Infrared Physics & Technology 66 (2014) 141–145

Contents lists available at ScienceDirect

Infrared Physics & Technology

journal homepage: www.elsevier.com/locate/infrared

Assessment of material blending distribution for electrospun nanofiber membrane by Fourier transform infrared (FT-IR) microspectroscopy and image cluster analysis

Jackapon Sunthornvarabhas [a], Kanjana Thumanu [b], Wanwisa Limpirat [b], Hyun-Joong Kim [c], Kuakoon Piyachomkwan [a], Klanarong Sriroth [d,*]

[a] Cassava and Starch Technology Research Unit, National Center for Genetic Engineering and Biotechnology, Bangkok 10900, Thailand
[b] Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima 3000, Thailand
[c] Laboratory of Adhesion and Bio-Composite, Program in Environmental Materials Science, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
[d] Department of Biotechnology, Kasetsart University, Bangkok 10900, Thailand

HIGHLIGHTS

- Modified cluster analysis for material spatial study within matrix of composite electrospun nanofibers.
- Polysaccharide electrospun composite development for medical application.
- Non-destructive technique was used to enable fast and low-cost product optimization.

ARTICLE INFO

Article history:
Received 4 February 2014
Available online 19 June 2014

Keywords:
Composite materials
Nanostructures
Computational techniques
Infrared spectroscopy (IR)

ABSTRACT

Electrospun nanofibers between starch and polyethylene oxide were successfully prepared to be used as a template for wound healing application. Material blending ratios and fabrication conditions were optimized to determine the ability to control material spatial for further development. A fourier Transform Infrared (FT-IR) mapping system and purposed modified image clustering analysis were adopted to evaluate the material homogeneity of a sheet of homogeneous composite nanofibers. The fabrication conditions and material blending ratios both have an influence on the material distribution and optimum points were observed from this technique. This study showed the possibility of using a quick and non-destructive technique and a modified image cluster analysis technique to evaluate the homogeneity of the electrospun nanofiber sheet.

1. Introduction

The electrospinning process has been greatly utilized for making sheets of polymeric nanofibers [1–3] due to its simplicity and the ability to create nanofibers of various sizes and characteristics and consequently many polymers were reported to have been successfully electrospun [4]. Because of the many variations in fiber features, electrospun nanofibers have been exploited by various applications including for filtration, protective clothing, and medical use. Electrospun nanofibers have been employed in medical applications because they have large surface area, high porosity, and interconnecting pore feature. Electrospun starch composite nanofibers have been developed for skin tissue scaffold application [5]. Starch has great potential to be mixed with other materials to promote cell growth [6]. Nevertheless, the development of materials with a better surface response is required to improve the overall product efficiency [7].

Material control in electrospinning technology is an important developmental aspect as it introduces new functions for electrospun nanofibers such as the fabrication of hollow nanofibers which impart material separation during article preparation. Polylactic acid and chitosan hollow nanofiber sheet were fabricated for antibacterial application [8]. This study reported that the optimum efficiency could be obtained within various hollow fiber structures through material spatial control. Another example that achieved benefits through material control is the fabrication of metal nanoparticles embedded in electrospun nanofibers

* Corresponding author. Tel.: +66 2 940 5634.
E-mail address: aapkrs@ku.ac.th (K. Sriroth).

http://dx.doi.org/10.1016/j.infrared.2014.06.004
1350-4495/

which was tailored by material blending and the fabrication conditions [9]. It appears that in the case of nanofibers with homogeneous appearance, the performance could be determined from a product specific testing protocol such as the antibacterial test. It is of interest to have other types of rapid evaluation method that can detect material distribution for the purpose of optimization. Another use of material control is in the field of material controlled release or encapsulation application. It was reported that the material release mechanism consists of two primary steps [10]. First step is the release of material on the surface while the second step is the release of material according to template matrix degradation. This concept requires the ability to control the material's position and concentration provides great potential to reveal material spatial control.

