

Box-Behnken Design Optimization Process of Electrospinning for Fabrication PLA/PVA Nanofibers

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ABSTRACT

The optimization of PLA/PVA nanofibers has been successfully performed using the Box-Behnken design method to produce nanofibers with small diameters. The fabrication process of the nanofibers was conducted using electrospinning. The electrospinning process parameters included PLA solution concentration, voltage, and flow rate. The optimal conditions for producing the nanofibers were found in sample N1, with variations of PLA solution at 9%, voltage at 14 kV, and flow rate at 16 kV, resulting in small and uniform nanofibers. The smallest fiber diameter achieved was 152.67 nm, which is quite close to the Box-Behnken predicted value of 144.45 nm. Statistical testing was conducted using analysis of variance (ANOVA), which indicated a p-value greater than 0.03 for the effect of the flow rate. This demonstrates that the flow rate has a significant influence on the diameter size. These results indicate that the Box-Behnken design can be an important tool for optimizing processes in electrospinning.

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INTRODUCTION

Nanofibers represent a nanotechnology material in the form of fibers that exhibit unique characteristics (Abdulhussain et al., 2023). These characteristics include a small size, a high surface area-to-volume ratio, high porosity, and the ability to manipulate their structure (Edikresnha et al., 2021; Li & Martin, 2001; Zulfi et al., 2018). Nanofibers have numerous applications, particularly in biomedicine, such as drug delivery, soft tissue engineering, and wound dressings (Haider et al., 2018; Partheniadis et al., 2020; Tanpichai & Oksman, 2016). Nanofibers can be fabricated using the electrospinning method, which is commonly used due to its relatively simple, flexible, and fast process for producing nanofibers. However, controlling the desired diameter and morphology remains a challenge (Huan et al., 2015).



Several factors affect the nanofiber fabrication process, such as solution parameters (conductivity, concentration, surface tension, and viscosity) and process parameters like flow rate, applied voltage, distance between the collector and the needle, humidity, and temperature (Dhakate et al., 2011; Latiffah et al., 2022). For instance, voltage influences fiber diameter—higher voltages lead to smaller diameters. However, voltage also affects the solution concentration. If the concentration is too high, it becomes difficult for the fibers to be drawn by the electric field, whereas low concentrations result in poor conductivity (Okutan et al., 2014). Therefore, nanofiber properties can be finely tuned to achieve optimal performance. One commonly used material in nanofiber fabrication is natural polymers. These materials are biocompatible and environmentally friendly. In this study, a combination of Polylactic Acid (PLA) and Polyvinyl Alcohol (PVA) was investigated due to their complementary properties, which are advantageous for biomedical engineering applications.

PLA is a polymer derived from renewable resources and exhibits biocompatibility, biodegradability, and is safe for medical and pharmaceutical use. It is often used for nanofiber production due to its ease and cost-effectiveness (Wang et al., 2024). However, PLA has some limitations, such as high stiffness and suboptimal mechanical properties in certain applications. Thus, combining it with other materials can address these shortcomings. PVA is frequently employed in biomedical applications due to its high flexibility, good hydrophobic properties, and water solubility (Khaleel et al., 2024). It also has the ability to bond with body tissues, making it ideal for such applications. In this context, PLA provides good mechanical strength and biodegradability, while PVA enhances flexibility and facilitates fiber formation. Therefore, this composite is expected to have optimal physical and mechanical properties for biomedical applications.

In recent years, optimization methods for the electrospinning process have utilized various statistical tools to explore the relationships between variables. Multivariate statistics, which analyze multiple variables simultaneously, have been employed to understand the interactions among variables. Multivariate approaches in electrospinning include methods such as Design of Experiments (DOE) (Ali et al., 2020), correlation analysis, Response Surface Morphology (Anindyajati et al., 2018) and Principal Component Analysis (PCA) (Younes et al., 2021). Among these, DOE, particularly the Box-Behnken Design (BBD) (Sarlak et al., 2012), is frequently used for its ease in optimizing the electrospinning process by varying three or more parameters simultaneously. The Response Surface Methodology (RSM) is an effective statistical approach for optimizing electrospinning parameters in nanofiber fabrication. Using the Box-Behnken Design (BBD), a predictive model was developed to estimate the diameter of titanium dioxide (TiO₂) nanofibers based on applied voltage, infusion rate, and electrode distance. Model validation through residual analysis confirmed its reliability. The optimized parameters 40 kV voltage, 32.5 cm electrode distance, and 0.6 mL h⁻¹ infusion rate produced a minimum predicted fiber diameter of 43.3 nm, demonstrating the effectiveness of the BBD method in refining electrospinning conditions (Ray & Lalman, 2011). The Box-Behnken Design (BBD) was applied to optimize electrospinning parameters for producing Nylon-6 nanofibers. Three key variables—solution concentration, applied

