Indian Journal of Chemistry
Vol. 58A, February 2019, pp. 288-293

Electrospun nano silver embedded polystyrene composite nanofiber as a possible water disinfectant

Nirmal N Patel, Mehdihasan I Shekh , Kaushal P Patel* & Rajnikant M Patel

Department of Advanced Organic Chemistry, P. D. Patel Institute of Applied Science,

Charotar University of Science and Technology, Changa 388 421, Gujarat, India

Email: kaus_chem@yahoo.com

Received 20 July 2018; revised and accepted 26 December 2018

Nano silver (Ag) embedded polystyrene (PS) composite nanofibers have been prepared by electrospinning technique using N,N-dimethylformamide (DMF) as solvent. Nanofibers have been characterized by Fourier transform infrared spectroscopy. Thermal properties of the fibers have been studied using thermogravimetric analysis (TGA). X-ray diffraction study showed silver nanoparticles to be of the size of 19 nm with a cubic structure. The size of silver nanoparticles have also been deduced from TEM analysis and found to be between 11–14 nm. The morphology of PS nanofiber has been demonstrated by scanning electron microscopy (SEM). Antibacterial activity of PS nanofiber and nano Ag embedded PS composite nanofiber against Gram-negative *Escherichia coli (E. coli)* and Gram-positive *Bacillus subtilis (B. subtilis)* microorganisms has been evaluated by well diffusion method and viable cell count method.

Keywords: Nano silver, Nanofibers, Thermal analysis, Water disinfectants

Styrene, obtained from petroleum is a useful monomer for many polymeric materials. Polystyrene is a polymer of styrene and is heat resistant, possesses practical toughness, strength and is light weight[1]. Because of its distinct physical, chemical and mechanical properties, polystyrene is widely used for the manufacture of polystyrene foam for food packaging objects[2,3], electronic appliances[4], and medical equipments[5].

Nanotechnology is a fast growing field, since it makes it possible to controll the size so as to perform functions which otherwise cannot be achieved by bulk sized materials. Electrospinning is a simple technique for the production of nanofibers and is widely employed. Nanofibers can also be obtained by the following techniques: drawing[6-8], template synthesis[6,7], phase separation[6,7], self-assembly[6,7] and melt-blown[9]; but electrospinning technique is the most preferred one for the production of nanofibers. It is a moderately easy technique for the continuous fabrication of polymer nanofibers. Wide range of polymers like polypropylene[10], polyvinyl alcohol[11], polyethylene[12], polyacrylonitrile[13], polylactic acid[14], polyurethane[15], chitosan[16] and cellulose acetate[17] have been employed to obtain nanofibers. The nanofibers find application in medical materials[18], for waste water treatment[19], and in the biomedical field[20]. They are also used for filtration purpose[21], chemical protection of clothes[22], tissue engineering[23], and as textile fabrics[24]. To procure nanofibers of choice, two important polymer characteristics which are solubility and molecular weight need to be considered.

Many reports are available on inorganic matrix/ polymer composite nanofibers. The polymer composites with nanoparticles of TiO_2, ZnO and other metal oxides are used in diverse fields such as electronics[25], biomedical[26], adsorption[27], catalyst[28] and filtration[29]. Amongst all such nanoparticles, silver nanoparticles have attracted the most attention because of their catalytic activity[30], high electrical conductivity[31], surface enhanced Raman scattering[32] and antimicrobial activity[33,34]. Antimicrobial agents, because of their ability to kill pathogens, find applications in many areas such as filtration[35], packaging[36], textiles[37] and in the field of medicine[38]. Because of their ability to get attached to cell wall, silver nanoparticles exhibit better antimicrobial activity. Nanofibers of nano silver embedded polymer composites are widely used in view of their antimicrobial activity. Interestingly, silver nano particles are nontoxic to humans.