FTIR spectroscopy combined with a microscope is known as FTIR microspectroscopy and has been used to identify and spatially resolve the chemical make-up at a high spatial resolution for two or more components [11,12]. By plotting the absorbance of a specific vibrational mode over the area in the x–y stage (2D) dimensions, a map of the chemical species relative concentration can be obtained. There are many publications using this technique to study the blend uniformity and potency of the blend mixture. With the use of IR images, the results have demonstrated that the quality of extruded–blend can be visualized by measuring the band ratio of OH stretching/C=O stretching. In addition, this technique can evaluate the blend homogeneity of drug-excipient mixtures and solid-state blended polymers [13,14]. Along with technique development and implementation in various applications, there has been no report on the determination of material blending within homogenous composite nanofibers.

This paper aimed to evaluate the starch distribution within Polyethylene oxide (PEO) nanofiber sheet by introducing FT-IR microspectroscopic imaging technique and taking statistical analysis into account. Optimum conditions will be used for further development of a skin tissue scaffold with a combination of nutrients and growth factors blending within starch matrix.

2. Experimental part

2.1. Material

Polyethylene oxide MW 1,000,000 (Sigma Aldrich, Singapore), commercial cassava starch in a form of fine powder was donated (Cholcharoen Group, Chonburi, Thailand), with distilled water being utilized as the solvent for both solutions in this study.

2.2. Sample preparation

Polyethylene oxide (5 g) was dissolved in distilled water (100 cc) and shaken at room temperature for 12 h. Cassava starch (2.5 g) was dissolved in distilled water (100 cc) in a hot bath at 90 °C. PEO solution and starch paste solution were shaken at room temperature for 12 h. The mixed solution was prepared according to Table 1 by shaking at room temperature for 1 h. The overall solid content of each mixed solution was adjusted by adding distilled water to achieve 3.50% solid content for fabrication purpose.

A single nozzle electrospinning setup was used to prepare electrospun nanofibers. A high voltage power supply (ES60P-10 W, Gamma High Voltage, Florida, USA) was attached to a metal nozzle to create a voltage difference between the metal nozzle and a grounded collector of 12–18 kV. A charged metal nozzle with 0.57 mm outer diameter was placed 20 cm directly above the center of the 20 × 20 cm aluminum flat grounded collector which was suspended on a frame of polyvinylchloride (PVC) pipe. A mechanical pump (NE-4000, Multi-Phaser, New York, USA) was used to deliver the solution from a plastic syringe to the metal nozzle at a rate of 20.0 μl/min.

Other fabrication conditions were fixed to avoid complexity in the study. The polymer solution pumping rate and the distance between nozzle and collector were proven in a preliminary study to have less influence in material distribution.

2.3. Scanning electron microscope

Scanning electron microscope (SEM) (JSM-6400, JEOL Ltd., Tokyo, Japan) was used to characterize electrospun fibers characteristic and size. Image J software (www.imagej.nih.gov) was used for measuring fiber diameter. The average fiber diameter and standard deviation (SD) were determined.

2.4. Microscopic FT-IR mapping and data processing

Measurements were performed at the IR end station, Synchrotron Light Research Institute (Public Organization), Thailand. The Bruker Hyperion 2000 microscope (Bruker Optics Inc., Ettlingen, Germany) equipped with a nitrogen cooled MCT (HgCdTe) detector coupled to the Bruker Tensor 27 spectrophotometer was used for IR data acquisition. Spectral acquisition and instrument control was performed using OPUS 6.5 software (Bruker Optics Ltd, Ettlingen, Germany) and analyzed by CytoSpec software (Bruker Optics Ltd, Ettlingen, Germany).

Spectra were collected from 36 square grids and detector area for each measurement was about 70 × 70 μm thus covering

Table 1
Material conditions and fabrication conditions.