voltage, and flow rate were varied across three levels, resulting in fifteen experiments. Statistical analysis using ANOVA showed that solution concentration had the most significant effect on fiber diameter. The optimal condition was achieved at 14 wt% concentration, 19.5 kV voltage, and 1 mL/h flow rate, yielding the smallest and most uniform nanofibers (Sukowati et al., 2023). Electrospinning has emerged as an effective technique for producing nanofibers from natural polymers with desirable structural and functional properties. In the fabrication of gelatin/sodium alginate nanofibers, the characteristics of the precursor solution play a crucial role in determining fiber morphology and diameter. A study explored how variations in gelatin concentration, alginate concentration, the ratio of alginate solution in the blend, and the amount of acetic acid in the gelatin solvent influence the resulting nanofiber size and distribution. Using the Response Surface Methodology (RSM) with a three-level, four-factor Box–Behnken Design (BBD), the research established the relationship between these solution properties and fiber diameter, accounting for both individual and interactive effects. Validation experiments confirmed the model's accuracy, with the produced fibers showing average diameters ranging from 68–166 nm without ethanol and 90–155 nm with ethanol. These findings demonstrate the importance of controlling solution composition in achieving nanofibers with tailored dimensions, particularly for tissue engineering applications (Gönen et al., 2016). Marziyeh et al. optimized PVA-PLA based on morphology, wettability, FTIR analysis, and mechanical properties to investigate the effects of composition (Ranjbar-Mohammadi et al., 2021). However, no studies have yet applied the Box-Behnken Design (BBD) method for optimizing the process parameters in PLA/PVA nanofiber fabrication. Thus, this research aims to fill that gap.

This study focuses on optimizing PLA/PVA nanofiber production using the Box-Behnken Design (BBD) in the electrospinning process. The parameters tested are PLA concentration, voltage, and flow rate, with experiments conducted at three levels for each variable, resulting in 15 test samples. ANOVA statistical analysis is used to examine the relationships among variables and to optimize the process, demonstrating the significant impact of these three variables on fiber diameter.

METHODS

1. Materials Preparation and Electrospinning Process

Poly(lactic acid) (PLA) and Poly(vinyl alcohol) (PVA) were purchased from Sigma-Aldrich, Singapore. PVA and PLA solutions with various concentrations were prepared by dissolving the polymer with aquadest at 80 °C temperature and continuously stirring for 2h. Electrospinning process were depicted in Figure 1. The PVA/PLA solution was then electrospun at the BBD Parameter using table 1 with varying concentration of PLA (%), voltage (kV) and flowrate (ml/min) with a fixed syringe-to-collector distance of 15 cm. The process was carried out at room temperature and humidity of 50%. The resulting of fiber were collected on the collector and left at dry box chamber before being observed and analyzed. The schematic process will find at Figure 1.

