Modelling of Average Pore Size and Porosity of Porous Polycaprolactone/Hydroxyapatite (PCL/HA) Composite Blends

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Abstract*: The objective of this paper is to develop mathematical relationship between average pore size and porosity of porous polycaprolactone/hydroxyapatite (PCL/HA) composite and investigate the combined effect of temperature and pressure of foaming process and presence of HA. Porous PCL/HA composite was prepared using supercritical carbon dioxide (ScCO2) solid state foaming process. Three different temperatures and pressures of foaming process were varied at 35°C, 40°C, 45°C and 10MPa, 20MPa, 30MPa respectively. Meanwhile weight of HA was varied at 10, 20, 30 and 40 wt%. The result from analysis of variance (ANOVA) using Microsoft Excel found that average pore size is reduced with higher pressure and content of HA presence does not significantly affect the average pore size due to poor distribution of HA. Meanwhile for porosity, higher temperature is more dominant in increasing of porosity compared to the HA content. In addition, both designed models have low values of Average Absolute Relative Deviation (%AARD) and high value of coefficient of determination (R^2) which reflects a good and satisfying result between the experimental values and model predicted values.

***Index Terms*: ANOVA analysis, Porous PCL/HA Composite, Average Pore Size and Porosity.**

I. INTRODUCTION

Pore size and porosity are two important physical properties in fabricating bone tissue engineering scaffold. The size of pore is an essential requirement that affect the quality and characteristics of new bone tissue formation [1]. It plays an important role in the rate and degree of new bone growth [1]. Meanwhile porosity is defined as a measure of the total pores spaces in a sample. It is a fraction of the volume of pores over the total volume of sample as percentage unit. According to [2], bone tissue engineering scaffold should have pore size between 100 to 500 micrometer and porosity more than 80% depending on the application of the scaffold. Reference [3] found that the ideal scaffold for bone tissue engineering are consist of macro pore size >100µm and micro pore size <20µm. Meanwhile for bone regeneration, [4] found the ideal mean pore diameter is between 100 µm and 1200 µm. In addition, [5] stated that pore size more than 100 µm is encourage for cell penetration, tissue growth and vasculation. On the other hand, [6] reported the range of porosity of cancellous bone is 50-90% and cortical bone is less than 10%. Moreover, [7], stated that porosity of cancellous bone is 75-90% and cortical bone is 5-10%.

One of the most common and widely technique that used to create pore structure and porosity is gas foaming due to use of supercritical fluid. This technique has been extensively studied for PCL/HA composite in recent years [8]-[12]. PCL/HA composite has received considerable attention as candidate of bone tissue engineering scaffold due to excellent biocompatibility, slow degradability, no toxicity and able to promote bone tissue cell growth [13]-[15]. In gas foaming technique, PCL will be saturated with supercritical CO_2 for a certain duration, and the, depressurized rapidly to atmospheric level. The release of pressure will result in the nucleation and growth of gas bubbles or cells within the PCL. Previous studies indicate that physical properties of foamed sample significantly influenced by foaming condition such as temperature, pressure and rate of depressurized. Nevertheless, the presence of filler such as HA also affected the physical properties of foamed sample. In addition, controlling the foaming conditions and presence filler will produce targeted physical properties of foamed sample.

Therefore, the main objective of this study is to develop mathematical relationship between the physical properties of the porous PCL/HA composite (average pore size and porosity) and the influential control factors (foaming conditions variables and presence filler) and interactions. In this study, porous PCL/HA composite was fabricate by using supercritical CO_2 foaming process without present any organic solvent. The temperature and pressure are varied at 35, 40 and 45°C and 10, 20 and 30MPa respectively. The mathematical models were developed using Microsoft Excel using analysis of variance (ANOVA).

II. EXPERIMENTAL

A. Mathematical Model

The mathematical models of average pore size and porosity of foamed samples were developed using Microsoft Excel. These mathematical models were developed to investigate the relationship between pore characteristic of foamed samples and the influence control factors (foaming temperature, foaming pressure and HA content) and interactions.

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Microsoft Excel is one of the statistical tool that uses the quantitative data from various experimental designs to determine and simultaneously solve the multivariate equations. Microsoft Excel explores the relationship between several independent variables towards one or more response variables. Fig. 1 shows the chronology on the Microsoft Excel procedure applied in this study.