Factors	Condition number								
	1	2	3	4	5	6	7	8	9
Mixing ratio[a]	4	4	4	1.5	1.5	1.5	0.85	0.85	0.85
Starch in solid content (%)	11.37	11.37	11.37	25.49	25.49	25.49	37.64	37.64	37.64
Applied voltage (kV)	12	15	18	12	15	18	12	15	18
Average fiber diameter (nanometer)	273 ± 45	328 ± 70	342 ± 49	273 ± 86	303 ± 51	346 ± 53	337 ± 71	343 ± 67	377 ± 73
Calculated values[b]									
Replicate 1	2.24	1.22	0.71	1.81	2.23	1.49	2.41	2.74	1.94
Replicate 2	2.07	1.39	0.74	1.76	2.33	1.51	1.86	2.50	1.17
Replicate 3	2.07	1.31	0.66	1.91	2.22	1.42	2.25	2.46	1.08
Qn	2.13	1.13	0.70	1.83	2.26	1.47	2.17	2.49	1.40
Qn deviation	0.10	0.09	0.04	0.07	0.06	0.05	0.28	0.25	0.47
Ql	4.62	6.65	6.07	3.99	2.69	3.12	13.08	9.91	33.71

[a] Mixing ratio = volume of PEO solution/volume of starch paste solution.
[b] Each calculated value was number average from multiplication between cluster indexes and amount of color grid, and then averaged statistically for each replicate.

sample area of 420 × 420 μm^2 for each sample. To make an integral area ratio, from integral area of the O—H band (3666–2996 cm^{-1}) for starch was divided by the integral area of the C—H band (2995–2692 cm^{-1}) for PEO at each square grid, IR scans were taken at 64 scans at 4 resolution (cm^{-1}) and then averaged to one spectrum per each area which represented the material ratio for selected measuring area. These ratios were then coded by color and used for construction of chemical image of spectra as shown in Fig. 1. Similar integral area ratio values were grouped into cluster index and resulted in cluster image.

To study material distribution, quantitative material distribution index (*Qn*) was introduced. Cluster index was multiplied by the corresponding number of color grids and statistically number averaged, then averaged again for all replicates to a *Qn* value for each condition. *Qn* deviation represents relative standard deviation of *Qn* value. The key parameter to determine material distribution is qualitative material distribution index (*Ql*). This value is a relative standard deviation which is a percentage of standard deviation, *Qn* deviation, in relation to the mean value, *Qn*. According to this purposed formula, high *Ql* value represents poor material distribution because it is relative standard deviation of material ratios.

$$Ql = [100 * Qn \text{ deviation}]/Qn$$

2.5. Statistical analysis

Three replicates of each condition were used for FT-IR mapping measurement. ANOVA statistics were used to evaluate factor effect.

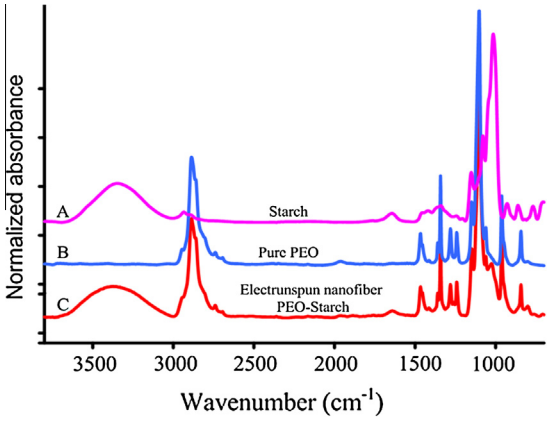
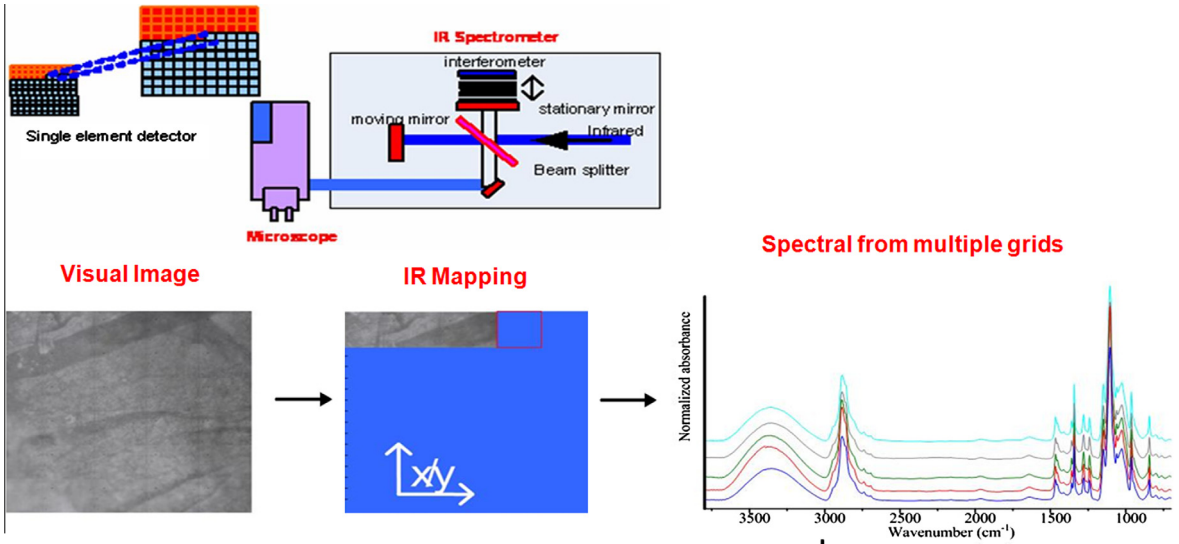


