experimental results.

While the very insignificant factor is factor C, so this factor is eliminated. The results after pooling up showed in Table 7. Then, the ANOVA calculation for the SNR value showed in Table 8.

Table 7. ANOVA calculation for mean value (pooling up)

Source	v	SS'	p%	SS	MS	F Ratio
A	1	0.0016	68.504	0.0036	0.0036	18.976
B	1	0.0012	51.524	0.0032	0.0032	16.840
C				0.0013		
Se				0.0156		
Pooled	9	0.0208	879.971	0.0169	0.0019	
SSt	11	0.0237	100.00	0.0237	0.0022	
Sm	1			0.0237		
ST	12			0.0120		

Table 8. ANOVA calculation for SNR value

Source	v	SS'	p%	SS	MS	F Ratio
A	1	165.530	326.952	242.446	242.446	33.675
B	1	61.087	120.658	131.003	131.003	18.737
C				69.916		
Se	1	279.665	552.390	69.916	69.916	10.000
SSt	3	506.282	1.000.000	506.282		
Sm	1			51.337.461		
ST	4			51.773.827		

The value of the F-table ($F_{0.05;1;8}$) is 5.3177. Based on the calculation results, the deviation of the data is 55.2390%. It means that this value is unacceptable. This is because the Taguchi data deviation requirement is a maximum of 50% (Damayanti, 2017). Verify the prediction of optimal conditions using the confirmation experiment stage. Confirmation experimental samples amounted to five test samples. This experiment uses the best combination of parameters from calculating the mean value. The data from the confirmatory experimental compressive strength test results showed in Table 9.

Table 9. Conformation experiment

No. Experiment	Compressive Strength Test Value
1	0.045
2	0.018
3	0.020
4	0.031
5	0.035
Total (y)	0.149
Mean ($\bar{y}$)	0.030
SNR	-32.01

Then, processing the data so that the confidence interval values for the mean and SNR founded. The comparison of the predicted and optimal confidence intervals showed in Table 10.

Table 10. The confidence intervals

Response (compressive strength of bone graft)		**Prediction**	**Optimal**	
			(-)	**(+)**
Taguchi Experiment	Mean	0.07	0.02	0.11
	SNR	-31.59	-41.44	-21.74
Confirmation Experiment	Mean	0.03	-0.04	0.10
	SNR	-32.01	-43.10	-20.92

Based on the comparison results, the data from the confirmation experiment for the mean and SNR values are within the confidence interval of the optimal value. So, the data is acceptable.

3.3. The Result of SEM-EDX-*Mapping*

The SEM-EDX-Mapping test used a magnification of 200, 500, 1000, and 2000. The aim is to determine the size of the sample pores and the shape of the macropores. The sample tested is the sample with the best combination of parameters. The results of the SEM-EDX-Mapping test showed in Figures 4 to 8.

