Modelling of Average Pore Diameter and Porosity of Porous PCL/HA Composite to the Foaming Conditions by Using ANOVA Analysis

Sofea Hanom Nordin[a], Suffiyana Akhbar[a]

[a]Faculty of Chemical Engineering, Universiti Teknologi MARA, Selangor, Malaysia

Abstract

Porous polycaprolactone/hydroxyapatite (PCL/HA) composite is said to be the most stable biodegradable scaffold for bone tissue regeneration. The objective of this study is to develop mathematical relationship between the average pore diameter and porosity of porous PCL/HA composite and investigate the correlation between temperature and pressure of foaming process with the presence of HA content. The composite was prepared through solid state foaming process of supercritical carbon dioxide ($scCO_2$). In this study, temperatures and pressures of foaming process were varies from 40℃ to 45℃ and 10MPa, 20MPa and 30MPa, respectively. In addition, HA content also were varied at 10, 20, 30 and 40 wt%, respectively. The analysis of variance (ANOVA) was done by using Microsoft Excel. The develop model shows that average pore diameter is increased with temperature and will decrease if the interaction between pressure and HA content increases. Meanwhile, develop model for porosity shows that temperature is a sole effect in increasing the porosity value. The develop model also indicated that the designated model for porosity have a high value of coefficient of determination (R^2) with 0.97 which means that it is highly fitted, while the develop model for average pore diameter is consistent with the theory which is towards the temperature and interactions between pressure and HA content. However, the fitness of the model is only 0.74 due to one data that is deviated far from the others. Therefore, validation with different values is recommended for future research.

Article Info

Article history:

Received date: DD Month 20XX
Accepted date: DD Month 20XX

Keywords:

PCL/HA
Composite
Foaming conditions
ANOVA
Bone scaffold
Average pore diameter
Porosity

1.0 Introduction

Tissue engineering (TE) is define as an interdisciplinary field that use the principles of engineering and life sciences towards the development of tissues' organ, which can regenerate, maintain, or improve the current tissue organ. For tissue engineering that applied to bone generation by tissue-engineered implant is called as bone tissue engineering (BTE) (Z. Chen, 2017). The main purpose of developing BTE is to protect the tissue inside the body and to increase the regeneration rate of bone tissue as well as act as temporary tissue to replace the damage tissue. Tissue implantation is usually applied to an area with a large damage tissue due to an accident that is almost impossible to recover.

In recent study, the researchers have further expanded the scope of BTE by developing a new tissue regeneration aid called biodegradable scaffolds. Scaffolds are 3D engineering materials that can stimulate desirable attachment and proliferation to contribute to the formation of new functional tissues (T. Ghassemi et al., 2018).

Pore diameter and porosity are two important physical properties in fabricating bone tissue scaffold. Pore diameter is crucial as it can affect the quality and characteristics of the new bone tissue formation (Y. Haiying et al., 2007). Meanwhile porosity is important due to its role in controlling cell functions and to guide formation of the new tissues (Y. Haiying et al., 2007).

According to T. Ghassemi et al. (2018), the proper structure of porosity and pore diameter in designing bone scaffolds are 80 to 95% and 100 to 402.5μm respectively. The structure arrangement is important for cell penetration, nutrients transfer, waste transfer and angiogenesis. In addition, B. Thavornyutikarn, and team also showed almost similar research, where they stated that the fabricated bone scaffolds need to be highly porous where the porosity should be more than 90% and interconnected with the pore diameter in the range of 300 to 500μm. The highly porous bone scaffold will make the cells to be easily penetrated into the pore structure and helps to promote the new bone formation, as well as vascularisation (B. Thavornyutikarn et al., 2014).

Porous PCL/HA composite has received considerable amount of attention to be the most suitable candidate of bone tissue scaffold in the BTE field due to its excellent biocompatibility, slow degradability, no toxicity and its ability to promote bone tissue cell growth (V. Brun et al, 2014). This porous PCL/HA scaffold is said to be obtained via solid-state supercritical carbon dioxide ($scCO_2$) foaming process which have a high porosity of ~87% and pore diameter of 150 to 200μm (K. Hae-Won et al., 2003).