Fig. 2. FTIR spectra of the cassava starch (A), pure PEO (B), and Electrospun nanofibers PEO-starch (C).



Qualitative index of material distribution calculation

Color code	Cluster index	Piece amount	
	Replicate 1		
blue	1.258	11	13.837
red	2.346	3	7.038
green	1.689	7	11.823
grey	1.169	3	3.507
light blue	1.462	12	17.543
	(A)	(B)	(A)x(B)
	Replicate 2		
	Replicate 3		

average	1.47
deviation	0.05
% deviation	3.12

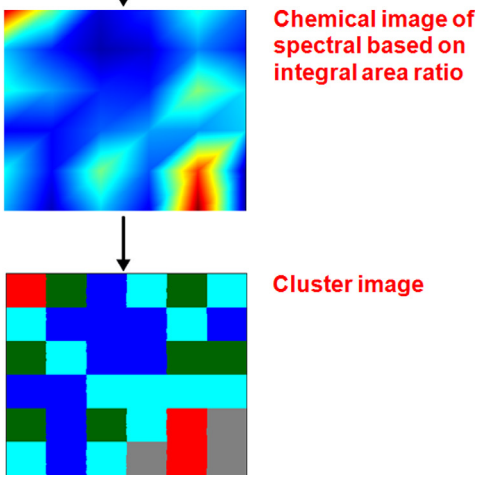


Fig. 1. FTIR microspectroscopy mapping with cluster analysis for studying material distribution of composite electrospun fibers sheet. Sample of *Qn* and *Ql* calculation is shown and use data from condition 6.

