Magneto-Thermal Effect in Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG Nanoparticles and Their Potential as Hyperthermia Therapy

Sunaryono$^{1,2,*}$, Kormil Saputra$^1$, Arif Hidayat$^1$, Chusnana Insjaf Yogyihat$^1$, Sigit Tri Wicaksono$^3$, Nurul Hidayat$^1$, Samsul Hidayat$^1$, Siriwat Soontaranon$^4$

$^1$ Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5, Malang 65145, Indonesia
$^2$ Center of Advanced Materials for Renewable Energy (CAMRY), Universitas Negeri Malang, Jl. Semarang 5, Malang 65145, Indonesia
$^3$ Department of Materials and Metallurgical Engineering, Faculty of Industrial Technology, Institut Teknologi Sepuluh Nopember Surabaya, Jl. Teknik Industri Kampus ITS Sukolilo Surabaya 60111, Indonesia
$^4$ Synchrotron Light Research Institute, Muang, Nakhon Ratchasima 30000, Thailand

*Corresponding author’s email: sunaryono.fmipa@um.ac.id

Abstract: Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG nanoparticles based on local iron sand were synthesized using the coprecipitation method. The characterization of sample was conducted using the X-ray Diffraction (XRD) instrument, Fourier Transform Infrared (FTIR), Small Angle X-Ray Scattering (SAXS), and Magneto-thermal each has a purpose to find out the formed phase structure, the adsorption pattern of sample functional group, nanoparticle distribution, and thermal effect of the sample. The results of characterization using XRD showing that the formed sample phase was in the form of the magnetite spinel structure. Through Rietica analysis and calculation using Debye-Scherrer, the sizes of nanoparticle samples were 7.9, 6.4, and 5.3 nm respectively with the addition of PEG concentration of 1000, 2000, and 4000. The adsorption of nanoparticle functional group was confirmed well with the appearance of Fe-O and Mn-O bound adsorption, at the wavenumbers of 430 cm$^{-1}$ and 482 cm$^{-1}$ respectively which were the representations of Mn$_{0.25}$Fe$_{2.75}$O$_4$ material. Furthermore, the SAXS data analysis using the two lognormal method showed that the primary size of the particle sample was around 3 nm. Meanwhile, the secondary sizes of the sample were 8.5, 7.1, and 5.9 nm with the addition of PEG concentration of 1000, 2000, and 4000. Interestingly, Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG nanoparticles of 1000, 2000, and 4000 characterized using the Magneto thermal instrument have the value of 1.079, 1.082, and 1.105 W/g respectively and was able to improve the temperature of 37 °C up to 38°C. These characteristics showed that the Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG nanoparticles have potentials to become a unique material which can function as the material for hyperthermia therapy.

Keywords: Magneto-thermal, Mn$_{0.25}$Fe$_{2.75}$O$_4$, PEG, Hyperthermia, SAXS
1. Introduction
Cancer is one of the types of diseases with an abnormal cell growth [1]. Cancer also has the potential to spread out or attack other cells in its nearby [2]. One of the solutions to kill the cancer cell is performing a hyperthermia therapy method to the organ attacked by the cancer cell. Hyperthermia is a therapy performed by inducing the heat to the cancer cells until a certain temperature around 113 °F/ 45 °C [3]. Many methods and materials are used as media to perform hyperthermia therapy, one of them is by approach method based on magnetite materials (Fe₃O₄).

Through the magneto-thermal properties of the utilized materials, the value of Specific Absorption Rate (SAR) from the material, it can be obtained and becomes one of the important parameters which functions as a cancer therapy agent [4]. The studies on the value of SAR from several materials have been reported by previous research.

The studies about the value of SAR from several materials have been reported by previous researchers. Lao and Ramanujan [5] report that Fe₃O₄ nanoparticle is a significant potential to be used as a material for hyperthermia therapy. The value of SAR obtained from the research is 8.7 W/g with the frequency of generator magnetic fields of 375 kHz. Furthermore, with the frequency of generator magnetic fields of 80 kHz, Gudoshnikov et al. [6] explain that the value of SAR in magnetic nanoparticles achieve the values of 60 W/g up to 80 W/g. Meanwhile, another report explains that the SAR value of Fe₃O₄ nanoparticles does not depend on the concentration of Fe₃O₄ nanoparticles [7]. The amount of concentration in Fe₃O₄ nanoparticles affects the change of temperature in the yielded sample. However, the thermal property of Fe₃O₄ nanoparticles is still relatively small to yield an optimal temperature which can be applied to the hyperthermia therapy.