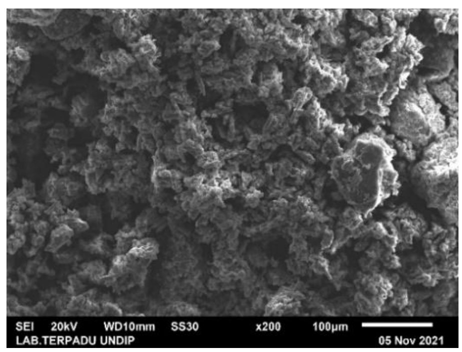

Figure 4. The resulf of SEM with 200x magnification

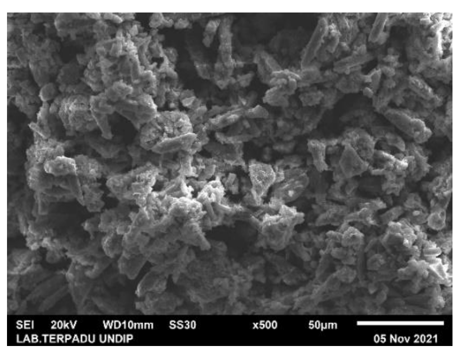

Figure 5. The resulf of SEM with 500x magnification

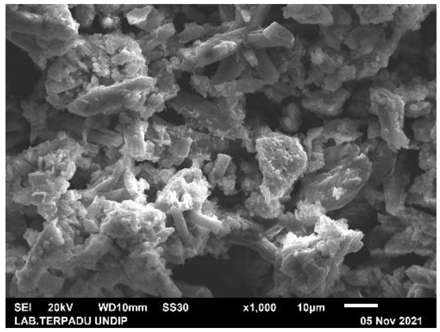

Figure 6. The result of SEM with 1000x magnification

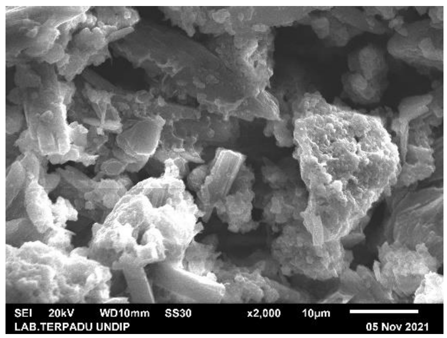

Figure 7. The result of SEM with 2000x magnification

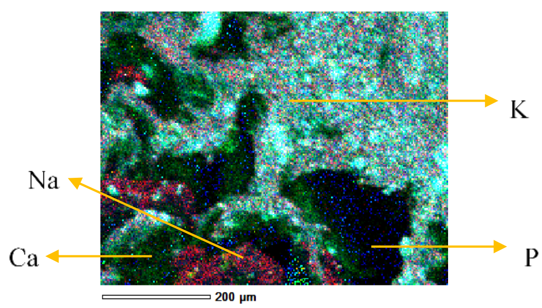

Figure 8. Mapping of components in samples

Based on the image of the SEM-EDX test results, a fine lump dense but brittle formed. The size of the pores is not uniform and large. It is because the formation of ice crystals is imperfect. In addition, the most content in the sample is that there are three components. The three components are carbon (C), phosphorus oxide (P_2O_3), and calcium oxide (CaO).

4. Analysis

Bone graft is a replacement material for patients with fractures. Based on the experimental results, the compressive strength values range from 0.008 to 0.176 MPa. This value does not meet the target of trabecular bone, which is in the range of 0.5 – 50 MPa. In addition, this value is also less than the previous research, which is in the range of 0.71 – 0.84 MPa. It is because of uncontrollable factors at the time of the experiment.

The calculation of the Taguchi method with ANOVA of mean values showed that factor A (ratio of collagen and hydroxyapatite) and factor B (freezing time) were less significant based on the value of F-Ratio. Meanwhile, factor C (ratio of collagen, CH_3COOH, and $Na_2HPO_4.H_2O$) is a very insignificant factor. Several factors caused this low compressive strength test value. There is a difference in the mixed material used from previous research. Purchases of materials are not at the same place due to limited costs, and the place of purchase is no longer producing. In addition, the relatively far distance from the freezer and the laboratory makes the sample when it enters the freeze dryer not completely frozen. Another factor that makes the compressive strength test values different is the different sediment yields.

The experimental results showed changes in the combination of parameters for the manufacture of bone grafts. Details of the latest parameter combinations are the ratio of collagen and hydroxyapatite of 1:2, freezing time of 3 hours, and the ratio of collagen, CH_3COOH, and $Na_2HPO_4.H_2O$ of 1:2:2. The test values for confirmation experiments ranged from 0.018 – 0.045 MPa. The difference in ranges occurs because there are factors beyond control, so it cannot reach 100% the same treatment for each replication.

The SEM test aims to support the reason for the low value of the compressive strength test. The results show that the pores are large. It causes a space so that the result is a brittle bone graft. In addition, ice crystals formed imperfectly. Thus, the bone graft density is not maximal. The EDX results show the content contained in the sample. Components that have a high value are carbon, phosphorus oxide, and calcium oxide. The respective percentages of these components are 35.27%, 33.89%, and 24.03%.

5. Conclusion

The compressive strength test value did not reach the target of the original bone or previous studies. It was due to several factors previously mentioned. The optimal level setting shows changes in composition and process procedures from previous research. Details of the process are a ratio of collagen and hydroxyapatite of 1:2, freezing time of 3 hours, and a ratio of collagen, CH_3COOH, and $Na_2HPO_4.H_2O$ of 1:2:2.

The SEM test results showed that the pore size was large, ice crystals were not perfect, and the collagen and hydroxyapatite composites were brittle. EDX test results show three components with the largest percentage in the sample. These components are 35.27% carbon, 33.89% phosphorus oxide, and 24.03% calcium oxide. Based on the Taguchi method, the factors used are less influential. The ratio factor of collagen, CH_3COOH, and $Na_2HPO_4.H_2O$ is a very insignificant factor. Based on the conclusion, the suggestions for further research are as follows.

1. Conducting experiments in one complete place regarding the tools and materials.
2. Adding more parameter factors so the research can get more accurate results.
3. Increase the number of sample replications to produce more accurate data.
4. Procurement of testing for toxicity to determine its compatibility with the human body.

Acknowledgements

During the preparation of this paper, the author received guidance from various parties. In this opportunity, the author would like to say my deepest gratitude to:

1. The LPPM (Institute for Research and Community Service) has provided grant funds for research in this Final Project.
2. Drs. Siswanto, M.Sc., and Mr. M. Nurur Rochman, as Field Supervisor, who guided when conducting experiments at the UNAIR Material Physics Laboratory during the research.

