

Preparation of magnetite nanoparticle and fatty acid incorporated poly(methacrylic acid-ethyl acrylate) nanowebs via electrospinning for magnetic hyperthermia application

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Abstract. Magnetic hyperthermia has been arising as a promising approach for treatment of cancer. When magnetic nanoparticles (MNPs) are locally injected through cancerous tissues and subjected to an appropriate alternating magnetic field, they generate heat due to the rotation of the nanomagnets, causing the destruction of the cancer cells. For repeated applications of magnetic hyperthermia, it is highly preferred to keep the temperature constant at about 41–46°C while preventing the leakage of MNPs, to minimize secondary effects on surrounding healthy tissues. In our study, we produced novel nanowebs provided with magnetic and thermal buffering properties by encapsulating MNPs and Lauric Acid in poly(methyl ethyl acrylate) matrix via uniaxial electrospinning. For the oleic acid functionalized magnetic nanoparticles, the magnetization for the corresponding nanowebs is higher by a factor of two for all concentrations than the ones with non-functionalized magnetic nanoparticles, indicating a more effective integration of the functionalized nanoparticles through the nanoweb. The heat absorption and release capacities of the nanowebs, incorporated with 1.25–2.50 % functionalized MNPs, vary between 70–75 Jg⁻¹ at 40–48°C. They also demonstrate thermal cycling ability and thermal stability.

1. Introduction

Despite the significant advances recorded in recent years, cancer is still one of the leading causes of human death worldwide [1]. Hyperthermia is an effective treatment method of general or regional warming of body tissue to kill tumor cells in the cancerous area [2] and [3]. Magnetite (Fe₃O₄) nanoparticles (MNPs) have been intensively investigated for biomedical applications due to their magnetic properties and dimensions similar to the biostructures. Recently, magnetic hyperthermia has also been arising as a promising approach for treatment of cancer [1] and [2]. In this method, when magnetic nanoparticles, locally injected through cancerous tissues, are subjected to an appropriate alternating magnetic field (AC), they generate heat due to the rotation of the nanomagnets themselves or the reversal of the direction of the magnetization vector μ of each single domain [2]. Cells are susceptible to heat damage because heat energy indiscriminately disrupts cellular pathways, especially through its effects on protein structure and function. For repeated applications of magnetic hyperthermia,



it is highly preferred to keep the temperature constant at about 41–46°C while preventing the leakage of MNPs, to minimize secondary effects on surrounding healthy tissues. It would be therefore highly advantageous if a material could be developed that would allow the repeated heating of the MNPs [3]. To maintain the biocompatibility of magnetic nanoparticles in human body and to prevent their sedimentation within the tissues, they should be suspended in suitable fluids and coated by biocompatible polymers [4].

Previous studies have suggested developing polymeric formulations to coat or to encapsulate MNPs prior to their hyperthermia application [4], [5], [6] and [7]. Combining MNPs and polymeric matrices not only can lead to significant enhancement in the surface characteristics of the particles, but also polymers may serve as carrier for particles, as coating materials and encapsulating shells, as direct targeting and stabilizing agents, or as linker to couple functional biomolecules [8], [9], [10], [11], [12], [13] and [14]. Recently, Huang *et al.* [15] described the incorporation of MNPs into electrospun polystyrene fibres for developing novel mediators for magnetic hyperthermia as an anti-cancer strategy. They suggested that the use of electrospun fibres for the encapsulation of MNPs has the additional advantage of fibres displaying a very high surface area over volume ratio, which may enhance their interactions with the surrounding tissues and the possible use of coated fibres for cell binding applications. Owing to the very small dimensions of MNPs, electrospinning of nanofibers presents itself as a simplistic method to decorate or incorporate MNPs into polymeric nanofibers to add functionality. Up to now, numerous MNPs including magnetite have been successfully encapsulated into polymeric nanofibers where the principle of electrospinning remains similar. Most of these previous studies focused primarily on the production and characterization of magnetic fibres [16], [17], [18] and [19]. However, studies taking advantage of the potential of local heat-generating capability of magnetic electrospun nanofiber composites upon application of alternating magnetic field for hyperthermia treatment are limited [15] and [20]. One study reports on a magnetic nanofiber that encapsulated MNPs being used for cancer therapy, which was delivered by surgical or endoscopic methods precisely to the tumour site. Another study explains the fabrication of electrospun polyurethane (PU) nanofibers decorated with superparamagnetic Fe_3O_4 NPs and their potential use as heat-generating substrates for magnetic hyperthermia application [21].