Accordingly, an in-depth study needs to be conducted in increasing the thermal property of the utilized materials. One of them is by synthesis and fabrication of Fe₃O₄ with the Mn doping. This research reports the distribution of particle size and the magneto-thermal analysis on the Fe₃O₄ nanoparticles modified with the Mn doping and the addition of PEG polymer. Mn doping chosen in this study is the Mn doping with the fraction composition of dopant of 25% wt. This is in accordance with the previous research report stating that 25% wt Mn dopant is the best composition in yielding the magnetic property from Mn₀.₂₅Fe₂.₇₅O₄ nanoparticles [8]. Besides that, the addition of PEG polymer is expected to decrease the aggregation between the obtained Mn₀.₂₅Fe₂.₇₅O₄ nanoparticles. Eventually, with the respective condition, it is expected that the thermal property of Mn₀.₂₅Fe₂.₇₅O₄-PEG nanoparticles can be better and representatives for the medication of hyperthermia therapy.

2. Methods

2.1. Synthesis Mn₀.₂₅Fe₂.₇₅O₄-PEG Nanoparticles
The synthesis of Fe₃O₄ nanoparticles was initiated by separating the iron sand from Sine Beach, Tulungagung, Indonesia using a permanent magnet. The 20-gram Fe₃O₄ powder as the results of separation was then collected to be reacted with HCl (12 M). The solution of Fe₃O₄ and HCl mixture was then filtered to obtain FeCl₂ and FeCl₃. The FeCl₂ and FeCl₃ solution was then mixed with MnCl with a certain composition and spun for 15 minutes at room temperature above hotplate magnetite stirrer. On the mixture, it was dripped with an NH₄OH solution (6.5M) also at room temperature so that it created sediment of Mn₀.₂₅Fe₂.₇₅O₄ particles. To remove the impurity materials, the Mn₀.₂₅Fe₂.₇₅O₄ sediment was then washed repeatedly until the sediment’s pH reaching 7 using distilled water. The sediment of the purification was then reacted with the PEG polymer (1000, 2000, and 4000) until yielding the Mn₀.₂₅Fe₂.₇₅O₄-PEG nanoparticles.

2.2. Characterization of Mn₀.₂₅Fe₂.₇₅O₄-PEG Nanoparticles
This research used the instrument of XRD X’Pert Pro Panalytical characterization with the wavelength specification of 1.5406 Å and the angles of reach between 10°-80° which is used to find out the crystal structure of the materials, phase composition, crystal size, and the lattice parameter. FTIR Shimadzu with the type of IR-Prestige 21 was used to know the functional group of Mn₀.₂₅Fe₂.₇₅O₄ after given PEG.
The magneto-thermal instrument with cooling duos with the frequency range of 533 Hz up to 1,357 kHz was utilized to find out the information of temperature change in Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG nanoparticles when given the magnetic field. Furthermore, this research also used the SAXS BL1.3W characterization with CCD detector (Rayonix SX165, LX170HS) on the range of 1200 mm up to 4200 mm used to find out the distribution of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG nanoparticles. The SAXS data analysis of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG nanoparticles employed Two lognormal method which was capable of calculating the primary and secondary particle size of the samples.

3. Results and Discussion

3.1. The Results of XRD Characterization

The XRD characterization results of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG are shown in Figure 1. The diffraction pattern of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles with the PEG variations of 1000, 2000, and 4000 was indicated with the existence of the magnetite phase in the diffraction pattern. The magnetite phase has the highest peak with the angle of 35.4° on the hkl field (311), while the other 2θ peaks were identified at 30.15°, 43.05°, 53.5°, 56.97°, 62.59°, and 74.28° each of them correlated with Miller index (111), (220), (400), (422), (511), and (440). These Results are in line with the previous research results [9]. However, the intensity obtained in this research was higher than the previous research. This was possibly due to the utilized composition of PEG polymer. In the previous work, the polymer composition being studied was PEG 6000 polymer until the chain diffraction of PEG 6000 polymer was more dominantly detected than the diffraction of the Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticle. Besides that, the data in Figure 1 also showed that the greater the composition of PEG given, the smaller the intensity of diffraction peak of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG sample.

