Application of Image Analysis in the Characterization of Electrospun Nanofibers

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ABSTRACT: *In this work, $CoFe_2O_4$ nanoparticles have been prepared by co-precipitation technique. The synthesized $CoFe_2O_4$ nanoparticles were applied in the preparation of $CoFe_2O_4$/Polyacrylonitrile fiber nanocomposites by the electrospinning process. The prepared nanoparticles and nanofibers were characterized using the Scanning Electron Microscopy (SEM) and X-ray diffraction methods. The results manifested that nanofibers of PAN and $CoFe_2O_4$ /PAN were successfully prepared with electrospinning method. Grayscale SEM images of nanofibers were analyzed by a new image analysis procedure for determination of fibers diameter, their diameter distribution and the compactness of electrospun nanofibers. It was found that the presence of $CoFe_2O_4$ nanoparticles in the PAN solution increases both of the compactness of electrospun nanofibers and their diameter. The prepared $CoFe_2O_4$/Polyacrylonitrile fiber nanocomposites have possible applications in fabrication of sensor and magnetic recording media.*

KEY WORDS: *Nanofiber, Electrospinning, Algorithm, Polyacrylonitrile, Image processing, Nanocomposite.*

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INTRODUCTION

Polymers and polymer nanocomposites have attracted a great attention for their special properties, potential applications, their easy processability and low-cost manufacturing [1-10]. Recently, nanostructures have attracted a lot of attention because of their potentially applications and their unique properties [11-14].

Cobalt ferrite is one of the important metal oxide magnetic materials which has inverse spinel structure. One half of the Fe cation occupies the tetrahedral sites while the remaining Fe cations besides the Co cations located in the octahedral sites and the O anion placed at Face-Centered Cubic (FCC) positions. This compound has high coercivity, moderate saturation magnetization, large magnetostrictive coefficient, high magneto-crystalline anisotropy and good chemical, mechanical and thermal stability [15,16]. These properties make cobalt ferrite an attractive material for different magnetic applications such as Magnetic Recording, Resonance Imaging (MRI) enhancement, pigments, magnetically guided drug delivery and storage media.

One dimensional (1D) nanocomposite fibers have attracted much attention owing to their often enhanced physical and chemical characteristics and wide potential applications in microelectronic devices, sensors, filtration membranes and biomedical applications [17-20]. Among the one-dimensional nanostructural materials that have taken special consideration due to their potential applications in nanodevices [21], nanofibers do have especial consideration because of their high surface-to-volume ratio.

It was reported that 1D cobalt ferrite has better properties than bulk and quantum dots for sensors and recording media applications [16]. Therefore production of cobalt ferrite fibers by electrospinning is very appealing. It is the best method for continuous mass production of nanofibers [22-25]. There were some reports which present the production of $CoFe_2O_4$ nanofibers with electrospining method. For example Sangmanee et al. [11] used the organic salt of Co and Fe as precursor. These sources were added to polymeric solution and then electrospunned. After the post annealing the $CoFe_2O_4$ nanofibers were produced. There is another methodology for production of ceramic nanofibers in which the previously prepared nanoparticles are dispersed in polymeric solution for electrospinning [15, 24, 26]. This method has some advantages to the former one because of its controllability on the sizes of nanoparticles and their properties. Using this technique, *Ju et al.* [15] successfully prepared the $CoFe_2O_4$/PVA nanocomposites. But up to now there is no report for production of $CoFe_2O_4$/PAN nanocomposites using the electrospinning.