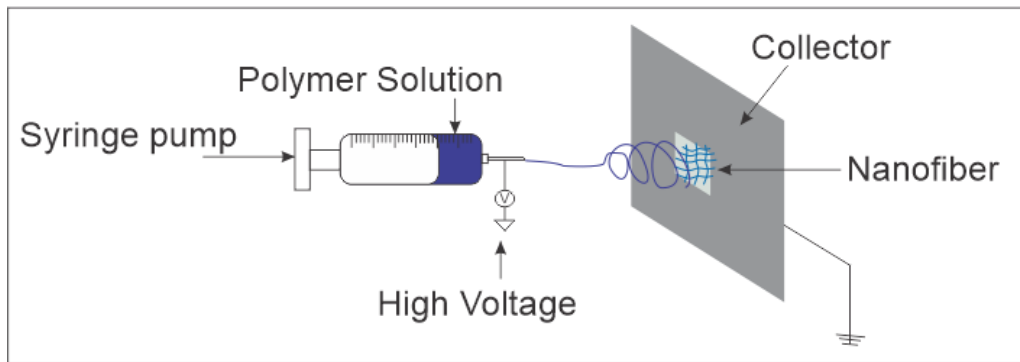


Figure 1. The Schematic Process of Electrospinning

2. Characterization

The nanofiber was characterized to observe its diameter and surface area. The morphology of the nanofibers was examined using a digital microscope and analyzed with Image-J software to measure the fiber diameters. Images of nanofibers were obtained using Scanning Electron Microscope (SEM) with maximum resolution capacity of the microscope was 1000x and the length of measurement is 1 μ m. Experimental data from the Box-Behnken Design were statistically analyzed using Minitab software, applying Analysis of Variance (ANOVA) to calculate the p-values, which indicate the significance level of the variables.

3. Parameter Optimization Using Box Behnken Design

To optimize the fiber formation process, three process parameters were selected: PLA concentration, voltage, and flow rate. The constant parameters maintained throughout the experiment were temperature, humidity, and the tip-to-collector distance. Optimization was carried out using three levels, with the variable ranges shown in Table 1.

Table 1. Independent Variable and Level at Box-Behnken Design

Variable	Variable Code	Levels		
		-1	0	1
Concentration (wt%)	K	9%	10%	11%
Voltage (kV)	V	14	15	16
Flow rate (ml/min)	L	14	15	16

RESULTS

1. Development of Nanofibers

Nanofibers were fabricated using the electrospinning method, which is one of the fastest methods for producing nanofibers, requiring only voltage and solution viscosity to generate small-sized fibers. The morphology of PVA/PLA nanofibers is shown in Figure 2. The results indicate that the nanofibers formed with a relatively smooth and homogeneous structure. The produced PLA/PVA nanofibers had diameters ranging from 152.67 to 206.16 nm.