This phenomenon can be explained from the interaction pattern of the PEG polymer chain on Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles. The PEG polymer chain has two different properties on the sides of the chain ends, both properties are hydrophilic on one end of the chain and hydrophobic on another end. From this property, the PEG polymer chain with hydrophilic property would stick to the surface of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles and the end of the polymer chain with hydrophobic property would move freely. The attachment of PEG polymer on the surface of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticle caused the nanoparticle included in the network of the polymer chain. The greater the composition of the polymer chain (from PEG 1000 to PEG 4000) which covered Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles, the greater the nanoparticles would be included in the polymer chain network so that the surface of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles would experience a decrease in the diffraction exposure and eventually caused the diffraction intensity of the crystal to shrink.

![Figure 1. The Refinement Results of Diffraction Pattern of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 1000-4000 nanoparticles](image-url)
An in-depth analysis of each maximum diffraction peak of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles is presented in Figures 2, 3, dan 4. From the figure, it can be seen that there was a decrease of the crystal diffraction intensity of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles along with the increase of molecule mass of PEG (1000 up to 4000) which was utilized. Through Rietica analysis, the crystal grain size of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 1000, 2000, and 4000 nanoparticles have values of 9.0, 5.1, and 4.2 nm respectively. The bigger the molecule mass of PEG, the smaller the particle size of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles. These results are in line with the research reported by Taufiq et al. [10] and Zakerhamidi et al. [11]. Similar to the previous discussion results, the decrease of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles’ size when added the greater PEG molecule mass was caused by the growth limitation of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles. The limitation of nanoparticle growth was caused by the area of the nanoparticle’s surface covered by the PEG polymer chain with hydrophilic property. The greater the PEG polymer molecule used, the greater the hydrophilic polymer chain covering the surface of nanoparticles and in the eventually it reduced the growth and aggregation on the respective nanoparticles.

![Figure 2. The Fitting Results of Diffraction Peak Pattern of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 1000 nanoparticles](image)

![Figure 3. The Fitting Results of Diffraction Peak Pattern of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 2000 nanoparticles](image)

![Figure 4. The Fitting Results of the Diffraction Peak Pattern of Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 4000 nanoparticles](image)
Furthermore, the XRD data analysis was performed using the Debye Scherrer equation approach through the Rietveld Method. The crystal grain size of $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG 1000 up to 4000 nanoparticles were approximately 7.9, 6.4, and 5.2 nm respectively. This result has a pattern similarity with the results obtained from the analysis using Rietica. Besides that, this result also experienced a decrease in aggregation of $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$ nanoparticles along with the increase in molecule mass of the used PEG polymer. The comparison results of crystal grain sizes of $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$ nanoparticles both using Rietica analysis and the Debye Scherrer equation can be shown in Table 1.

| No | Sample                      | Lattice Parameter $a = b = c$ (Å) | Crystal Grain Size (nm) |
|----|------------------------------|-----------------------------------|-------------------------|
|    |                              | Rietica  | Bragg  | Rietica  | Debye-Scherrer |
| 1  | $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG 1000 | 8.38     | 8.38   | 9.0       | 7.9           |
| 2  | $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG 2000 | 8.38     | 8.38   | 5.1       | 6.4           |
| 3  | $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG 4000 | 8.38     | 8.40   | 4.2       | 5.2           |

3.2. The Results of FTIR Characterization

The results of FTIR characterization of $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG are shown in Figure 5. These characterization results were used to find out the absorption power of the functional group of $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$ nanoparticles after given PEG polymer. The PEG polymer has the chemical structure of -OH[-CH$_2$-CH$_2$]$_n$OH adjusted with the chain length of the PEG polymer. $N$ index showed the average total of oxyethylene group or the solution thickness. Index $n$ can amount to $n = 2, 3, 4$ and maximum $n$ amounted 180. The more the index value, then the longer the polymer chain length of PEG.

![Figure 5. The results of FTIR characterization for $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG 1000-4000 samples](image-url)
The results of FTIR characterization shows there is a stretching at the wavenumber of 430 cm\(^{-1}\) and 482 cm\(^{-1}\) each representing the bounds of Fe-O and Mn-O. These results explained that Mn doping can succeed well in Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\) nanoparticles. The appearance of the bond was similar to the research report performed by Veisi et al. [12] and Zhang et al. [13] that is there were bounds of Fe-O and Mn-O at the wavenumber of \(\leq 700\) cm\(^{-1}\). Although, Meng and Zhang [14] and Sundari et al. [15] reported that for Fe-O and Mn-O are located at the wavenumber of \(\geq 685\) cm\(^{-1}\). However, Singh et al. [16], Wang et al. [17], and Vamvakidis et al. [18] explained that the bounds of Fe-O and Mn-O would experience stretching vibration at the wavenumbers of 430 – 580 cm\(^{-1}\).