The electrospinning method was first reported in 1934 [25]. However it has attracted much attention since 1990. Electrospinning is an efficient and inexpensive fabrication method for the deposition of polymers by applying an electric field. In electrospinning process, a polymer solution or melt is injected with an electrical potential to create a charge imbalance and placed in proximity to a grounded target. At a suitable voltage, the charge imbalance begins to overcome the surface tension of the polymer solution or melt, forming an electrically charged jet. The jet within the electric field is directed toward the grounded target, solvent evaporates or melts solidifies and fibers are formed. Electrospinning makes continuous filaments that collect on the target as a nonwoven fabric [27]. Notably, it is possible to fabricate filaments on the nanometer scale with this technique [28]. Also, it has been used to fabricate hybrid nanofibers by incorporating nanomaterials into different polymer matrices. The electrospun nanofibers have several remarkable advantages including: large specific surface area, high aspect ratio, unique physicochemical properties, and design flexibility for surface functionalization [25, 29]. In addition to the complex pore structure and easy fiber surface modification, electrospun nanofibers are ideal for various applications [30].

Polyacrylonitrile (PAN) and polyvinylalcohol (PVA) are ideal candidates to fabricate nanofibers using the electrospining method [31]. In some application such as sensor, catalyst and magnetic devices which demand high stability, the PAN nanofibers are prominent than PVA. PAN also has lower price which makes it outstanding material for electrospinning.

In this work, first the $CoFe_2O_4$ nanoparticles were synthesized by simple coprecipitation method which characterized by X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) analysis. Then, $CoFe_2O_4$/PAN nanofibers were prepared by

electrospinning method. Owing to comparison, the pure PAN nanofibers were also prepared. In order to determine the average fiber diameter, distribution and the compactness of nanowovens, the SEM images of electrospuned nanofibers were investigated by image analysis method.

EXPERIMENTAL SECTION

Materials

PAN (polyacrylonitrile, Mw 80 kg / mol) was prepared from Isfahan Polyacryle Co. (Isfahan-Iran). Since N, N-dimethylformamide (DMF) is the common solvent of PAN which can evaporate during the electrospinning, so in this study, the DMF was selected as a solvent. It was purchased from Merck. Doubly distilled water was obtained by purification through a Millipore water system and used throughout the work. All experiments were carried out at 25 ± 2 ºC temperature.

Synthesis of $CoFe_2O_4$ nanoparticles

The mixed solution containing 50 mL of 80 mM $FeCl_3.6H_2O$ and 40 mM $Co(CH_3COO)_2.4H_2O$ was prepared in distilled water. This solution was added very slowly (drop-by-drop) into the 50 mL of 640 mM sodium hydroxide solution under vigorous stirring. The temperature of the reaction beaker more or less maintained at 60 ºC. With the slowly addition of the solution containing Fe^{3+} and Co^{2+} cations to the alkaline solution, the precipitation of dark brown nanoparticles occurred. The precipitation was completed at 60 ºC for 120 min. Afterward, the nanoparticles were filtered and washed with water and ethanol. Finally, the obtained nanoparticles were heated at 200 ºC for 1h in ambient atmosphere.

Preparation of polymer solutions

The solutions of both pure PAN and suspension of $CoFe_2O_4$ in PAN solutions (PAN/$CoFe_2O_4$) were prepared by dissolving of polymer in dimethylformamide (DMF). A solution containing 12 wt% of PAN in DMF was prepared under strong stirring at room temperature for 2 h. For the preparation of $CoFe_2O_4$ suspended solution, 0.4 wt% of synthesized nanoparticles was added to the above mentioned solution.

Electrospinning apparatus

Polymeric solutions were fed through a capillary tip with the diameter of 0.7 mm using a 10 mL syringe. The anode of the high voltage power supply was clamped to a syringe needle tip and the cathode was connected to a metal collector. During electrospinning, the applied voltage was 20 kV, the distance between the tip and collector was 13 cm, and the flow rate of the spinning solution was 0.5 mL / h. The electrospun fibers were collected on aluminum foil wrapped on a metal drum rotating at approximately 100 rpm, and were dried in vacuum at 60 ºC for 12 h before further use.