References

Damayanti, M. K. (2017). Desain Parameter Eksperimen Untuk Optimasi Nilai Frangibility Factor Material Komposit Dengan Metode Taguchi Dan Neural Network. (Skripsi, Institut Teknologi Sepuluh November, 2017). Retrieved from http://repository.its.ac.id/1760/

Ficai, A., Andronescu, E., Voicu, G., & Ficai, D. (2011). Advances in Collagen/Hydroxyapatite Composite Materials. (D. B. Attaf, Ed.) *Advances in Composite Materials for Medicine and Nanotechnology*, 3-32. Retrieved from https://www.intechopen.com/books/advances-in-composite-materials-for-medicine-and-nanotechnology/advances-in-collagen-hydroxyapatite-composite-materials

Grimm, M. J. (2004). Orthopaedic Biomaterials. In T. Mcgraw-Hill, *Standard Handbook Of Biomedical Engineering And Design* (pp. 15.1-15.22). Michigan: Digital Engineering Library McGraw-Hill. Retrieved from http://ptik.unhas.ac.id/tahir/BAHAN-KULIAH/BIO-MEDICAL/NEW/HANBOOK/15_Orthopedic_materials.pdf

Ichsan, M. Z. (2012). Sintesis Makroporus Komposit Kolagen-Hidroksiapatit Sebagai Kandidat Bone Graft. (Skripsi, Universitas Airlangga, 2012). Retrieved from http://repository.unair.ac.id/25703/1/ICHSAN%2C%20MIRANDA%20Z.pdf

Ichsan, M. Z., Siswanto, & Siswanto, D. (2013, Januari). SINTESIS KOMPOSIT KOLAGEN-HIDROKSIAPATIT SEBAGAI KANDIDAT BONE GRAFT. *Jurnal Fisika dan Terapannya, 1*, 89-103. Retrieved from http://journal.unair.ac.id/download-fullpapers-Miranda.pdf

Jebahi, S., Oudadesse, H., Wers, E., Elleuch, J., Elfekih, H., Keskes, H., . . . Elfeki, A. (2013, July). Effect of pH and Ionic Exchange on the Reactivity of Bioglass/Chitosan Composites Used as a Bone Graft Substitute. *World Academy of Science, Engineering and Technology International Journal of Materials and Metallurgical Engineering, 7*. doi:doi.org/10.5281/zenodo.1088500

Kementerian Kesehatan RI. (2015). Data & Kondisi Penyakit Osteoporosis di Indonesia. *Infodatin Pusat Data dan Informasi Kementerian Kesehatan RI*, 1-6.

Santhi, & Mutiara, P. K. (2020). Gambaran Asuhan Keperawatan Pada Pasien Post Operasi Open Reduction Internal Fixation Fraktur Ekstremitas Bawah dengan Nyeri Akut di Ruang Sandat BRSU Tabanan Tahun 2020. Denpasar: Poltekkes Denpasar.

Saraswati, M. D., Siswanto, & R., D. I. (2015). Pengaruh Variasi Suhu Pembekuan terhadap Sifat Fisis dan Sifat Mekanik Komposit Kolagen-Hidroksiapatit sebagai Bone Graftdengan Metode Freeze-Drying.

Taufik, A., Zuhan, A., Kusdaryono, S., & Rohadi. (2017). Karakterisasi Hydroxyapatite Alami yang Dibuat dari Tulang Sapi dan Cangkang Telur sebagai Bahan untuk Donor Tulang (Bone Graft). *Kedokteran Unram*, 9-13.

Biography

Tri Wibawa is lecturer at Industrial Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta. He graduated M.T. (Magister Teknik) in Industrial Engineering and Management, from the Institute of Technology Bandung (ITB), Indonesia in 2005 and a B.Sc in Mechanical Engineering from the University of Gadjah Mada (UGM), Indonesia in 1997. He has been teaching at UPN specializing in Industrial Engineering courses, especially in product design and manufacturing processes. He supervises undergraduate students as part of his academic responsibilities.

Firdaus Fitri graduated B.Sc from Industrial Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta. She graduated in 2021, especially in product design and manufacturing processes.

Gunawan Madyono Putro is lecturer at Industrial Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta. He graduated B.Sc and M.T. (Magister Teknik) Industrial Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta. He has been teaching at UPN specializing in Industrial Engineering courses, especially in product design and manufacturing processes.

Agris Setiawan is lecturer at Metalurgy Engineering, Faculty of Mineral Technology, Universitas Pembangunan Nasional Veteran Yogyakarta. He graduated M.Eng. (Magister Engineering) from from Mechanical Engineering, University of Gadjah Mada, Indonesia in 2016 and B.Sc from Deparment of Mechanical Engineering Education, State University of Yogyakarta (UNY), Indonesia in 2010.

Prijoto is lecturer at Geophysic Engineering, Faculty of Mineral Technology, Universitas Pembangunan Nasional Veteran Yogyakarta. He graduated Magister from University of Sebelas Maret, Indonesia and B.Sc from State University of Yogyakarta (UNY), Indonesia.