The purpose of adding HA to the PCL is to improve the bone growth or bone regeneration for the damaged tissue or bone. However, the presence of HA in PCL will hinder the diffusivity of supercritical carbon dioxide ($scCO_2$) to the PCL/HA composite in the foaming process and consequently will affect its pore diameter and porosity. On top of that, foaming condition; temperature (T) and pressure (P) also will influence the pore diameter and porosity of the PCL/HA composite (A. Salerno et al., 2014). Therefore, this study is conducted to investigate the correlation between porous PCL/HA composite's pore diameter and porosity towards HA content, as well as foaming process parameter (T, P) and its interactions by developing mathematical model. The mathematical model was developed using Microsoft Excel using analysis of variance (ANOVA).

2.0 Methodology

2.1 Mathematical Model

The mathematical model from Microsoft Excel is used in this study. The purpose of developing these mathematical models are to study the relationship between average pore diameter and porosity of porous PCL/HA composite with HA content and the foaming conditions (temperature and pressure) of solid state supercritical carbon dioxide ($scCO_2$) interactions.

Microsoft Excel is one of the statistical tools that use the quantitative data from various experimental designs to determine and simultaneously solve the multivariate equations (G. Harvey, 2006; G. Harvey, 2007). Microsoft Excel explores the relationship between several independent variables towards one or more dependent variables. Figure 1 shows the chronology on the Microsoft Excel procedure applied in this study.

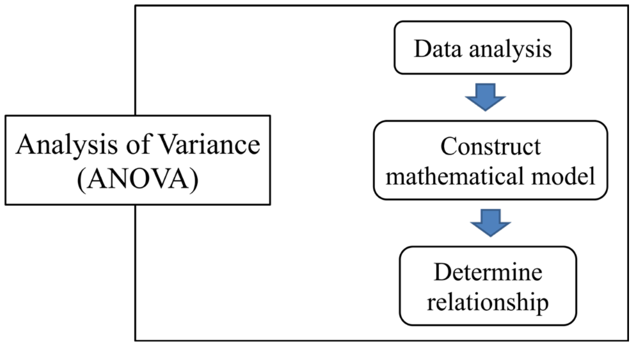

Figure 1: Microsoft Excel procedure.

2.2 Variables Selection

Table 1 shows the summary of all variables used in this study which are hydroxyapatite (HA) content inside the scaffold, temperature and pressure of the foaming conditions. These variables will be the potential predictor towards the dependent variables which are pore size and porosity of the scaffold.

Table 1: Description of variables

Variables	Code
Pore diameter (μm)	Dependent (Y1)
Porosity (%)	Dependent (Y2)
Pressure (MPa)	Independent (A)
Temperature (℃)	Independent (B)
HA content (%)	Independent (C)

The table shows that the dependent variables which are pore size and porosity of scaffold are coded as Y1 and Y2 respectively while the independent variables such as pressure, temperature and HA content are coded as A, B and C respectively. The purpose of this coding is to make the analysis work faster and efficient.

2.3 Design of Experiment

The data used in the experimental design are acquired from the foaming conditions of solid state supercritical carbon dioxide ($scCO_2$) in a slow depressurization process (A. Salerno et al., 2014; J. Ivanovic et al., 2016) such as:

1) Foaming pressure (MPa): 10, 20 and 30
2) Foaming temperature (℃): 40 and 45
3) HA content (%): 10, 20, 30 and 40

Table 2 shows the experimental design and the results of average pore diameter and porosity of foamed samples that will be used in this study.