Phase change materials (PCMs), which absorb and release a great amount of heat repeatedly during phase conversions between solid-liquid phases over a certain temperature range, have been receiving great attention in the last decades particularly to develop efficient thermal energy storage systems. A wide variety of PCMs are available with different heat storage capacities and phase change temperature intervals. Among them, fatty acids derived from the fats and oils, have recently attracted attention as potential bio-based and renewable PCM candidates in consequence of their attractive characteristics, such as high heat storage densities, low vapour pressure in the liquid phase, chemical and thermal stability, non-toxicity, biocompatibility and commercial availability at a relatively low cost [22]. In the last decade, a few research groups performed studies on the preparation of magnetic nanoparticle-PCM mixtures for testing their magnetic hyperthermia properties. Pradhan *et al.* prepared lauric acid-coated, superparamagnetic, nanoparticle-based magnetic fluids of different ferrites suitable for hyperthermia application [23]. Zhao *et al.* studied magnetic and inductive heating properties of Fe_3O_4 /polyethylene glycol composite nanoparticles with core-shell structure [24].

The aim of this study is to develop novel nanowebs provided with magnetic and thermal buffering properties by encapsulating MNPs and Lauric Acid (LA) in poly(methyl ethyl acrylate) (PMEA) matrix via uniaxial electrospinning. Structural and thermal characterizations of the electrospun nanowebs were then carried out throughout the Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), Thermogravimetry (TG) analyses. Magnetic properties of the NWs were characterized as a function of temperature and of applied magnetic field.

2. Experimental

2.1. Materials

The oleic acid functionalized Fe_3O_4 magnetic nanoparticles (MG3) and non-functionalized Fe_3O_4 magnetic nanoparticles (MG6) were synthesized by M D Carvalho, L P Ferreira and M M Cruz through the project UID/MULTI/04046/2013 and UID/MULTI/00612/2013 [Portuguese FCT foundation].

Lauric Acid [$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$] (LA), chloroform and methyl alcohol as solvents were all supplied from Sigma-Aldrich Chemicals (USA). For encapsulating LA and MNPs mixtures by uniaxial electrospinning, the copolymer of methacrylic acid and ethyl acrylate (PMEA) with the methacrylic acid groups of 46.0–50.6%, glass transition temperature of $96 \pm 5^\circ\text{C}$ and the molecular weight of $320,000 \text{ g mol}^{-1}$, was kindly supplied from Evonik Company (USA) under the trade name of Eudragit L100-55® in powdered form.

2.2. Methods

The nanowebs of PMEA, LA and MNPs were manufactured using uniaxial apparatus of the electrospinning device with 15 cm collector distance (Yflow Co.; Spain). The whole procedure was as follows. PMEA and LA were dissolved in chloroform-methanol (7 mL: 3 mL) solvent mixture. The final concentration of PMEA and of LA were calculated as 14%. Then, different amounts of MNPs with respect to the mass of electrospinning mixtures (either MG3 or MG6) were added to those solutions while stirring by a shaker for 5 minutes. The resultant MNP percentages were 1.25%, 1.75% and 2.50%. From then on, each mixture, instantly drawn into the pump injector, was electrospun under the processing parameters given in Table I. As spun nanofibers were collected on an aluminium sheet of 15 cm x 15 cm.

Table 1 Composition of the NW Samples and their electrospinning conditions

Sample	PMEA (g)	LA ^a (g)	MNP ^b (%)	η^c (Pas)	Pump rate (mLh ⁻¹)	Injector Voltage (kV)	Collector Voltage (kV)	Time (h)
PMEA-LA	2.5	2.5	-	0.84	0.040	+5.0	-5.0	1.0
PMEA-LA-MG3-1.25	2.5	2.5	1.25	0.87	0.030	+10.0	-10.0	1.0
PMEA-LA-MG3-1.75	2.5	2.5	1.75	0.95	0.030	+10.0	-10.0	1.0
PMEA-LA-MG3-2.50	2.5	2.5	2.50	1.02	0.030	+10.0	-10.0	1.0
PMEA-LA-MG6-1.25	2.5	2.5	1.25	0.89	0.040	+10.0	-10.0	1.0
PMEA-LA-MG6-1.75	2.5	2.5	1.75	1.06	0.030	+5.0	-5.0	1.0
PMEA-LA-MG6-2.50	2.5	2.5	2.50	1.11	0.030	+4.0	-4.0	1.0