In the present investigation, nanofibers of PS and nano silver embedded polystyrene composites have been prepared by electrospinning technique. Silver nanoparticles were characterized by XRD and TEM analyses. Antimicrobial activity of the nanofibers was studied by well diffusion[39] and viable cell count methods[40], both Gram-positive and Gram-negative bacteria were used for the study.

Experimental

N,N-Dimethyl formamide (DMF), 2,2-azobisisobutyronitrile (AIBN), silver nitrate ($AgNO_3$) and methanol were purchased from

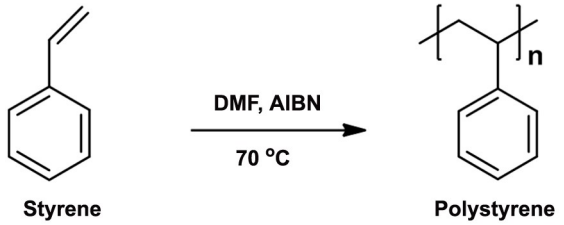
Loba Chemie Pvt. Ltd, India. Styrene monomer was procured from Sigma-Aldrich. The solvents DMF and methanol were distilled for purification. Other chemicals were used as obtained.

Polystyrene was prepared by the free radical polymerization of styrene monomer[41]. Styrene (1 mol) was taken in a round bottom flask (RBF) fitted with a reflux condenser. DMF was used as a solvent and AIBN (0.5% *w*/*w* of total monomer) as free radical initiator (Scheme 1) .The reaction was carried out at 70±2 °C for 6 h with constant stirring. After completion of the process, the reaction mixture was cooled to room temperature and the resultant polymer solution was poured into a large amount of methanol with stirring and the polymer precipitated out. It was then filtered and washed with methanol. The polymer was purified by repeated precipitation using methanol from solution of PS in DMF and then dried.

Preparation of PS solution containing nano silver was carried out as follows. A 15% *w*/*v* solution of PS was prepared in DMF by stirring the mixture overnight. Thereafter $AgNO_3$ (1% *w*/*w* calculated on the basis of weight of PS) was added into the solution which was kept overnight in dark and then the solution was refluxed for 1 h to reduce $AgNO_3$. The solution became dark yellow because of the formation of Ag[42].

Nanofibers of PS and nano Ag embedded PS composite were fabricated by electrospinning technique. Appropriate solutions were transferred into a syringe equipped with needle and the fiber was collected on an aluminium collecting plate using a voltage of about 20 kV. The distance between the needle and collecting plate was kept at 12 cm and a flow rate of 25 µL/min was maintained.

The products were characterized by X-ray diffraction (XRD) using the model, Bruckner D2 Phaser 3600 X-ray diffractometer having Cu-kα radiation source, λ = 0.154 nm. Nicolet 6700 FTIR spectrophotometer was employed to obtain IR spectra of the fibers in KBr pellets. GPC instrument equipped with WATERS 1525 binary pump, manual injector and R.I. detector (WATERS 2414) was used for GPC analysis. Flow rate of THF was 1.0 mL/min and temperature of the column (styragel HR4) was 30 °C. The sample size was 20 µL of a 0.1% *w*/*v* solution in mobile phase (THF).



Synthesis of polystrene (PS)
Scheme 1

Thermal properties of nanofibers were studied by TGA/DSC in nitrogen atmosphere at 10 °C/min heating rate using Mettler toledo thermogravimetric analyser. The surface morphology of PS nanofiber was obtained from scanning electron microscope (FEG-SEM, Nova Nano Scanning Electron Microscope 450, accelerating voltage of 20 to 30 kV). The morphology of Ag nanoparticles in polymer composite nanofiber was found by transmission electron microscope (TEM), (model Tecnai 20, Philips, Holland) with an electron source of W emitter and LaB6 and accelerating voltage 200 kV.

Bacteria *E. coli* (Gram negative) and bacteria *B. subtilis* (Gram positive) were used for the antimicrobial activity. All microorganism cultures were prepared from their respective slants. Cultures were grown for 24 h at 37 °C and the optical density was adjusted to 0.1 which corresponds to 10^8 CFU (colony forming unit)/mL at 600 nm. About 100 µL bacteria were added to 100 mL of a nutrient broth solution to give bacteria concentration of about 10^8 CFU/ mL.