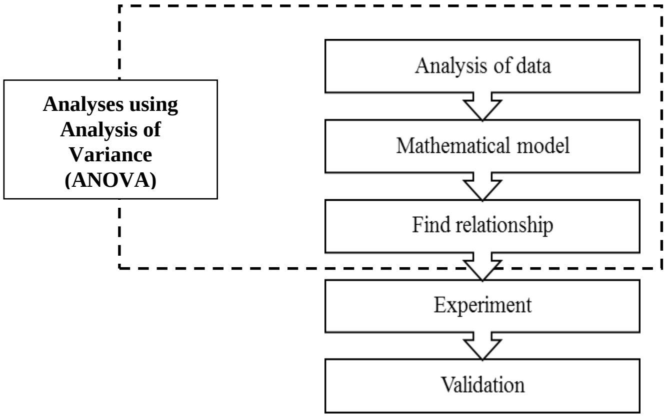


Fig. 1: Flow chart of Microsoft Excel Steps

B. Variable Selection

Three independent variables were selected to be investigated based on previous studies [8]-[12]. The effects of these independent variables were investigated towards average pore size and porosity which were chosen as response variables. Table I shows the independent variables defined as factor and its code that were used throughout the statistical analysis.

Table I Factors and its code

Factors	Code
Foaming pressure (MPa)	A
Foaming temperature (°C)	B
HA content (wt%)	C

C. Design of Experiment

The initial condition of experiment was design based on critical condition of supercritical carbon dioxide which is critical temperature, T_C is 31.1°C and critical pressure, P_C is 7.8MPa. Therefore this study select initial condition of experiment at temperature of 35°C and pressure of 10MPa. Thus from previous studies the range of foaming temperature and pressure are 35°C -50°C and 10-30 MPa respectively [8]-[12]. Therefore, Table II shows the experimental combination used in this study including result of average pore size and porosity of foamed samples.

Table II Design of Experiment and Result of Average Pore Size and Porosity

Run	A	B	C	Average Pore Size, (μm)	Porosity (%)
1	10	35	0	117.82	62.19
2	10	40	0	71.09	69.44
3	10	40	10	100.79	67.21
4	10	40	20	125.55	67.00
5	10	40	30	111.93	65.19
6	10	40	40	150.14	63.52
7	10	45	0	133.69	68.75
8	10	45	10	130.12	69.72
9	10	45	20	174.87	66.69
10	10	45	30	155.19	65.86
11	10	45	40	116.78	61.53
12	20	35	0	76.29	62.19
13	20	40	0	131.09	75.4
14	20	40	10	100.89	65.53
15	20	40	20	98.99	70.4
16	20	40	30	101.08	71.07
17	20	40	40	106.18	59.48
18	20	45	0	104.85	78.25
19	20	45	10	110.4	78.42
20	20	45	20	127.12	76.00
21	20	45	30	128.7	72.87
22	20	45	40	115.14	79.04
23	30	35	0	79.83	56.58
24	30	40	0	95.55	62.28
25	30	40	10	57.72	62.36
26	30	40	20	70.82	60.61
27	30	40	30	63.98	59.59
28	30	40	40	59.48	51.85
29	30	45	0	48.97	76.09
30	30	45	10	64.08	74.66
31	30	45	20	56.69	73.32
32	30	45	30	76.42	71.82
33	30	45	40	47.53	76.58

D. Evaluation of the Fitted Model

A polynomial linear regression model was used to represent the relationship between the predicted outcome variables (average pore size and porosity) and the predictor variables (factors and their interactions) using multiple regression analysis. This model was considered because linear behaviour usually occurs in physicochemical analysis of ingredient mixture [16]. For the experimental design used in this study, the general form of the polynomial linear regression model is expressed as:

$$Y = b0 + b1A + b2B + b3C + b4AB + b5AC + b6BC + b7ABC \quad (1)$$

Referring to the Equation (1), Y is the predicted outcome variable which are average pore diameter or porosity for this study, A, B and C are the factors as mentioned in Table I. AB, AC, BC and ABC are the interaction factor. Meanwhile b0 is the intercept of the regression equation, b1 to b3 are known as linear regression coefficients and b4 to b6 are the second order interaction coefficients.