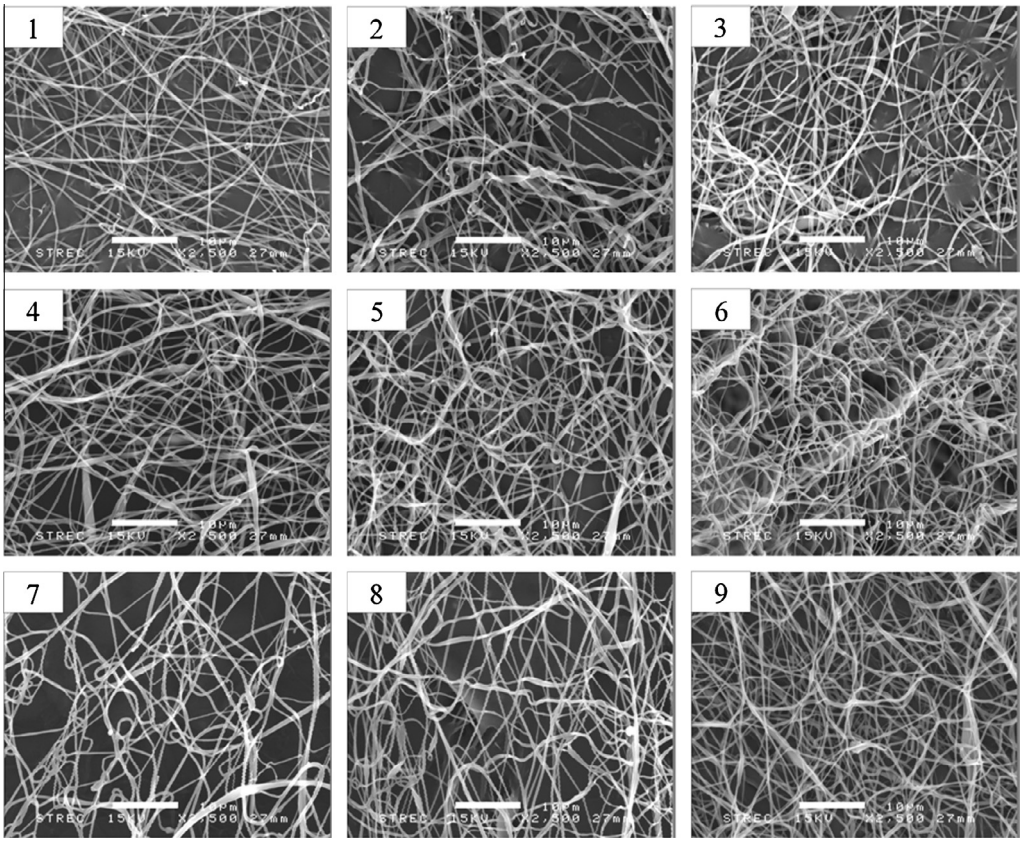


Fig. 3. Electron micrographs of electrospun nanofibers from polyethylene oxide (PEO) and cassava starch composite at different ratios prepared under different fabrication conditions.

The test adopted null hypothesis, H_0: $\mu_i = \mu_j$ with confidence level $\alpha = 0.050$.

3. Results and discussion

Fig. 2 shows IR spectra of material component of electrospun nanofibers composite between starch and PEO. Due to the nature of material preparation and IR result, physical blending was assumed to represent material blending behavior. Further analysis with gas chromatography may reveal more accurate character of material blending which can be considered by future work.

3.1. Effect of fiber characteristics

Fabrication conditions in this study were selected to obtain condition suitable for statistical analysis and yield smooth fibers. Fibers from all fabrication conditions were smooth fiber as shown in Fig. 3 to avoid effect of material segregation that might occur from non-smooth fiber. Applied voltage and starch content were factors that significantly affected fiber diameter as confirmed by a Two-way ANOVA. *P*-value of both factors was 0.002 and 0.015, respectively. *P*-value less than 0.050 in this study indicated significant influence of factor to response. Similar fabrication condition effects were observed in other works [5,15].

3.2. Effect of material distribution

Effect of fabrication conditions and material blending ratio of these composites to material distribution were evaluated from both quantitative and qualitative material distribution indexes in Table 1. Statistical analysis reported distinguishable different between *Qn* indices as influenced by applied voltage and material blending proportion which yielded *P*-value = 0.000 < 0.050 for both factors. This indicates that two primary studied factors have significant influence on material distribution but did not contribute to the optimization of material spreading.

Ql values were introduced as a potential tool to identify material distribution for homogenous fibrous matrix. It revealed that at conditions (7–9) not only starch content is high based on *Qn* values, poor starch distribution was also detected according to high *Ql* values. Starch retrogradation was suspected to be the cause of poor starch distribution. This phenomenon initiated hydrogen bonding and expels water from the starch solution and thus forming gel structure [16]. Due to nature of electrospinning, solvent will be evaporated and dried fibers are final product as confirm by SEM images. This bonding is believed to promote starch segregation within fiber matrix during fiber forming thus yielded poor starch distribution that explained high *Ql* values. Starch segregation, poor starch distribution, increases possibility to detect PEO and decrease *Qn* value which explained condition 9. Optimum points between applied voltage and starch content to achieve optimal material distribution were observed. Current technology of FT-IR microspectroscopy technique and cluster image analysis technique allow assessment of material distribution at smallest area of $70 \times 70\ \mu m^2$ but this purposed technique shows promising potential for further characterization analytical routine in the field of nanofibers product.