Characterization of sample

The morphology of the nanofibers and nanoparticles was examined using Scanning Electron Microscopy (SEM Philips XL30 Netherlands). The X-ray diffraction patterns of samples are recorded by Philips DW3710 instrument with the Cu kα X-Ray tube operating at the voltage of 50 kV and current of 250 mA in 2θ range of 15-75°. The crystallite sizes of sample are estimated using the Scherrer's method [29, 32-34] (Eq. (1)).

$$D = \frac{0.89 \times \lambda}{\sqrt{\beta_{obs}^2 - \beta_{std}^2} \times \cos(\theta)} \quad (1)$$

The parameters denotations in equation 1 are as below:

D = Crystallite size (nm)

βobs= Full Width in Half Maximum (FWHM)

βstd = The broadening (FWHM) of standard sample

λ = Wavelength of X-ray (in this work Cu kα which is 0.1541874 nm)

θ = Angle of diffraction

RESULTS AND DISCUSSION

Determination of fiber diameters, fiber diameters distribution and the compactness of electrospun nanofibers are the important characterization process which are necessary for optimization of electrospinning process in different applications. Routine measurements of fiber diameters and its distribution were carried out manually by selecting 100 fibers and measuring their respective diameters which was a very time consuming method and is completely based on the operator's accuracy. In this work, we utilized the modified method

which is previously reported by *Ziabari et al.* [35, 36]. The following paragraphs describe the various steps of image analysis.

Step 1 conversion of the real image into a grayscale image

At first in order to process the image, in part (a) of Fig. 1 the SEM image with the original magnification of 10000× is read and converted to grayscale.

Step 2 image contrast enhancement

Contrast enhancement is carried out via adjusting the image intensity values and mapping them to new values in a new image in a way that 1% of data is saturated at low and high intensities of the grayscale image. It can be seen in part (b) of Fig. 1.

Step 3 image thresholding

Suppose that light objects (fibers) are on dark background. For making simplicity in calculation it is common to produce the binary image from the gray scale image. For this purpose, local thresholding algorithm [37] is applied in this work which provides acceptable binary image in the case of uneven background (part (c) of Fig.1).

Step 4 Distance transformed image

By using the process of thinning, the skeleton of binary image was determined. The spurs of the skeleton were removed using the pruning procedure (part (d) of Fig.1) [38, 39]. Then by applying the algorithm (1) the intersection point on skeleton was distinguished. In this algorithm the sum of neighbor pixels of each point in the skeleton was computed. In the intersection this value was larger than 3 because of crossing of at least 2 line.

If SC(i+1,j)+ SC(i,j+1)+ SC(i-1,j)+ SC(i,j-1)+ SC(i+1,j+1)+ SC(i-1,j-1)+ SC(i+1,j-1) + SC(i-1,j+1)>3, intersection point (algorithm 1), SC = skeleton image and the value of pixels are shown with SC (x,y).

After that, in part (e) of Fig. 1 the intersection points were removed from the skeleton image and the end points of skeleton were removed two times in order to get rid of the neighbor of intersection. It should be noted that the thickness of fibers could not accurately determined in the intersections therefore it is necessary to exclude the intersections from calculations. Then, the distance map of binary image was computed (part (f) of Fig.1). As can be seen in parts (e) and (f) of Fig. 1, all of the intersections have successfully omitted using the mentioned algorithms.

Subsequently, the values of distance map at any pixel on the skeleton were recorded. These values are equal to fiber radii (part (g) of Fig.1). By application of the scale bar, the diameter of fibers in pixel unit can be converted to nanometer scale bar. Finally, the fiber diameter distribution histogram (part (h) of Fig.1), the Gaussian width and mean value of fiber diameter were computed. The compactness of electrospun nanofibers can also be calculated by using the binary image and ratio of white area to the black area.

Step 5 calculation of nanofibers compactness

For determination of compactness of nanofibers, the binary image of electrospun nanofibers was produced from the SEM images using local thresholding. The ratio of white area to black area in binary image was used as compactness identifier.

Fig. 2 shows the XRD pattern of $CoFe_2O_4$ nanoparticles prepared by co-precipitation method. The XRD pattern is well matched with the inverse spinel structure of $CoFe_2O_4$ compound with Fd3m space group and lattice parameter of 0.839 nm. The crystallite size of sample is estimated to be about 26 nm using the Scherrer's equation for the main reflection (311).