The obtained results were analysed by analysis of variance (ANOVA) to assess the model fitness. The ANOVA was carried out on the model for a confidence level of 95% ($p \leq 0.05$). The fitted model was analysed with the aim to ensure that it provides adequate approximation to the true system and also to verify that none of the least squares regression assumptions are violated.

Table III shows how the ANOVA was applied in this study in fitting the mathematical model to the experimental data set using multiple regression.

The number of terms in the model or equation is denoted as P and N is referred as the number of experiment. On the other hand, sum of square regression (SS_{reg}) is obtained by the difference of total sum of square (SS_{total}) and the residual (SS_{res}). From the table, the ratio between the mean square of regression (MS_{reg}) and the mean square of residuals (MS_{res}) was employed to evaluate the significance of regression (F-value). F-value less than 0.05 shows that the results are reliable or statistically significant.

From the ANOVA, the test for significant model was performed in order to select significant factors and interactions only in order to obtain the final regression model (revised model). In order to ensure the goodness of the fit of the revised regression model obtained, the test for lack of fit, the normal probability plot and the analysis of the residuals were performed [17].

Table III ANOVA for Fitted Mathematical Model to an Experimental Data Set

Source of variation	Sum of Square (SS)	Degree of Freedom (d.f)	Mean Square (MS)	F-value
Regression (SSR)	SS regression (SS_{reg})	P-1	(SS_{reg})/ (P-1)	F=MS_{reg}/ MS_{res}
Residual	SS residue (SS_{res})	N-P	(SS_{res})/ (N-P)	-

E. Validation of the Developed Model

In order to evaluate the appropriateness of the revised model (developed model), experimental was carried out and the predicted value was compared to the actual value obtained from the experimental. In addition, two methods were applied to validate the model which are coefficient of determination (R^2) value and Average Absolute Relative Deviation (%AARD). R^2 value was obtained using Equation (2) [18]. Meanwhile, for calculating %AARD, the general equation is defined as Equation (3) [19].

$$R^2 = 1 - \frac{\Sigma[(y_{pre} - y_{exp})]^2}{\Sigma[(y_{pre} - \bar{y}_{exp})]^2} \quad (2)$$

Where,

y_{exp} = Experimental value
y_{pre} = Predicted value
$\bar{y}_{exp}$ = Mean of experimental value

$$\%AARD = \frac{1}{N}\sum_{1}^{N}\frac{|y_{exp} - y_{pre}|}{y_{exp}} X\ 100 \quad (3)$$

Where,

N = Number of data
y_{exp} = Experimental value
y_{pre} = Predicted value

III. RESULT AND DISCUSSION

A. Analysis of Variance (Test for significant of model)

The initial multiple linear regression model from ANOVA, showing the coded relationship between predicted outcome variables and the predictor variables are as follow:

$$\text{Average pore size} = 3.3953A+3.3968B-1.1671C-0.1345AB-0.01409AC+0.05265BC-0.0007402ABC \quad (4)$$

$$\text{Porosity} = 0.2449A+1.7158B+4.6253C-0.007258AB-0.2582AC-0.1139BC+0.006155ABC \quad (5)$$

These initial regression models for average pore size and porosity were developed based on the ANOVA result in Table IV and Table V respectively.

There are two matters must be evaluate in significant model test which are the degree of fit of the models and removing insignificant factors and interactions. The degree of fit of the models which is interpreted by value R^2. R^2 is defined as the ratio of the explained variation to the total variation [20]. From the Table IV and V, it is found that the R^2 of the initial model for average pore size and porosity are 0.9718 and 0.9969 respectively. These high value of R^2 suggesting that the modelling equations are highly reliable. The closer R^2 to unity (=1) indicate a model with satisfactory predictable outcome. However, a high R^2, does not necessarily indicate that the model is adequate. Therefore, at 95% confidence, the equations models must be improved by removing insignificant factors and interactions.

Significant factors and interactions are analysed from ANOVA result. Table IV presented the ANOVA result for the initial regression model for the average pore size of porous PCL/HA composite. Examination of the table shows that only temperature (B) is considered to be the statistically significant factor that influence on the average pore size of the porous PCL/HA composite, as *p* is less than 0.05 (95% confidence). Also the interaction (AB) influence the average pore size of porous PCL/HA composite. However, pressure (A), HA content (C) and HA content interaction (AC, AC and ABC) did not influence the average pore size of porous PCL/HA composite.