Table 2: Design of Experiment and Results of Average Pore Diameter and Porosity of PCL/HA composite

Run	A	B	C	AB	AC	BC	Average Pore Diameter (μm)	Porosity (%)
1	10	40	0	400	0	0	159.37	48.12
2	10	45	0	450	0	0	271.18	51.75
3	20	40	0	800	0	0	128.94	70.91
4	20	45	0	900	0	0	137.12	74.83
5	30	40	0	1200	0	0	98.85	70.48
6	30	45	0	1350	0	0	117.46	78.42
7	10	40	10	400	100	400	195.87	45.38
8	10	45	10	450	100	450	300.67	47.3
9	20	40	10	800	200	400	100.43	65.54
10	20	45	10	900	200	450	116.69	70.59
11	30	40	10	1200	300	400	48.54	63.36
12	30	45	10	1350	300	450	42.8	76.08
13	10	40	20	400	200	800	307.22	45.84
14	10	45	20	450	200	900	213.08	55.87
15	20	40	20	800	400	800	105.23	66.29
16	20	45	20	900	400	900	157.21	73.38
17	30	40	20	1200	600	800	34.82	61.71
18	30	45	20	1350	600	900	45.43	73.95
19	10	40	30	400	300	1200	290.84	46.4
20	10	45	30	450	300	1350	484.57	57.73
21	20	40	30	800	600	1200	119.42	64.44
22	20	45	30	900	600	1350	158.06	64.67
23	30	40	30	1200	900	1200	45.54	59.22
24	30	45	30	1350	900	1350	44.7	70.03
25	10	40	40	400	400	1600	281.71	41.7
26	10	45	40	450	400	1800	257.82	47.14
27	20	40	40	800	800	1600	117.57	60.14
28	20	45	40	900	800	1800	141.95	66.39
29	30	40	40	1200	1200	1600	53.11	65.91
30	30	45	40	1350	1200	1800	43.07	68.43

2.4 Analysis of Variance (ANOVA)

A polynomial linear regression model was used to represent the relationship between the output variables (average pore diameter and porosity) and input variables (pressure, temperature, HA content and its interactions). This model was chosen because it shows linear behaviour which usually occurs in the physicochemical analysis of mixture ingredients (D. Granato & V. M. de Araujo calado, 2014).

The general formulas (W. Kenton, 2019) that will be used for this model are:

1) For average pore diameter of scaffold:

$$y_1 = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC \quad (1)$$

2) For porosity of scaffold:

$$y_2 = \beta_o + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC \quad (2)$$

Based on the formula, y_1 (average pore diameter) and y_2 (porosity) are the dependent variables while A (pressure), B (temperature) and C (HA content) are the independent variables or parameters, along with AB, AC and BC which indicates the correlation or interactions between A, B and C parameters. Next, β_o is the intercept of regression equation (set to zero) and β_1 to β_6 are the regression coefficients.

Analysis of variance (ANOVA) was used to investigate the effect of parameters together with its interactions with each other towards the average pore diameter and porosity of the PCL/HA composite. The coefficient of determination (R^2) will be used in this study, where it is defined as a statistical tool that is used to measure the variance in the outcome which can be explained by the independent variables and followed by adjusted R^2 as the better version or revised version of R^2 (Stephanie, 2014). For this study, R^2 and adjusted R^2 need to be higher than 80% (0.8) to show that the correlation between the model and the data used are strong enough (T. Bock, 2017). Nevertheless, a high R^2 does not necessarily indicate that the model is adequate.

ANOVA also helps to study the reliability or the contribution of the data to the precision of models used with confidence level of 95% (p-value$\leq$0.05. Only the parameters and interactions that showed a significant effect will be chosen as the final regression model. Then, test for goodness of fit for the regression model was done; which comprises of test for significance of the regression model (Significance F≤0.05), analysis of residuals, normal probability plot and test for lack of fit (F. Andy, 2013).

3.0 Results and discussion

3.1 Analysis of Variance (ANOVA)

The results of the average pore diameter and porosity of the 30 runs on the PCL/HA composite samples are shown in Table 2. The initial linear regression model which showed the interrelationship between the dependent and independent variables are shown below:

1) For average pore diameter of scaffold:

$$y_1 = -1.5313A + 6.8987B + 3.1964C - 0.1389AB - 0.1784AC + 0.0201BC \quad (3)$$

2) For porosity of scaffold:

$$y_2 = 0.2451A + 0.9868B + 0.0756C + 0.0212AB - 0.0073AC - 0.0021BC \quad (4)$$

The results of ANOVA for initial regression model for both average pore diameter and porosity of the composite are presented in Table 3 and Table 4, respectively. From the results, an analysis will be done, which is evaluating the degree of fit of the regression model and to remove the insignificance parameters and interactions from the model. Basically, this analysis can be called as the test for significance of model.