^a LA= Lauric Acid, ^bMNP%0 w.r.to the mass of electrospinning mixture, ^c η =dynamic viscosity of the electrospinning mixture

The Fourier transform infrared (FTIR) transmission spectra of the electrospun nanowebs were recorded between 4000 and 650 cm^{-1} at a resolution of 4 cm^{-1} using a Perkin Elmer Spectrum 100 FTIR spectrometer equipped with a universal attenuated total reflection (ATR) accessory. The thermal properties of the materials and nanoweb (NW) samples were investigated under nitrogen atmosphere (20 mL min^{-1}) using a Perkin Elmer DSC 4000 differential scanning calorimeter (DSC). Temperature scans were run on samples that had been placed in a closed pan and that weighed 10 – 15 mg for ten successive heating and cooling cycles. All samples were brought to thermal equilibrium at the starting temperature. Then, the samples were heated to the final temperature at a heating rate of $10^\circ\text{C min}^{-1}$. Finally, the samples were cooled to the starting temperature at a cooling rate of $10^\circ\text{C min}^{-1}$.

Thermogravimetric (TG) analyses of the samples were performed from 20 to 650°C at a heating rate

of $10\text{ }^{\circ}\text{Cmin}^{-1}$ under a dry nitrogen atmosphere purged at a rate 20 mLmin^{-1} by a SEIKO EXSTAR 6200 Model TG/DTA instrument. Magnetic properties of the NWs were characterized using a QD-MPMS SQUID magnetometer as a function of temperature, between 10 and 380 K, and of applied magnetic field up to 5.5 T. The temperature dependence was studied in an applied magnetic field of 2 mT after cooling from 300 K in zero magnetic field (zero field cooled–ZFC) and after cooling under the measurement field (field cooled–FC).

3. Results and Discussion

The FTIR transmission spectra of the electrospun PMEAL-LA NWs, including different percentages of MG3 and MG6, as well as that of PMEAL-LA are shown in Figure 1 (a) and (b). All distinctive bands of the PMEAL and Lauric Acid are observed in the FTIR spectra of the NW samples. Asymmetric and symmetrical stretching vibrations of $-\text{CH}_3$ and $-\text{CH}_2$ groups of LA are observed at 2954, 2917 and 2849 cm^{-1} . The stretching bands for the ester bonds of the methacrylic acid and ethyl acrylate groups of the matrix PMEAL, namely $\text{O}-\text{C}=\text{O}$ ($-\text{COO}$) and the $\text{O}-\text{C}$ and $\text{C}=\text{O}$, as well as those of Lauric Acid are observed at 1731, 1697 and 1159 cm^{-1} . The scissoring deformation band of long chain $-\text{CH}_2$ groups is observed at 1470 cm^{-1} . Consequently, the distinctive IR bands of PMEAL-LA-MG3 and PMEAL-LA-MG6 nanowebbs and the IR bands of PMEAL-LA NW sample are all overlapped, suggesting that the addition of MNPs (MG3 or MG6) does not have any adverse effect on the electrospinnability and physicochemical properties of PMEAL-LA mixtures.