PEG polymer with the chemical structure of -OH[-CH\(_2\)-CH\(_2\)-]n OH which became the template of Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\) experienced stretching vibration at the wavenumbers of 921, 1905, 1251, 1352, 1472, 1629, and 2879 cm\(^{-1}\) which were the representations of C-H functional group, in addition the appearance of stretching vibration a the wavenumber of 1300 cm\(^{-1}\) referred to C=O functional group. This result is in accordance with the study conducted by Catauro et al. [19]. They reported that C=O functional group experienced stretching vibration at the wavenumber of 1250 cm\(^{-1}\), while the C-H functional group would experience stretching vibration at the wavenumbers of 1298, 1350, 1456-1471, and 2879-2791 cm\(^{-1}\).

Meanwhile, the stretching vibration for O-H functional group located at the wavenumber of 3346 cm\(^{-1}\) which also represented the existence of functional group uptake of PEG polymer. This is confirmed well by the research conducted by Vamvakidis et al. [18], Ghosh et al. [20], and Sun et al. [21]. They reported that the stretching vibration for O-H functional group was located at the wavenumber of \(\sim 3439\) nm\(^{-1}\). And these results were strengthened in a report by Singh et al. [16] reporting that the stretching vibration for O-H functional group was located at the wavenumber of \(\sim 3500\) cm\(^{-1}\) range.

### 3.3. The Results of SAXS Characterization

SAXS is an advanced instrument which is able to characterize the fractal structural pattern of a particle, particle distribution, particle aggregation, particle shape, the distance between particles and others. Figures 6, 7, and 8 are the refinement results of SAXS characterization of Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\)-PEG 1000-4000 samples using SASfit Software. The fitting results generated the value of \(D\) fractal dimension determined by matching the exponential between the fractal are intensity scattering curve \(I(q)\), form factor \(P(q)\), and \(S(q)\) factor structure [22]. The structural pattern of a factor from the particle distribution generated the correlation length \(\xi\) or the distance of particle aggregation and the dimension of \(D\) fractal [23]. In addition, the patterns of particle distribution were in the form of particle primary size \((R_1)\) and particle secondary size \((R_2)\) which are concluded in the system of intensity scattering curve \(I(q)\) can be stated in the following Equation 1 [24].

\[
I(Q) \propto \int_0^\infty N_1(R_1)^2 (Q.R_1) dR_1 S(Q,\xi,D,R_1) + \int_0^\infty N_2(R_2) F_N^2(Q,R_2) dR_2 S(Q,\xi,D,R_2)
\]  

(1)

The model employed in analyzing the SAXS data in this research was two lognormal model. This model was able to represent the patterns of particle distribution and form factor from the structured particle. The fitting results are as shown in Figures 6, 7, and 8 showing a high matching between the experimental results data (signified with a black-colored rounded curve) and the model data (signified with a red-colored lined curve). From the fitting results, the information obtained was the primary size of Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\)-PEG 1000 – 4000 nanoparticles were 3.08 nm. This result is in accordance with the primary size of Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\) nanoparticles without PEG polymer as reported by Taufiq et al. [8]. Furthermore, the secondary sizes of Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\)-PEG 1000–4000 nanoparticles were 8.5, 7.1, and 5.9 nm respectively. The results of these particle secondary sizes are not significantly different from the refinement results of Rietica and the calculation results using Debye Scherrer method from the aforementioned XRD characterization results. Therefore, the correlation of nanoparticle size between SAXS and XRD data analysis can be concluded that the secondary size of Mn\(_{0.25}\)Fe\(_{2.75}\)O\(_4\)-PEG 1000-4000 nanoparticles obtained from SAXS analysis were the representations of the particle aggregation which was also confirmed by XRD data analysis.
3.4. The Results of Magnetothermal Characterization

The results of magneto-thermal characterization $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$-PEG nanoparticles without PEG polymer are shown in Figure 9. The variations of PEG 1000, 2000, and 4000 molecule mass given to $\text{Mn}_{0.25}\text{Fe}_{2.75}\text{O}_4$ nanoparticle causing a different increase in temperature alteration ($\Delta T$) generated by the samples. The alteration of the given PEG molecule mass does not positively correlate with the increase of the temperature alteration.
Figure 9. The results of magneto thermal characterization for Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 1000-4000 sample