Table 3: ANOVA Results for Testing the Significance of Regression Model for Average Pore Diameter

Factor and interactions	Coefficients	p-value
Intercept	0	null
A	-1.531321777	0.896465324
B	6.898702822	7.03569E-07
C	3.196438075	0.759865209
AB	-0.138901205	0.618564192
AC	-0.1783634	0.042970926
BC	0.020112932	0.9342366
Standard Error	52.90249521	
Total SS	1040635.022	
Significance F	9.76182E-13	
R Square	0.935454627	
Adjusted R Square	0.880341007	

Table 4: ANOVA Results for Testing the Significance of Regression Model for Porosity

Factor and interactions	Coefficients	p-value
Intercept	0	null
A	0.245093898	0.846728
B	0.98679939	5.253E-09
C	0.075641317	0.9464167
AB	0.021248909	0.4807098
AC	-0.00727075	0.4266109
BC	-0.00209036	0.936548
Standard Error	5.698987657	
Total SS	117638.6474	
Significance F	4.51227E-24	
R Square	0.99337392	
Adjusted R Square	0.95032682	

3.1.1 Average Pore Diameter of PCL/HA Composite

Based on the ANOVA results (Table 3), the average pore diameter of PCL/HA composite is influenced by the foaming temperature and the combined effect of foaming pressure and the presence of HA content; as both of the p-value is less than 0.05. These results are supported by the significance F of less than 0. However, the foaming temperature (B) has a more dominant effect towards the average pore diameter as a result of poor distribution of HA content (J. Ivanovic et al., 2016). From the results also, the foaming pressure (A), HA content (C) and the interactions of AB and BC does not influence the average pore diameter as the p-value is very far from 0.05. In addition, the value of R^2 and adjusted R^2 are more than 0.8 which are 0.94 and 0.88, respectively. These values indicated that the model is reliable to use.

3.1.2 Porosity of PCL/HA Composite

Next, ANOVA results for porosity are shown in Table 4. The results showed that the foaming temperature (B) is the sole influencer of the porosity of PCL/HA composite as it is the only parameter that has p-value less than 0.05 and was supported with the significance F value of less than 0.

The R^2 and adjusted R^2 also are very high which are 0.99 and 0.95, respectively. As these values are very close to '1', the model is considered reliable as it was predicted to have a satisfactory outcome. Since the p-value, R^2 and adjusted R^2 complies with the assumptions of a reliable model conditions, the model is adequate enough to be used to simulate the bone tissue formation in the BTE field.

Therefore, the proposed model for average pore diameter consists of B and AC while porosity consists only B. Hence, all the other parameters and interactions are eliminated for the revised model due to the incompliance of p-value (should be less than 0.05).

3.2 Development of the Model

ANOVA results showed that the values of R^2 for both of the models are more than 0.8; however, it does not necessarily means that the model is reliable. Therefore, test for lack of fit is performed and the test shows that not all the p-value is less than 0.05. These parameters and interactions were eliminated and a new model was developed as shown below.

1) For average pore diameter of scaffold:

$$y_1 = 4.8107B - 0.1258AC \quad (5)$$

2) For porosity of scaffold:

$$y_2 = 1.4522B \quad (6)$$

Equation (5) indicates that the average pore diameter will increases with the temperature but will decrease if there is an interaction with combined effect of HA content and pressure. This might happen due to the poor distribution of HA content. This model concurred with A. Salerno et al. (2014) statement, where he mentions that the average pore diameter will increase when temperature increase as the pore will be smaller in size. In addition, the average pore diameter is supposedly reduced when the amount of HA content increases as it will produce more pore at the end of the experiment.