Fig. 3 shows the SEM images of synthesized $CoFe_2O_4$ nanoparticles. This image shows slight agglomeration of particles. The average size of cobalt ferrite nanoparticels is less than 100 nm considering the SEM images.

Part (a) of Fig. 4 shows the SEM image of PAN nanofibers, while part (b) shows the SEM image of PAN/$CoFe_2O_4$ nanofibers. Nonwoven mats of uniform fiber with smooth surface were obtained in the absence and presence of nanoparticles. As it is obvious, there are no beads in PAN/$CoFe_2O_4$ nanofibers which indicate a good dispersion of $CoFe_2O_4$ nanoparticles in the fiber structures.

The SEM images of PAN nanofibers and $CoFe_2O_4$/PAN nanofibers are investigated by new distance transformed image analysis method. The resultant fiber diameter distribution histograms are presented in Fig. 5. The average fiber diameter, Gaussian width of histogram, and compactness of fibers are listed

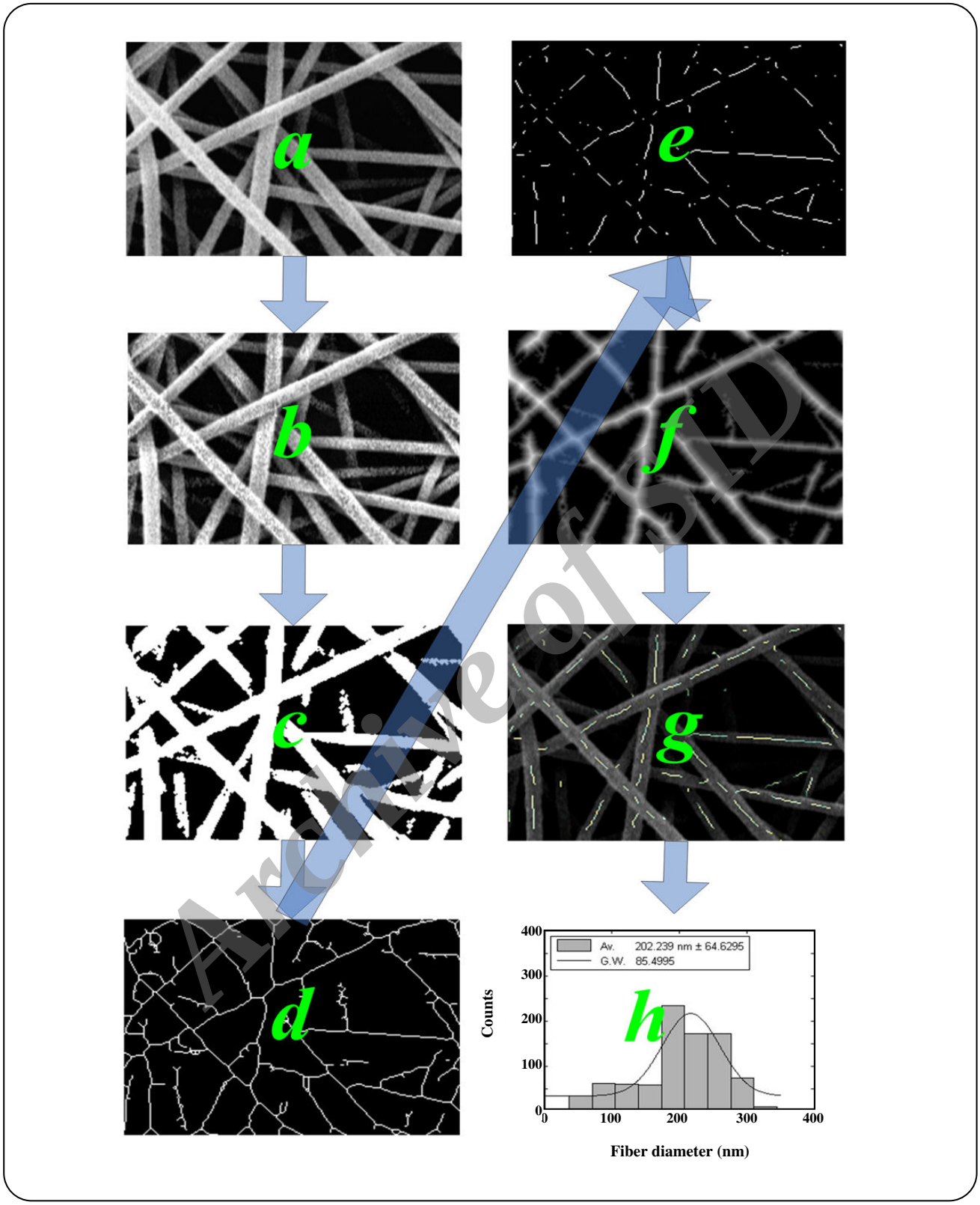

Fig. 1: (a) The first SEM image, (b) adjusted image, (c) binary image, (d) the skeleton of Fig. 1a after pruning, (e) the skeleton of Fig. 1a after deleting the intersections, (f) distance transformed image of binary image, (g) the final skeleton superimposed on the first image and (h) the final histogram.

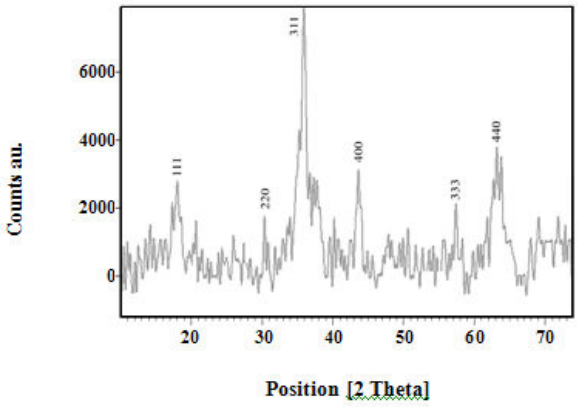

Fig. 2: XRD pattern of $CoFe_2O_4$ nanoparticles.

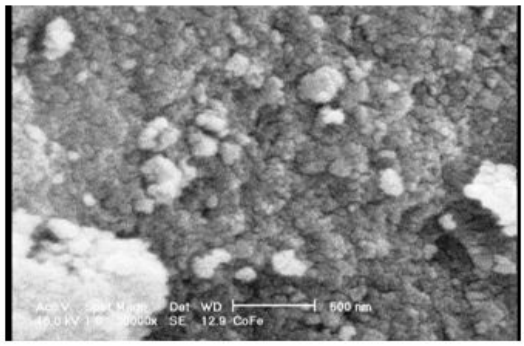

Fig. 3: SEM image of $CoFe_2O_4$ nanoparticles.

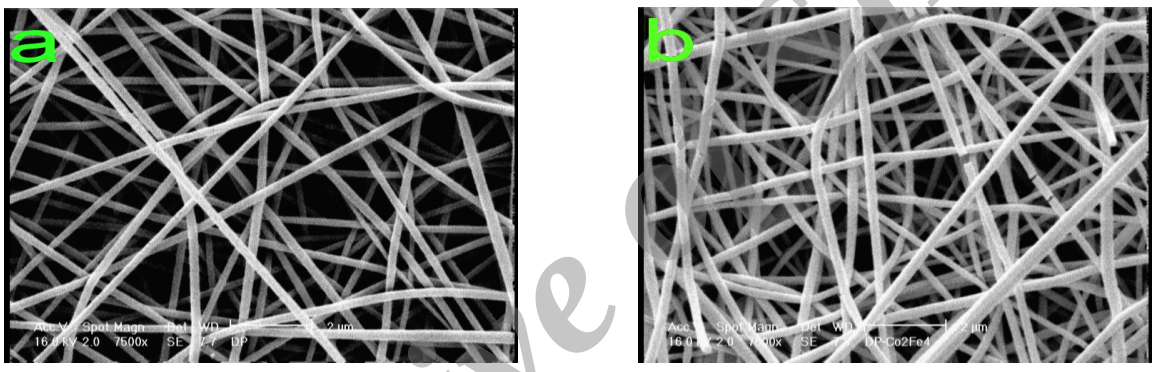

Fig. 4: SEM images of (a) PAN nanofibers and (b) $CoFe_2O_4$/PAN nanofibers.