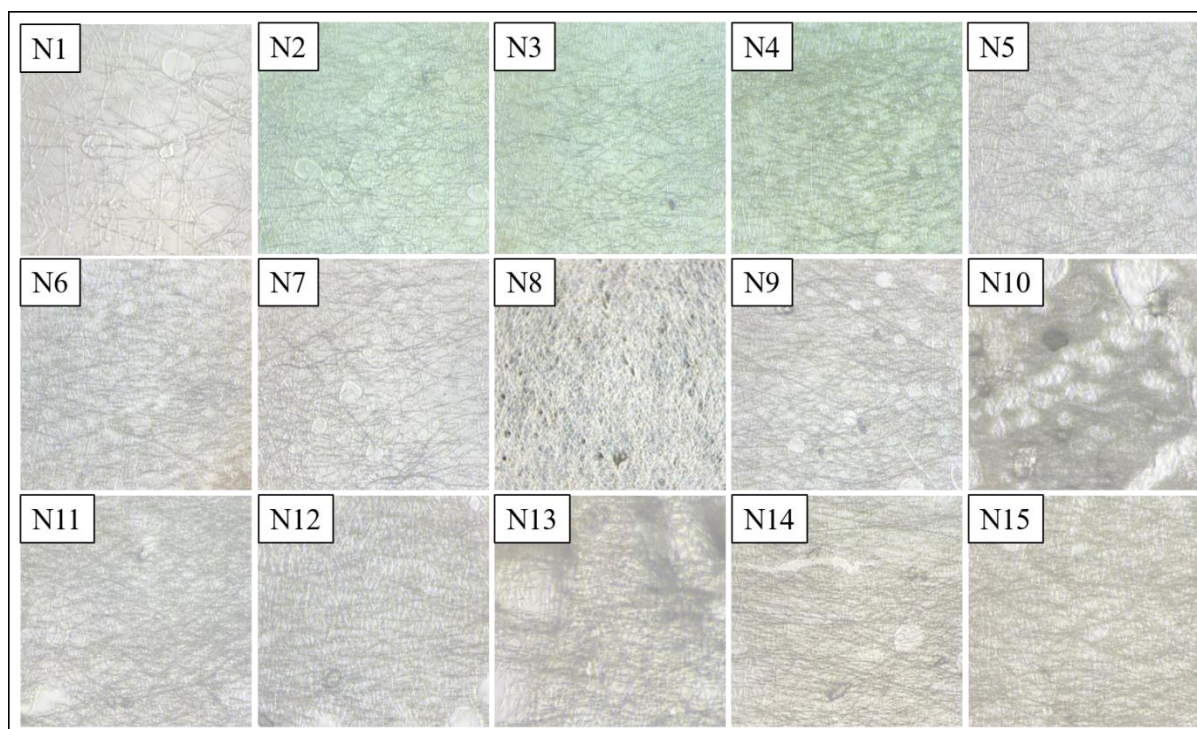


Figure 2. Digital Microscope Result for Nanofiber Sampel N1 - N15 (The details name of N1 – N15 are shown in Table 2)

2. The Influence of Process Parameters Using Box-Behnken Design (BBD)

The results of the BBD design optimization are shown in Table 2. A total of 15 samples were tested, yielding the smallest nanofiber diameter of 152.67 nm and the largest of 206.16 nm. The smallest diameter was obtained in sample N1, with a concentration of 9%, voltage of 14 kV, and flow rate of 16 $\mu\text{L}/\text{min}$, while the largest diameter was observed in nanofiber N12, with a concentration of 10%, voltage of 16 kV, and flow rate of 16 $\mu\text{L}/\text{min}$. These results demonstrate that concentration significantly affects fiber diameter. As PVA concentration increases, the nanofiber diameter also increases. This is due to the rise in solution viscosity, which makes the solution more difficult to stretch during fiber formation, resulting in larger diameters. Additionally, a higher concentration implies a greater number of polymer molecules in the solvent, leading to stronger intermolecular forces that affect polymer chain stability during fiber formation. A higher solution concentration enhances molecular interactions and cohesion, ultimately resulting in larger nanofiber diameters.

The smallest fiber diameter was achieved with a voltage of 14 kV, lower than the other voltage variations. The electric field voltage affects fiber formation—higher voltages result in smaller nanofiber diameters due to Coulombic forces on the syringe surface. At low concentrations, the fibers are easily drawn by the electric field at lower voltages, likely due to the low surface tension. In addition, with low solution concentration, fewer polymer molecules are drawn towards the electric field, and the Coulombic forces at low voltage are sufficient to stretch the solution, forming thinner fibers.

Flow rate also influences fiber formation. A higher flow rate tends to produce larger nanofiber diameters due to polymer accumulation at the tip surface and reduced pulling force from the electric field. The flow rate also impacts the productivity of nanofibers, as reflected in the experimental results.

Optimization of the process using BBD was analyzed through Analysis of Variance (ANOVA), which is highly effective for optimizing manufacturing processes, particularly in evaluating the effects of various process parameters. The ANOVA results are presented in Table 3. The analysis focused on p-values, where a p-value less than 0.05 indicates a significant effect of the parameter. In this study, fiber diameter was the response variable, and concentration, voltage, and flow rate were the factors. The results showed that flow rate had a significant impact on nanofiber diameter, with a p-value of 0.039. In contrast, concentration had a p-value of 0.603, indicating it did not significantly affect nanofiber diameter, and voltage had a p-value of 0.409.

Table 2. Table of Parameter Box-Behnken Design for 15 Sample

Name of Sample	Concentration of PLA (%)	Voltage (kV)	Flow Rate ($\mu\text{L}/\text{min}$)	Average Diameter (nm)
N1	9	14	16	152.67
N2	9	16	15	167.26
N3	11	14	15	173.08
N4	11	16	15	157.41
N5	9	15	14	202.09
N6	9	15	16	173.78
N7	11	15	14	200.52
N8	11	15	16	163.46
N9	10	14	14	199.4
N10	10	14	16	166.56
N11	10	16	14	188.15
N12	10	16	16	206.16
N13	10	15	15	175.68
N14	10	15	15	183.56
N15	10	15	15	184.5111

This finding is particularly interesting, as it reveals that flow rate plays a crucial role in determining nanofiber diameter. The PVA/PLA solution composite increases viscosity, which in turn affects the nanofiber formation process. Due to the high viscosity, greater input pressure is required for the syringe to generate a stable jet.

This process requires a sufficiently high flow rate to produce the jet, which leads to smaller nanofiber diameters. Moreover, there needs to be a balance between flow rate and solution viscosity. As solution viscosity increases, the flow rate must also be increased to produce smooth, small, and uniform nanofibers.