This condition might happen due to an increase of viscosity of HA content and low diffusivity of CO_2 into PCL (M. J. Kim & Y. H. Koh, 2013) which means a higher viscosity would restrain the formation of pores and pores growth. In addition, HA content can affect by the $scCO_2$ solubility depends on its integration in polymer matrix since HA is very stiff filler which can hindered the CO_2 gas diffusion. The dispersion of filler in the matrix (the polymer 'wets' the filler particles) will result in decreases of sorption and uneven dispersion of the filler throughout the matrix (J. Ivanovic et al., 2016).

In contrast, Equation (6) showed that porosity only affected by temperature. As the temperature increase, porosity will increase as well. This means that all other interactions such as AB, AC and BC do not have a significance effect on the model as the temperature effect is the most dominant compared than the other parameters.

3.3 Checking the Adequacy of the Developed Model

The summary of results of the analysis for the revised models is shown in Table 5. The summary shows that both of the significance F for average pore diameter and porosity were approximately zero and adequate at 95% confidence. The standard error for porosity is acceptable which are 10.12; however, it is very high for average pore diameter at 97.57 and it might affect the reliability of the developed model.

Table 5: Test of Model vs. Residual

Response	**Average Pore Diameter (µm)**		**Porosity (%)**	
Test	Model	Residual	Model	Residual
df	2	28	1	29
SS	774070.2	266564.9	114668.9	2969.704
F	40.6542	-	1119.775	-
Significance F	7.17E-09	-	4E-24	-
P-value				
B	3.23E-08	-	1.01E-24	-
AC	0.020717	-	-	-
R Square	0.743844032		0.974755709	
Adj. R Square	0.698981319		0.94027295	
Standard Error	97.57137887		10.11946613	

The goodness of fit of the models was also tested by evaluating R^2 and adjusted R^2. R^2 and adjusted R^2 for average pore diameter is 0.74 and 0.7, respectively, while for porosity is 0.97 and 0.94, respectively. The revised regression model for porosity is fit as the R^2 value is still above 0.8 even after being adjusted. Unfortunately, it is not the case for average pore diameter. The model is not fit after being adjusted. This might cause from some casualty that happen during experiment.

In order to further test the adequacy of the model, normal probability plots was done. The normal probability plots for the average pore diameter and porosity are shown in Figure 2(a) and Figure 2 (b).

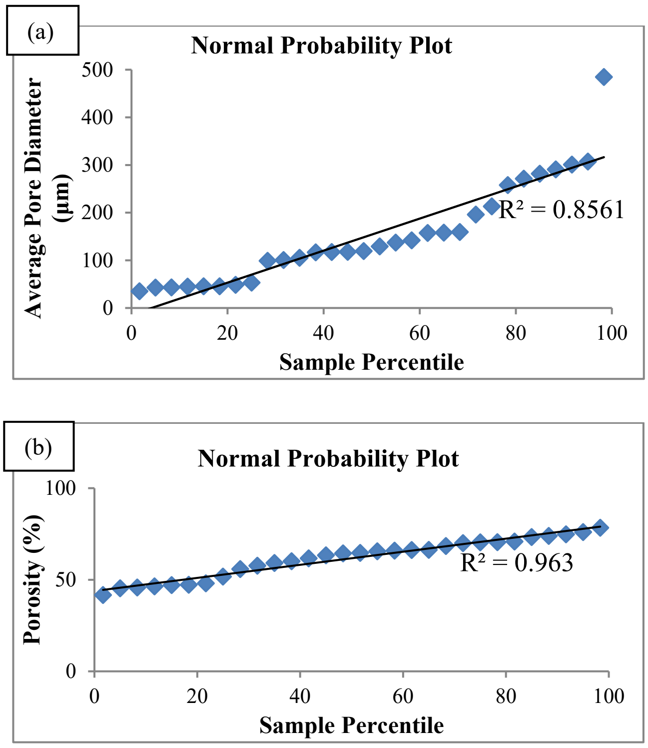

Figure 2: Normal probability plot for (a) average pore diameter; (b) porosity

Figure 2 shows that most of the data falls on a straight line which implies that the error was equally distributed within the data (M. S. Khan et al., 2014). The model usually will be considered as applicable in predicting the composite's properties. However, Figure 2(a) shows that one of the data deviated far from the others. This answer is expected as the standard error is very high (97.57) in the initial development of the model (refer Table 5). Therefore, it is suggested to repeat the experiment for that data to get a more appropriate result.