Meanwhile the result of ANOVA for the porosity of porous PCL/HA composite are presented in Table V. Here, temperature and HA content has significant influence on the porosity of the porous PCL/HA composite. Furthermore, all the main effect interactions exclude AB are considered significant as their p value is less than 0.05.

Table IV ANOVA Table for Testing the Significance of Regression Model for Average Pore Size

Factor and interactions	*Coefficients*	*p-value*
Intercept	0	-
A	3.3953	0.2233
B	3.3968	$5.83x10^{-10}$
C	-1.1671	0.8628
AB	-0.1345	0.05393
AC	-0.01409	0.9655
BC	0.05265	0.7408
ABC	-0.0007402	0.9233
Standard Error	19.9096	
Total SS	365966.5669	
Significance F	$5.5961x10^{-18}$	
R Square	0.9718	
Adjusted R Square	0.9269	

Table V ANOVA Table for Testing the Significance of Regression Model for Porosity

Factor and interactions	*Coefficients*	*p-value*
Intercept	0	-
A	0.2449	0.6784
B	1.71580	$1.58x10^{-18}$
C	4.6253	0.003401
AB	-0.007258	0.6241
AC	-0.2582	0.000941
BC	-0.1139	0.002359
ABC	0.006155	0.00086
Standard Error	4.2730	
Total SS	153780.8	
Significance F	$5.83x10^{-30}$	
R Square	0.9969	
Adjusted R Square	0.9577	

Therefore, all the main factors and the interactions that has *p* less than 0.05 are considered and *p* greater than 0.05 are eliminated in the next revised model.

B. Development of the Model

The revised regression model for each predicted outcome in terms of the coded factors after eliminating insignificant factor and interaction are obtained as following;

$$\text{Average pore diameter} = 3.8706B-0.0738AB \quad (6)$$

$$\text{Porosity} = 1.6897B+4.6253C-0.25AC-0.1130BC+0.005913ABC \quad (7)$$

Equation (6) indicates that, within the range of the experiment, the average pore size is reduced with higher pressure. Thus, content of HA presence does not significantly affect the average pore size due to poor distribution of HA. Meanwhile porosity is increased with higher temperature and higher HA content as indicate in Equation (7). However porosity was reduced by two interactions (AC and BC) which involve factor of HA content. This shows that, higher temperature is more dominant in increasing of porosity compared to the HA content.

C. Checking the Adequacy of the Developed Model (Revised Model)

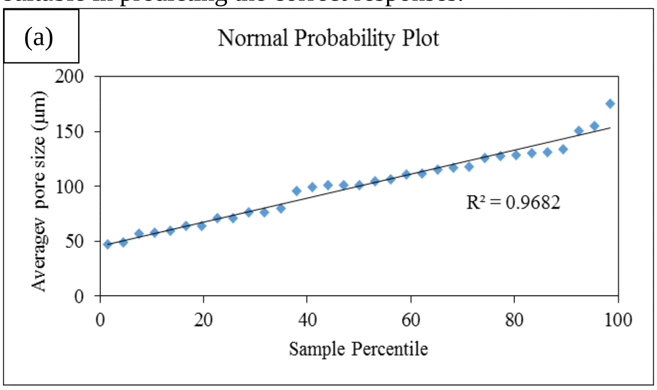
Beside of R^2, adjusted R^2 was also tested in determining the goodness of fit of the developed or revised models. Here, R^2 is the proportion of variance in the observed values of average pore diameter and porosity values that is accounted for by the factors and interactions in the regression model, while the adjusted R^2 makes adjustment for the number of factors and interactions in the model.

For average pore size, R^2 and the adjusted R^2 are 0.9668 and 0.9334 respectively, while for porosity, the values are 0.9969 and 0.9607 respectively. The adjusted R^2 for revised model are increased compared to initial model, shows that the factors and interactions entered in the model are significantly to the model fit [17]. Therefore, the terms in the models are appropriate.