Through the reference of the initial temperature of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles without PEG polymer template, the particle changes of Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticle sample with the variations of PEG namely 1000, 2000, and 4000 respectively have the temperature change of 37, 38, and 38 $^\circ$C as presented in Figure 10. The highest temperature change was obtained when the Mn$_{0.25}$Fe$_{2.75}$O$_4$ nanoparticles given the polymer template of PEG 2000 and PEG 4000 reached the temperature change up to 38 $^\circ$C. The results of the temperature change are categorized as low compared to the temperature change reported by the previous researcher that is approximately 43 up to 47 $^\circ$C [5]. One of the factors affecting the small temperature change in this research was the utilized magnetic field. The ability of frequency from the magneto-thermal instrument utilized in this research was approximately 965 Hz. Ramanujan and Lao [5] reported that the temperature change of magnetite nanoparticles will reach the optimal value when the magneto-thermal of the magnetite sample is characterized using the alternating frequency up to 375 kHz. In fact, several other researchers use the alternating frequency with the range of 188-375 kHz [25] and 123-430 kHz [26].

Hyperthermia therapy is one of the cancer therapies by increasing the heat performed on the cancer cell with an optimal temperature of 43 up to 47 $^\circ$C [5]. On the temperature change, the specific absorption rate (SAR) from the sample is classified into an optimal and effective category to kill the cancer cell. The SAR of the magnetic nanoparticle can achieve the value of 3.7 up to 325 W/g Fe in the influence of certain magnetic field with the frequency range of 123-430 kHz [26]. The SAR value analysis of the nanoparticles samples and the magnetic hydrogel is determined by the increase of temperature on the time duration as formulated in Equation (2).

$$\text{SAR} = c \left( \frac{\Delta T}{\Delta t} \right)$$

where, $c$ is the heat capacity of Mn$_{0.25}$Fe$_{2.75}$O$_4$–PEG, $\Delta T$ is the temperature change, and $\Delta t$ is the time duration. From the graphic of correlation between the temperature and the time duration, then the slope of the temperature change curve on the time duration can be calculated to determine the SAR value [27]. The graphic of correlation between the temperature of Mn$_{0.25}$Fe$_{2.75}$O$_4$–PEG nanoparticles on the time duration is shown in Figure 11, through the graphic of the slope, the SAR value of Mn$_{0.25}$Fe$_{2.75}$O$_4$–PEG 1000, 2000, and 4000 nanoparticles respectively have the values of 1.079, 1.082, and 1.105 W/g. the bigger the PEG template used, the greater the SAR value of Mn$_{0.25}$Fe$_{2.75}$O$_4$–PEG.
Figure 10. The magneto thermal characterization for Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG 1000-4000 sample.

Figure 11. Linear magneto-thermal fitting for Mn$_{0.25}$Fe$_{2.75}$O$_4$-PEG sample.
These results are in line with the results obtained by Shah et al. [26]. They characterized maghemite sample magneto-thermal with several variations of particle size namely 8, 11, 13, and 18 nm. From the research, they reported that the highest value of SAR was 325 W/g experienced by the maghemite particle which has the size of 18 nm. Accordingly, the particle size and PEG template used in the synthesis of Mn₀₂₅Fe₂₇₅O₄ nanoparticles can affect the value of SAR in the respective particles.

4. Conclusion
The synthesis of Mn₀₂₅Fe₂₇₅O₄-PEG nanoparticles with the variation of PEG template composition (1000, 2000, and 4000) has been successfully conducted using a coprecipitation method. Through the Rietica analysis and calculation using Debye-Scherrer, the size of Mn₀₂₅Fe₂₇₅O₄-PEG nanoparticles PEG 1000, 2000, and 4000 are 7.9, 6.4, and 5.3 nm respectively. Meanwhile, through SAXS data analysis, the primary particle of Mn₀₂₅Fe₂₇₅O₄-PEG sample has a value of 3 nm with the secondary particle of each sample with the values of 8.5, 7.1, and 5.9 nm with the addition of PEG concentration of 1000, 2000, and 4000. Furthermore, the particle size and PEG template can affect the value of SAR of Mn₀₂₅Fe₂₇₅O₄-PEG nanoparticles. The SAR value of the nanoparticles have the values of 1.079, 1,082, and 1,105 W/g respectively with the addition of PEG template molecule mass of 1000, 2000, and 4000, as well as able to improve the temperature from 37-38 °C.

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