Furthermore, the residuals for both models were also analysed. The standard residuals against the predicted values was plotted to evaluate the feasibility of the revised model. In statistic, residuals are defined as the differences between the observed (measured) values and predicted values. The plots in Figure 3 shows that residuals are randomly scattered and indicates that is does not contradict with the linear assumption.

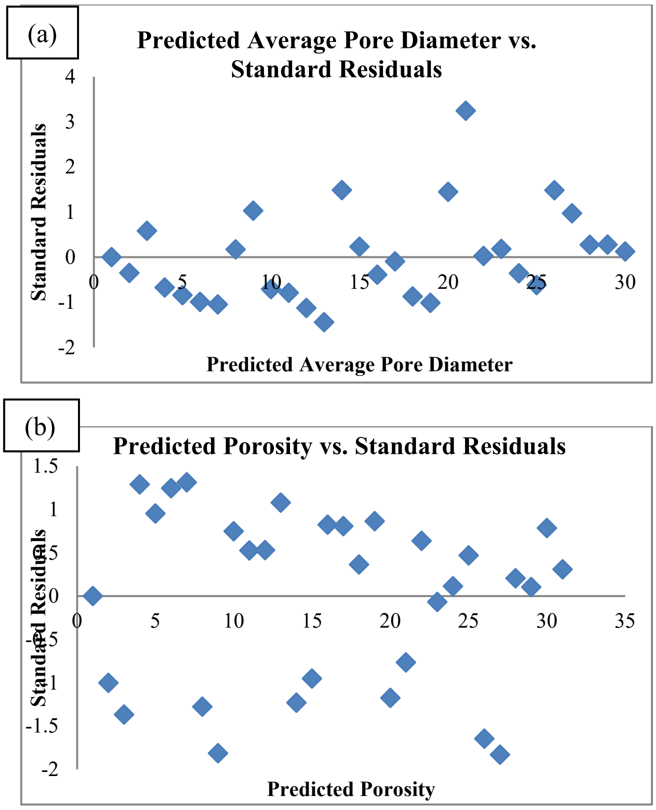

Figure 3: Plot of predicted value vs. standard residuals for (a) average pore diameter; (b) porosity

4.0 Conclusions

The study was run for 30 times to develop multiple linear regression (MLR) equations in order to predict the average pore diameter and porosity of different combinations of polymer PCL/HA composite; based on pressure, temperature and HA content. ANOVA was used to determine the significant parameters and interactions for the models and the adequacy of the models developed was tested using goodness of fit test and scattered diagram.

At the end of the study, the designed model of average pore diameter is found to be consistent with the theory where the average pore diameter is affected by temperature and the interactions between pressure and HA content. However, the fitness of the model is only 0.74 due to one data that is deviated far from the others. Meanwhile, develop model for porosity shows a high value of coefficient of determination (R^2) with 0.97 which means that the correlation between the model and data used are strong and will produce a good experimental results. In addition, these models can be used as a preliminary reference and guidance for fabricate other porous polymer composite using supercritical CO_2 solid state foaming process.

5.0 Recommendations

1. Validation of the model should be done to test the reliability of model with different variables and conditions.
2. For further research, variables for foaming temperature should be added to three or more instead of two.

Acknowledgment

This study was carried out by Sofea Hanom Nordin with the support of references. The researcher would like to thank Madam Suffiyana Akhbar, senior lecturer of University Technology Mara (UiTM) Shah Alam, for the excellent guidance throughout the whole process.