Table VI Test of Model vs Residual

Response	Average Pore Size (μm)		Porosity (%)	
Test	Model	Residual	Model	Residual
df	2	31	5	28
SS	353801.3986	12165.1683	153300.4	480.4115
MS	176900.6993	392.4248	30660.08	17.1576
F	450.7888		1786.973	
Significance F	$4.1546x10^{-23}$		$3.92x10^{-33}$	
R^2	0.9668		0.9969	
Adjusted R^2	0.9334		0.9607	
Standard Error	19.8097		4.1422	

In addition to the lack of fit test, this revised regression model are also be checked by normal probability plot of the models. The normal probability plots for the average pore size and porosity are shown in Fig. 2(a) and Fig. 2(b), respectively. From Fig. 2 it can be seen that the observed value generally fall on a straight line implying that the errors are normally distributed [21]. Hence, the regression models appear to be suitable in predicting the correct responses.



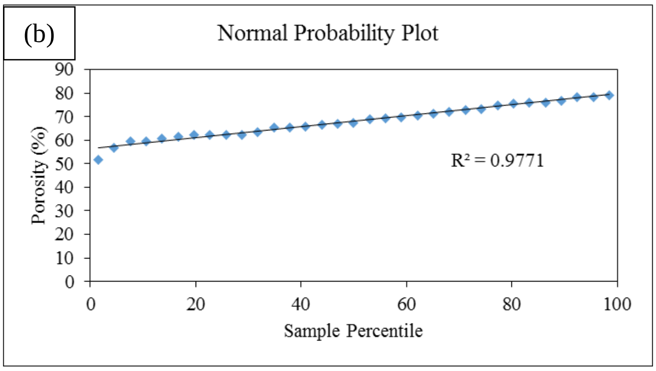


Fig. 2: Normal probability plot for (a) average pore size; (b) porosity

Besides, the residuals of the models were also analysing. The standard residuals against the predicted values was plotted to evaluate the appropriateness of the revised model. The residuals are define as the differences between the observed (measured) values and predicted values. The plots in Fig. 3 shows the residuals are randomly scattered. These show that the residuals do not contradict the linear assumption.

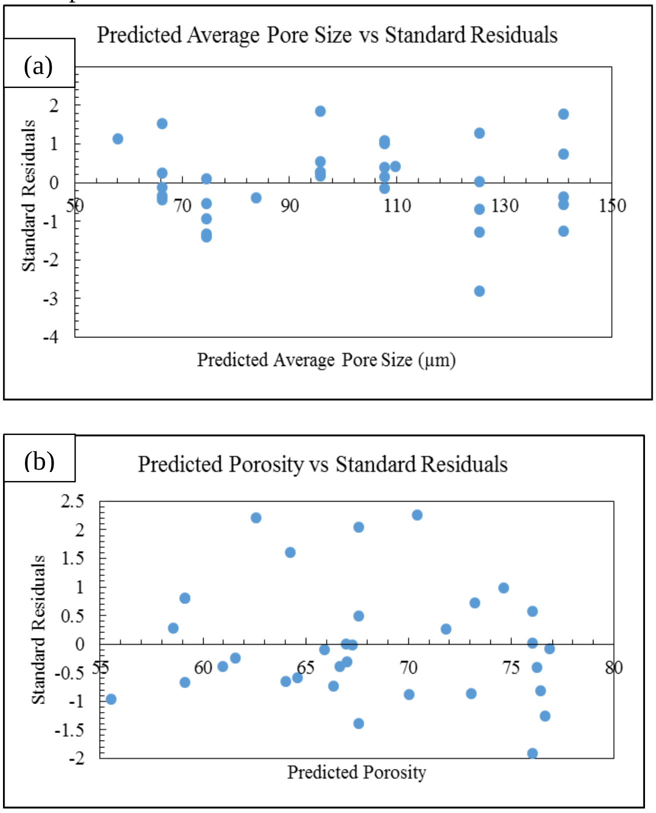


Fig. 3: Plot of predicted value vs standard residuals for (a) average pore size; (b) porosity