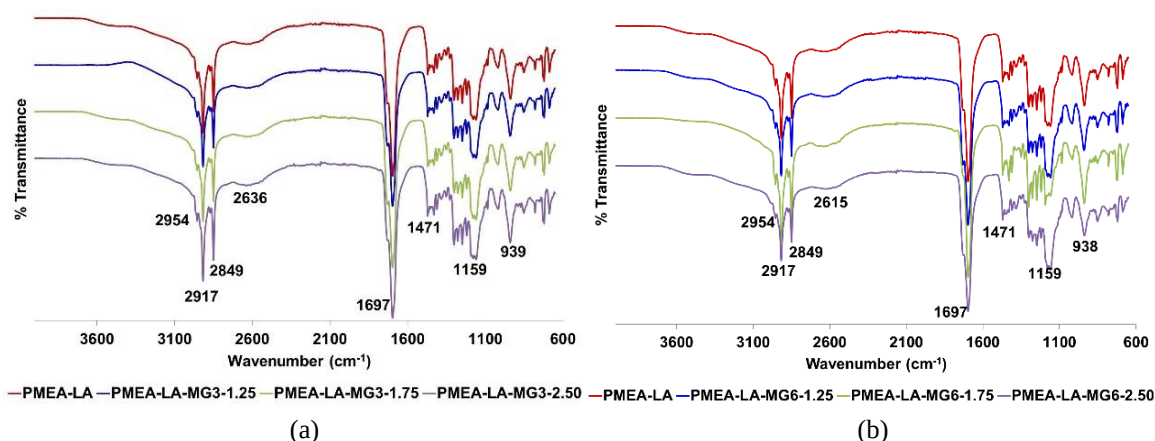


Figure 1. FTIR transmission spectra of (a) PMEAL-LA and MG3 incorporated NWs of PMEAL-LA-MG3; (b) PMEAL-LA and MG6 incorporated NWs of PMEAL-LA-MG6.

Table 2 summarizes the phase transition temperatures and enthalpies of all the NWs. Figure 2 illustrates DSC results for the PMEAL-LA-MG3 NW samples obtained from the 10th heating-cooling cycles. The DSC results are consistent with the FTIR results. As seen from Table 2 and Figure 2, the onset, peak and end temperatures of the phase transitions of the PMEAL-LA-MG3 and PMEAL-LA-MG6 nanowebbs, incorporated with increasing percentages of MG3 and MG6, mainly overlap with those of PMEAL-LA.

The heat absorption capacities of the magnetite-containing nanowebbs are measured as $70\text{--}82\text{ Jg}^{-1}$ between $42\text{--}48\text{ }^{\circ}\text{C}$. In the cooling cycles, the crystallizations slightly shift to the lower temperature interval of $41\text{--}36\text{ }^{\circ}\text{C}$, the heat release capacities are about $65\text{--}81\text{ J.g}^{-1}$. As expected, there is a slight decrease in Lauric Acid encapsulation efficiency of PMEAL-LA-MG3 and PMEAL-LA-MG6 NWs with respect to PMEAL-LA NW depending on the amount of MNP incorporated into the structure. Whatever happens, the phase change enthalpies of PMEAL-LA-MG3 and PMEAL-LA-MG6 samples are all remarkable regarding the related concentrations used in electrospinning as the evidence of successful

encapsulation of LA in the presence of MNPs, MG3 and MG6.

Table 2. Phase transition characteristics of LA and the NWs obtained from the DSC analyses ($10^{\circ}\text{Cmin}^{-1}$).

Sample	10 th Heating				10 th Cooling			
	Phase transition ($^{\circ}\text{C}$)			ΔH (Jg^{-1})	Phase transition ($^{\circ}\text{C}$)			ΔH (Jg^{-1})
	T_{onset}	T_{peak}	T_{end}		T_{onset}	T_{peak}	T_{end}	
LA	44	49	53	185	41	37	34	181
PMEA-LA	44	46	48	82	41	40	34	79
PMEA-LA-MG3-1.25	43	45	47	75	41	40	36	72
PMEA-LA-MG3-1.75	43	45	48	71	41	40	36	70
PMEA-LA-MG3-2.50	43	45	48	74	41	40	36	71
PMEA-LA-MG6-1.25	43	46	48	82	40	39	36	81
PMEA-LA-MG6-1.75	42	44	46	70	40	39	37	65
PMEA-LA-MG6-2.50	44	46	47	74	42	41	26	68