References

Z. Chen, (2017). *Fabrication and research of 3D complex scaffolds for bone tissue engineering based on extrusion deposition technique Fabrication and Research of 3D Complex Scaffolds for Bone Tissue Engineering Based on Extrusion Deposition Technique*. Thesis. 211.

T. Ghassemi, A. Shahroodi, M. H. Ebrahimzadeh, A. Mousavian, J. Movaffagh, & A. Moradi, (2018). *Current Concepts in Scaffolding for Bone Tissue Engineering*. The archives of bone and joint surgery. 90-99.

Y. Haiying, W. M. Howard, H. W. Paul, Y. Shang-You, (2007). *Effect of Porosity and Pore Size on Microstructures and Mechanical Properties of Poly-e-Caprolactone-Hydroxyapatite Composites.* Biomedical Materials Research Part B: Applied Biomaterials. 541-547.

B. Thavornyutikarn, N. Chantarapanich, K. Sitthiseripratip, G. A. Thouas, & Q. Chen, (2014). *Bone tissue engineering scaffolding: computer-aided scaffolding techniques*. Progress in biomaterials, 3(2-4). 61-102.

V. Brun, C. Guillaume, S. Mechiche Alami, J. Josse, J. Jing, F. Draux & F. Velard, (2014). *Chitosan/hydroxyapatite hybrid scaffold for bone tissue engineering*. Bio-Medical Materials and Engineering, 24(1), 63–73.

K. Hae-Won, C. K. Jonathan & K. Hyoun-Ee, (2003). *Hydroxyapatite/Polycaprolactone composite coatings on hydroxyapatite porous bone scaffold for drug delivery.* Biomaterials 25. 1279-1287.

A. Salerno, M. A. Fanovich & C. D. Pascual, (2014). *The effect of ethyl lactate and ethyl-acetate plasticizers on PCL and PCL–HA composites foamed with supercritical CO_2*. Journal of Supercritical Fluids (Vol. 95). 394-406.

G. Harvey, (2006). *Excel 2007 For Dummies*. Wiley. ISBN 978-0-470-03737-9.

G. Harvey, (2007). *Excel 2007 Workbook for Dummies* (2nd ed.). Wiley. p. 296 *ff.* ISBN 978-0-470-16937-7.

J. Ivanovic, S. Knauer, A. Fanovich, S. Milovanovic, M. Stamenic, P. Jaeger, I. Zizovic & R. Eggers, (2016). *Supercritical CO_2 sorption kinetics and thymol impregnation of PCL and PCL-HA*. The Journal of Supercritical Fluids, 107, 486-498.

D. Granato and V. M. de Araujo Calado, (2014). *The use and importance of design of experiments (DOE) in process modelling in food science and technology.* Mathematical and Statistical Methods in Food Science and Technology, John Wiley & Sons, Ltd., New York. 3-18.

W. Kenton, (2019). `*Multiple Regression–MLR Definition*'. Retrieved from :*https://www.investopedia.com/terms/m/mlr.asp*.

Stephanie, (2014). '*Excel Regression Analysis Output Explained'*. Retrieved from: https://www.statisticshowto.datasciencecentral.com/excel-regression-analysis-output-explained/

T. Bock, (2017), *'Tips for Interpreting R-Squared'*. Retrieved from: https://www.displayr.com/8-tips-for-interpreting-r-squared/

F. Andy, (2013). *Discovering Statistics Using IBM SPSS Statistics*. 4th Edition. SAGE Publications Inc. ISBN 978-1-4462-4917-8.

M. J. Kim and Y. H. Koh, (2013). *"Synthesis of aligned porous poly(epsilon-caprolactone) (PCL)/hydroxyapatite (HA) composite microspheres,"* Mater Sci Eng C Mater Biol Appl, vol. 33, no. 4. 2266-2272.

M. S. Khan, Z. Hasan, and Y.A. Ansari, (2014). *Statistical analysis for the abrasive wear behavior of Al 6061*. Journal of Minerals and Materials Characterization and Engineering. 292-299.