D. Experimental and Validation of Models

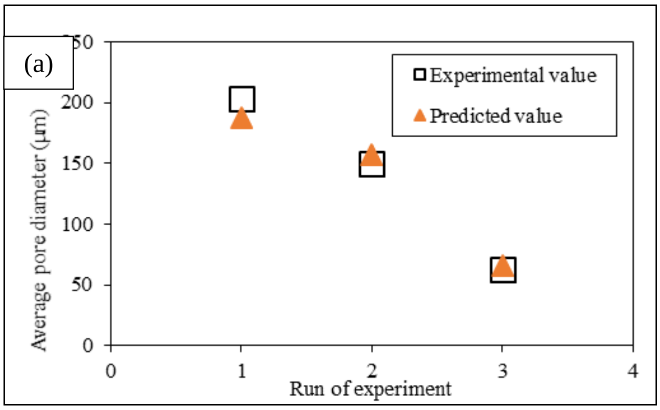
The evaluation of revised model appropriateness was carried out, where three experimental runs were conducted. The data from these experimental were used to compare with the prediction of the revised model [22]. Table VII shows the experimental design for the validation where consist of the experimental result value, predicted value by the revised model and the residuals.

Table VII Design of Validated Runs and Results

Coded Factors				Average Pore Size (μm)			
Run	A	B	C	Experimental (μm)	Predicted (μm)	Residual (μm)	$\frac{\lvert y_{exp} - y_{pre}\rvert}{y_{exp}}$
1	10	60	10	203.01	187.96	-15.05	0.0742
2	10	50	20	149.56	156.63	7.07	0.0473
3	30	40	30	62.69	66.26	3.57	0.0570
							∑= 0.1785
							%AARD= 5.95

Coded Factors				Porosity (%)			
Run	A	B	C	Experimental (%)	Predicted (%)	Residual (%)	$\frac{\lvert y_{exp} - y_{pre}\rvert}{y_{exp}}$
1	10	60	10	78.90	90.31	11.41	0.1447
2	10	50	20	73.39	73.12	-0.27	0.0037
3	30	40	30	60.05	58.62	-1.44	0.0239
							∑=0.1722
							%AARD= 5.74

The comparison between predicted values and experimental values is shown in Fig. 4. A very least deviations are found between the predicted and experimental value and they conform the best fits of experimental values. This shows that the develop model gives good agreement with experimental values.



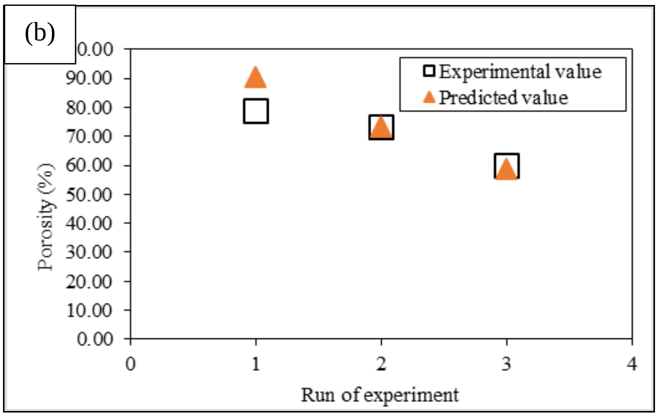


Fig. 4: Comparison between predicted value and experimental value for (a) average pore size; (b) porosity

There are two method used in this study to investigate the model validation which is by using R^2 value [18] and Average Absolute Relative Deviation (%AARD) [19]. R^2 was implemental to check the goodness of fit of the model between the experimental value and the predicted value by the revised model. In this case, R^2 for average pore size and porosity are 0.9986 and 0.9977 respectively which indicate a good agreement between the experimental and predicted value obtain from the model. The R^2 should be at least 0.80 for the good fit of a model [23]. According to the data, the proposed model for average pore size and porosity seem to have good correlation with %AARD of 5.95% and 5.74% respectively. According to [24] and [25] the percentage of AARD value of 6.07% and 3.4% respectively is acceptable and consider as a good result. Therefore the obtained %AARD results also should be consider as satisfied. The small %AARD value reflect a consistency between experimental and predictable data.

IV. CONCLUSION

In conclusion, both methods applied in verifying the model appropriateness reflects a good and satisfying result between the experimental values and model predicted values. Both models have low values of %AARD and high value of R^2. Therefore, the designed model of average pore size and porosity are applicable to predict the average pore size and porosity of PCL/HA composite within the variables ranges. In addition these models can be applied as a preliminary reference and guidance for fabricate other porous polymer composite using supercritical CO_2 solid state foaming process.

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