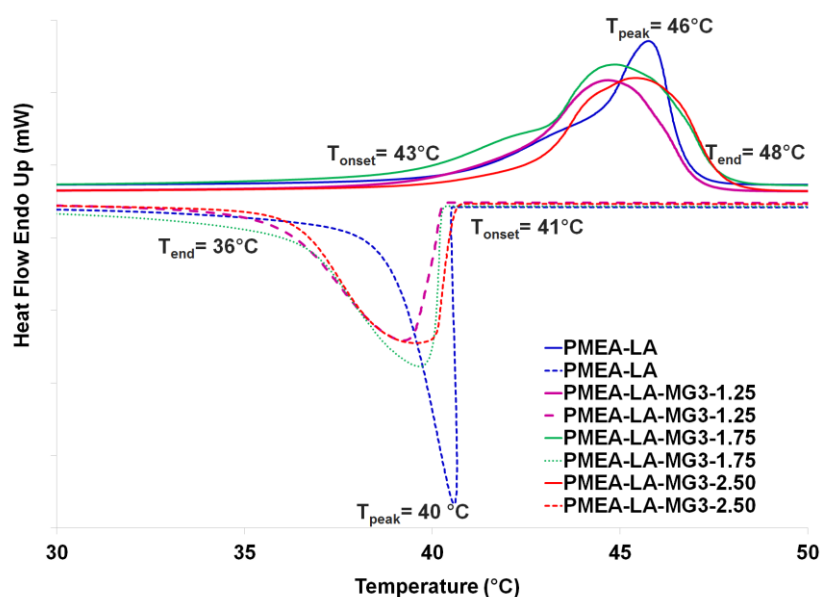


Figure 2. DSC curves of PMEA-LA and MG3 incorporated PMEA-LA-MG3 NWs for 10th heating-cooling cycle at $10^{\circ}\text{Cmin}^{-1}$.

The thermograms of the NW samples are shown in Figure 3 and Figure 4. The PMEA-LA-MG3 and PMEA-LA-MG6 samples exhibit a two-step decomposition, starting at $175\text{--}179^{\circ}\text{C}$ and ending at $409\text{--}426^{\circ}\text{C}$. The residues of the NWs at 650°C are related to the non-volatile hard segments of the polymer chains and magnetite nanoparticles incorporated to their structures. These results confirmed that the PMEA-LA-MG3 and PMEA-LA-MG6 NWs are thermally stable.

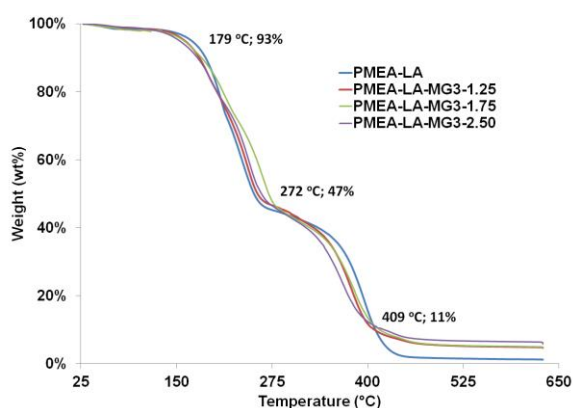


Figure 3. TG curves of PMEA-LA and MG3 incorporated NWs of PMEA-LA-MG3.

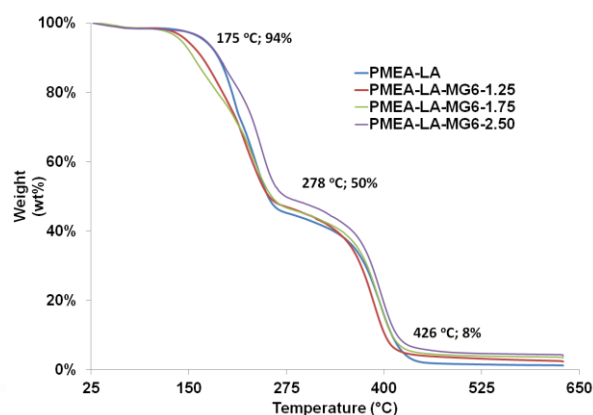


Figure 4. TG curves of PMEA-LA and MG6 incorporated NWs of PMEA-LA-MG6.

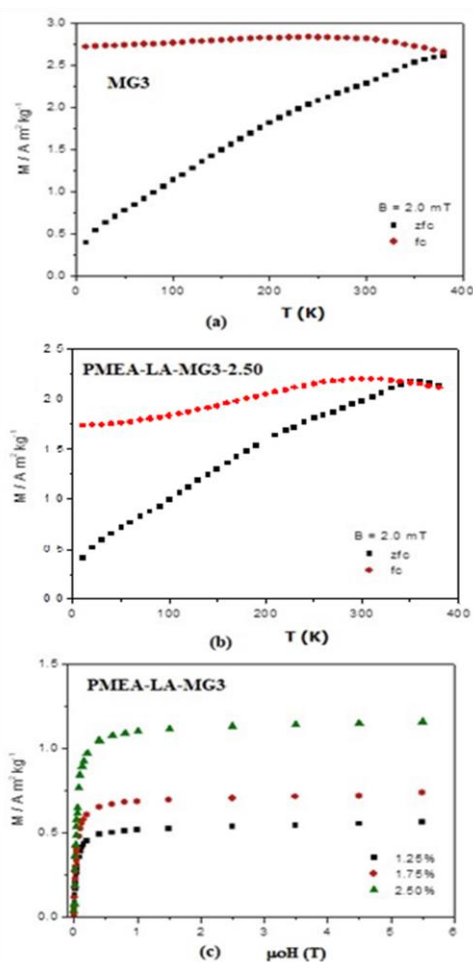


Figure 5. (a) Magnetization of oleic acid covered MG3 as a function of temperature; (b) Magnetization of PMEA-LA-MG3-2.50 as a function of temperature; (c) Magnetization of PMEA-LA-MG3 samples as a function of applied magnetic field.