4. Conclusion

Material distribution between two materials within sheet of nanofibers was examined by applying FT-IR microspectroscopy

technique and modified cluster analysis technique for the purpose of improving product surface response. Results indicated optimum conditions of material concentration, starch, and fabrication condition, applied voltage, which promoted better starch distribution and these were recorded for further optimization. This non-destructive technique can be applied to evaluate blending efficiency nanofiber sheet with two or more components.

Conflict of interest

The authors have declared no conflict of interest in this work.

References

[1] D.H. Reneker, A.L. Yarin, Electrospinning jets and polymer nanofibers, Polymer 49 (2008) 2387–2425.
[2] Jackapon Sunthornvarabhas, G.G. Chase, Novel technique for mass production of electrospun fibers: design and experiment, Publisher: VDM VerlagDr. Müller, ISBN 978–3639322989.
[3] G.G. Chase, Jackapon Sunthornvarabhas, D.H. Reneker, New methods to electrospin nanofibers, J. Eng. Fibers Fabr. 6 (2011) 32–38.
[4] W. Kataphinan, Electropsinning and Potential Application, The University of Akron, Polymer Science Department Doctoral Dissertation, 2004.
[5] Jackapon. Sunthornvarabhas, Pathama. Chatakanonda, Kuakoon. Piyachomkwan, Klanarong. Sriroth, Electrospun polylactic acid and cassava starch fiber by conjugated solvent technique, Mater. Lett. 65 (2011) 985–987.
[6] A. Rodrigues, M. Emeje, Recent applications of starch derivatives in nanodrug delivery, Carbohydr. Polym. 87 (2012) 987–994.
[7] J. Lanutti, D.H. Reneker, T. Ma, D. Tomasko, D. Farson, Electrospinning for tissue engineering scaffolds, Mater. Sci. Eng., C 27 (2007) 504–509.
[8] T.T.N. Thuy, H.C. Ok, S.P. Jun, Coaxial electrospun poly(lactic acid)/chitosan (core/shell) composite nanofibers and their antibacterial activity, Carbohydr. Polym. 86 (2011) 1799–1806.
[9] B. Jie, L. Yaoxian, L. Meiye, W. Shugang, Z. Chaoqun, Y. Qingbiao, Electrospinning method for the preparation of silver chloride nanoparticles in PVP nanofibers, Appl. Surf. Sci. 254 (2008) 4520–4523.
[10] F. Zhongxiang, B. Bhandari, Encapsulation of polyphenols – a review, Trends Food Sci. Technol. 12 (2010) 510–523.
[11] Kanjana Thumanu, T. Waraporn, L. Chanchao, H. Philip, P. Rangsun, FTIR microspectroscopic imaging as a new tool to distinguish chemical composition of mouse blastocyst, J. Mol. Struct. 933 (2009) 104–111.
[12] B. Rohit, W. Shi-Qing, K.L. Jack, FTIR Microspectroscopy of polymeric systems, Adv. Poly. Sci. 163 (2003) 137–191.
[13] L. Ting-Huei, L. Shan-Yang, Microspectroscopic FT-IR mapping system as a tool to assess blend homogeneity of drug–excipient mixtures, Eur. J. Pharm. Sci. 23 (2004) 117–122.
[14] S.J. Ryan, M.S. Brian, Synchrotron infrared microspectroscopy characterization of heterogeneities in solid-state blended polymers, Mater. Lett. 61 (2007) 2151–2155.
[15] Jackapon Sunthornvarabhas, G.G. Chase, D.H. Reneker, Electrospun nanofibers from a porous hollow tube, Polymer 49 (2008) 4226–4229.
[16] F.B. William, Kuakoon Piyachomkwan, Klanarong Sriroth, Tapioca/cassava starch: production and use, in: B.M. James, W. Roy (Eds.), Starch, third ed., Academic Press, 2009, pp. 541–568.