Table 3. ANOVA Result of Nanofibers Diameter

Source	DF	F-Value	P-Value
Concentration	2	0.54	0.603
Voltage	2	1.00	0.409
Flow Rate	2	4.98	0.039

3. Analysis of Contour Plot

Figure 3 shows the estimated surface plot with contour lines to understand the relationship between the response function and the factor parameters. The impact of these parameters is evaluated through the surface plot, illustrating the influence of concentration, voltage, and flow rate on nanofiber diameter. The effect of concentration and voltage on diameter is depicted in Figure 3.a. The results indicate that the fiber diameter decreases with lower concentration. The light green contour suggests that lower voltage and lower concentration produce smaller fiber diameters. A low concentration results in reduced viscosity, which also requires a lower voltage (Khaleel et al., 2024). This indicates weaker interaction with the electric field at low solution concentrations, leading to thinner fibers.

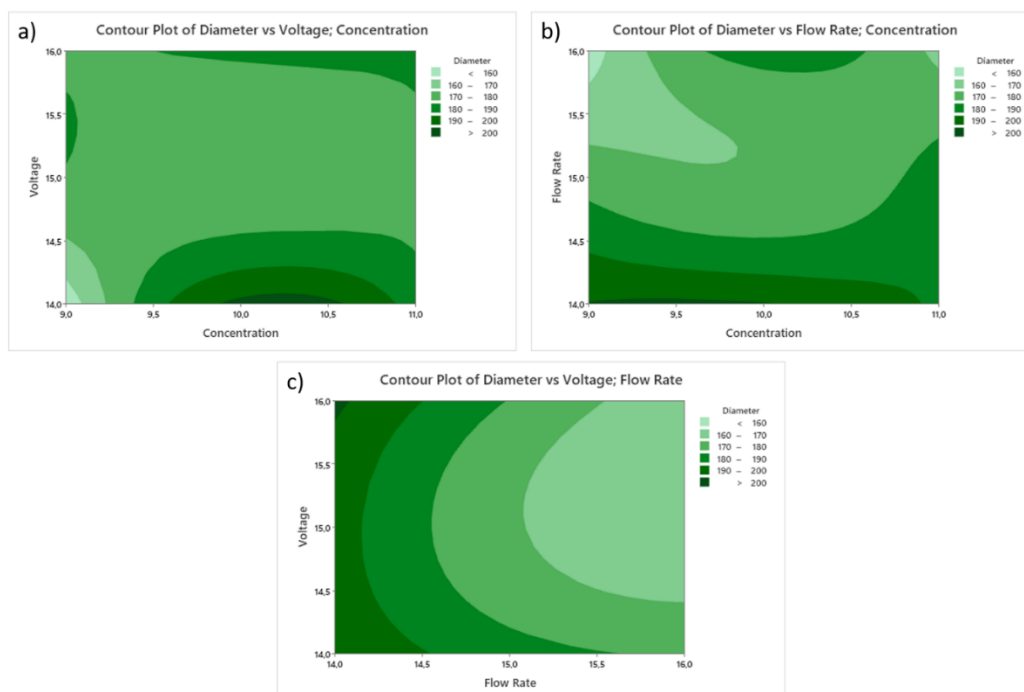


Figure 3. Analysis of Contour Plot of Diameter, a) Voltage vs Consentration, b) Flow Rate vs Concentration, c) Voltage vs Flow Rate

Figure 3.b illustrates the influence of voltage and flow rate, showing that higher flow rates and voltages result in smaller nanofiber diameters. This finding is consistent with the ANOVA test, which identified flow rate as a significant factor in determining nanofiber diameter. A higher flow rate requires a higher voltage, producing thinner and smoother nanofibers (Kang et al., 2021). This is indicated by the light green color in the Figure 3, observed at higher flow rates and voltages.

Figure 3.c highlights the effect of flow rate and concentration, showing that higher concentrations require a greater flow rate. Moreover, a higher flow rate results in smaller nanofiber diameters when concentration is low. Another observation is that smaller nanofibers are obtained with both high flow rates and high concentrations. This occurs due to the balance between flow rate, concentration, and voltage. Thus, the relationship between concentration, voltage, and flow rate is proportional. When concentration is low, the flow rate and voltage should also be low. Conversely, higher concentrations demand higher flow rates and voltages (Dhakate et al., 2011).