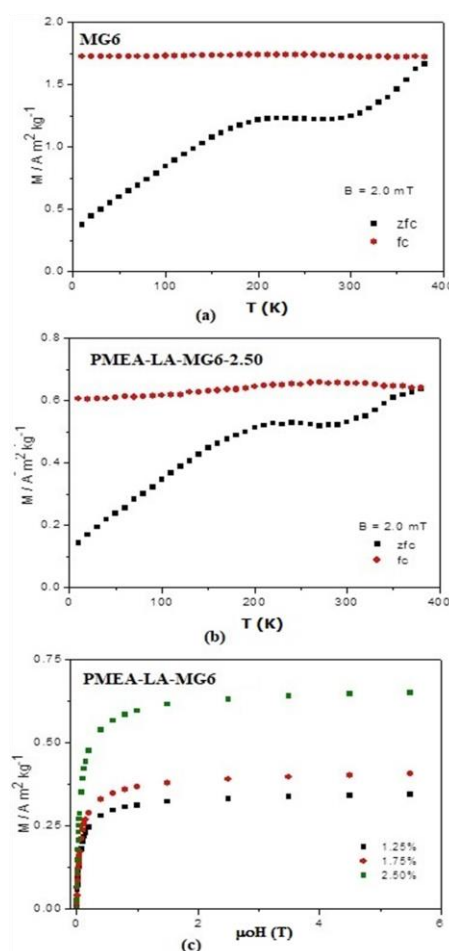


Figure 6. (a) Magnetization of non-functionalized MG6 as a function of temperature; (b) Magnetization of PMEA-LA-MG6-2.50 as a function of temperature; (c) Magnetization of PMEA-LA-MG6 samples as a function of applied magnetic field.

Figure 5 and Figure 6 display the magnetizations of MG3, MG6 and of PMEALA-MG3-2.50 and PMEALA-MG6-2.50 as a function of temperature and as a function of applied magnetic field. The magnetization results show that the nanoweb produced without magnetic nanoparticles, namely PMEALA, is diamagnetic with a magnetic susceptibility of $-7.0 \times 10^{-9} \text{ m}^3 \text{ kg}^{-1}$ and that all the nanowebs became magnetic when fabricated with integrated MNP. The comparison of ZFC/FC curves for the MNPs and for the NWs with MNP shows that the production process does not affect the nanoparticles magnetic behavior. For two types of MNPs, namely MG3 and MG6, the ratio of obtained spontaneous magnetization is consistent with the ratio of the nominal mass concentration. For the oleic acid functionalized magnetic nanoparticles, MG3, the magnetization for the corresponding nanowebs is higher by a factor of two for all concentrations than the ones with non-functionalized magnetic nanoparticles MG6, indicating a more effective integration of the functionalized nanoparticles in the nanoweb.

4. Conclusion

We developed novel nanowebs provided with magnetic and thermal buffering properties by encapsulating oleic acid functionalized MNPs or non-functionalized MNPs and Lauric Acid in PMEALA matrix via uniaxial electrospinning. There are numerous benefits of the NWs produced in this study. These are as follows: Lauric Acid, used as the PCM, and PMEALA, used as the polymer matrix, are bio-based and biocompatible compounds that do not mainly pose human health hazards. The encapsulation of MNPs and LA in PMEALA matrix prevents the leakage of MNPs and LA, which in turn minimizes their secondary effects on surrounding healthy tissues in prospective magnetic hyperthermia applications. They exhibit solid-liquid phase transitions between 41–48 °C as required in magnetic hyperthermia. The nanowebs have high enthalpies of fusion and crystallization, thermal cycling ability and thermal stability as well as the considerable magnetization capacity. Consequently, the as-spun nanowebs of MNPs, oleic acid covered MG3 or uncovered MG6, and LA in PMEALA matrix, along with many other applications in biomedicine, are highly advantageous in their potential applications in the field of magnetic hyperthermia.

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