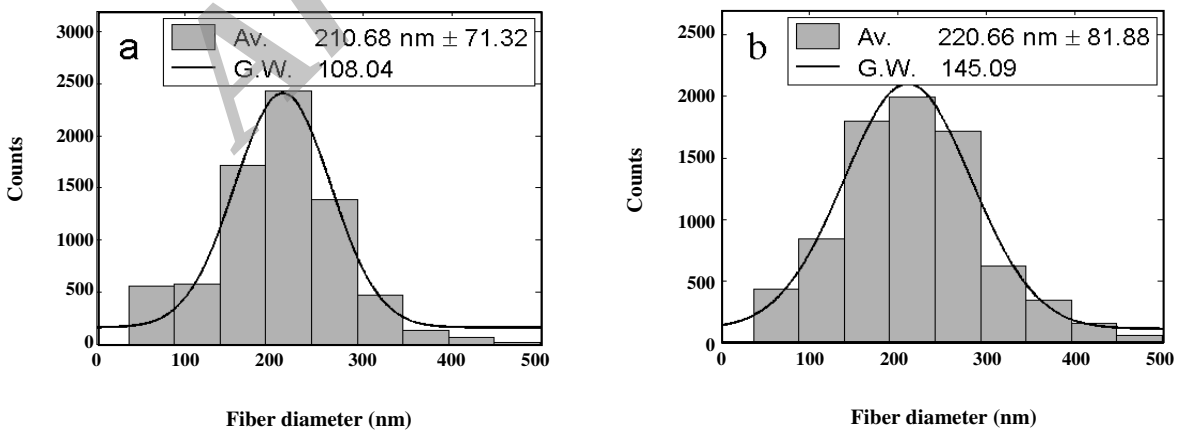

Fig. 4: SEM images of (a) PAN nanofibers and (b) $CoFe_2O_4$/PAN nanofibers.

Table 1: Compactness, the average fiber diameter (AVE), Gaussian width of fiber diameter histogram (G.W.) for both PAN nanofibers and $CoFe_2O_4$/PAN nanocomposites.

Sample	Compactness %	AVE (nm)	G.W. (nm)
PAN	45.0	210.68	108.04
$CoFe_2O_4$/PAN	51.6	220.66	145.10

in Table 1. It can be seen that the addition of $CoFe_2O_4$ nanoparticles in the polymeric solution caused the increasing of average fiber diameter (from 210 nm to 220 nm), its distribution (from 108 to 145 nm) and compactness of fibers (from 45% to 51.6%). With incorporation of nanoparticles the viscosity of polymeric solution is increased which resulted in boosting of fiber diameter and compactness. To get the best results and accurate comparison, application of image analysis is demanding as the conventional comparison may not accurate due to hand errors and qualitative comparison.

CONCLUSIONS

Pure PAN and $CoFe_2O_4$/PAN nanofibers were produced by the electrospining process. The resultant nanofibers and nanoparticles were characterized by XRD and SEM techniques. The SEM images of nanofibers were investigated by new distance transformed image analysis for determination of fiber diameter and its distribution. The results indicated that the prepared $CoFe_2O_4$/PAN composites have larger fiber diameters and compactness than electrospunned PAN nanofibers. The prepared $CoFe_2O_4$/PAN fiber nanocomposite shows magnetic properties and has been strongly absorbed by magnet which indicates its high paramagnetic properties.

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