Scanning Electron Microscopy (SEM) analysis was performed to examine the correlation between the experimental results and the statistical model obtained using the Box–Behnken Design (BBD). The SEM images (Figure 4) revealed that the nanofibers exhibited uniform and elongated structures. The nanofibers display a smooth morphology without visible bead formation and maintain a consistent diameter along their length. The fiber diameters were measured using ImageJ and OriginLab software, yielding an average diameter of 154.69 ± 4.51 nm. This result indicates that the experimentally measured nanofiber diameters are in close agreement with the values predicted by the BBD model. Overall, the findings confirm a strong positive correlation between the SEM observations and the BBD-based predictions.

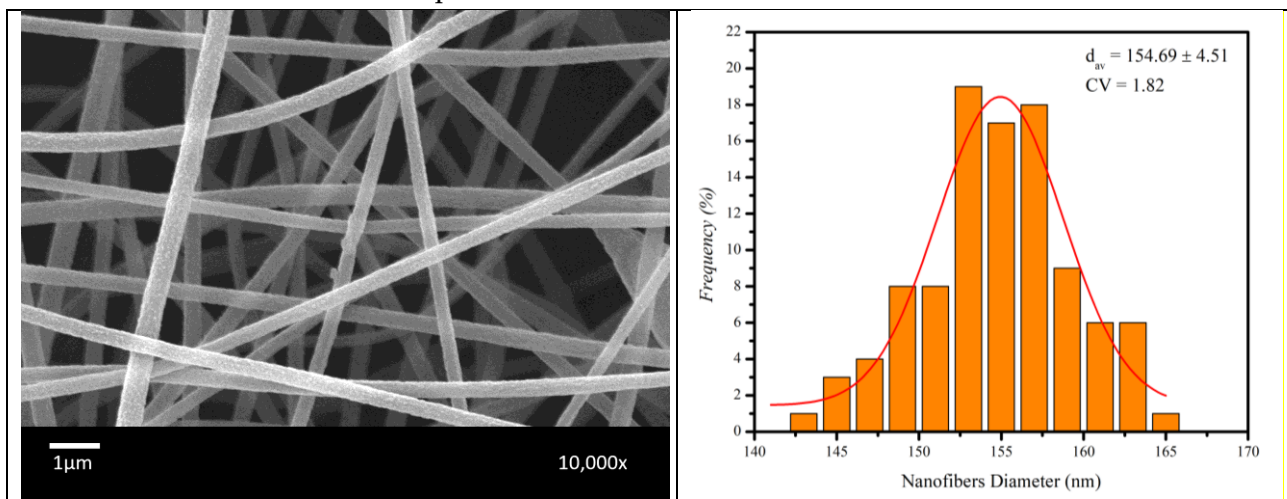


Figure 4. The SEM Images with Diameter Distributions of Sample N1 Represents the Optimal Process

4. Regression Equation

$$\begin{aligned}
 \text{Nanofiber Diameter} &= 181.55 - 5.46K1 + 3.60K2 + 1.85K3 + 6.22T1 - 6.14T2 \\
 &\quad - 0.08T3 + 16.63L1 + 4.69L2 + 11.94L3
 \end{aligned}
 \tag{1}$$

Where K represents solution concentration, T is voltage, and L is flow rate. This mathematical model predicts the impact of these parameters on fiber diameter. According to the predictions, the optimal parameters for achieving the smallest nanofiber diameter are 144.45 nm.

CONCLUSIONS

In this study, PLA/PVA nanofibers were successfully fabricated. Process parameters were optimized using the Box-Behnken Design to determine their effect on nanofiber diameter. The study found that the optimal parameters for the smallest fiber diameter were obtained in sample N1, with a PLA concentration of 9%, voltage of 14 kV, and flow rate of 16 $\mu\text{L}/\text{min}$. The resulting nanofibers had a very small diameter of 152.67 nm with a smooth and uniform distribution. The predicted value from the regression model based on the optimal parameters yielded a nanofiber diameter of 144.45 nm, which is close to the experimental result. Nanofibers with smaller diameters have a larger specific surface area, making them suitable for a wide range of applications.

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