Well diffusion method was employed to assay antimicrobial activities of pure PS nanofiber and Ag nano particle doped PS composite nanofibers. The bacterial suspension (100 µL 10^8 CFU) was spread uniformly on the nutrient agar plate and 50 µL solution of each nanofiber of PS and nano Ag embedded PS composite in DMSO was loaded in each well on nutrient agar plate. DMSO was used as solvent as PS/Ag nanofiber was soluble in DMSO, further DMSO does not interfere with the microbes. The plate was then put in an incubator for 24 h at 37 °C and then the inhibition zone was measured[39].

Antimicrobial activity of PS nanofiber and Ag nanoparticle embedded PS composite nanofibers was also measured by viable cell counting method. Bacterial solution (2 mL) was added to 8 mL sterilized water to which 30 mg of each nanofiber was added and the solution was kept in a shaker at ambient temperature for 15 min. After the stipulated time, 100 µL of this solution was spread into nutrient agar plate and kept in an incubator for 24 h at 37 °C. The number of surviving colonies was counted. The same procedure was repeated for duration of 30 min, 45 min, 60 min, 1 h and 24 h

under shaking. These results were compared with the number of bacterial colonies of the untreated control[40]. Under control experiment, the same procedure was followed in the absence of nano Ag embedded PS composite.

Results and discussion

IR spectra of PS and Ag nanoparticles doped PS composite nanofiber are shown in Fig. 1. There are no major differences between the IR spectra of nanofibers of PS and Ag/PS composite, but intensity of the peaks in nano Ag /PS composite nanofiber decreased in comparison to the same in PS nanofiber. In these IR spectra the CH out of plane bending of the mono substituted benzene ring are seen at ~700 and ~750 cm^{-1}. The peaks due to breathing vibration in benzene ring are attributed to the absorptions at 1600–1400 cm^{-1}. The =C-H stretching of aromatic ring is traced to the peak at ~3000 cm^{-1}. The number average molecular weight of PS is 25317 dalton.

Presence of Ag in nanofiber was confirmed by XRD (Fig. 2) of the polymer composite. Four reflections from (111), (200), (220) and (311) planes were identified. The JCPDS number is 01-1167. The peaks in XRD suggested that Ag nanoparticles have cubic structure. The size of silver nanoparticle was ~19 nm.

The SEM images of PS nanofibers are shown in Fig. 3. The diameter of PS nanofiber was found to be in the range 800 nm to 1.1 μm. The TEM images of Ag nanoparticles embedded PS composite nanofiber are presented in Fig. 4. The diameters of the Ag nanoparticles varied from 11 to 14 nm. The nanoparticles of Ag are clearly seen in the TEM images and some of them are aggregated.

Thermogravimetric analysis (TGA) provided information regarding thermal stability of PS and Ag NPs/PS composite nanofiber. TGA traces of the nanofibers are shown in Fig. 5. The activation energy (E_a) of nanofibers was calculated by Broido method[43]. The values of activation energy are presented in Supplementary Data, Table S1. PS nanofiber demonstrated more thermal stability compared to Ag NPs/PS composite nanofiber. This can be traced to the catalytic effect of Ag nanoparticles on thermal degradation of Ag NPs/PS composite.

As indicated earlier, the antimicrobial activity of PS nanofiber and Ag NPs/PS composite nanofiber was investigated by employing two different methods. By the well diffusion method, the PS nanofiber and Ag NPs/PS composite nanofiber were treated with *E. coli* and *B. subtilis*. The results are shown in Supplementary Data, Fig. S1 & Table S2. It is seen that while nano Ag embedded PS composite nanofiber was able to inhibit the growth of bacteria, nanofiber containing only PS could not inhibit the bacterial growth. The antibacterial property of Ag doped PS nanofiber was traced to the presence of Ag nano

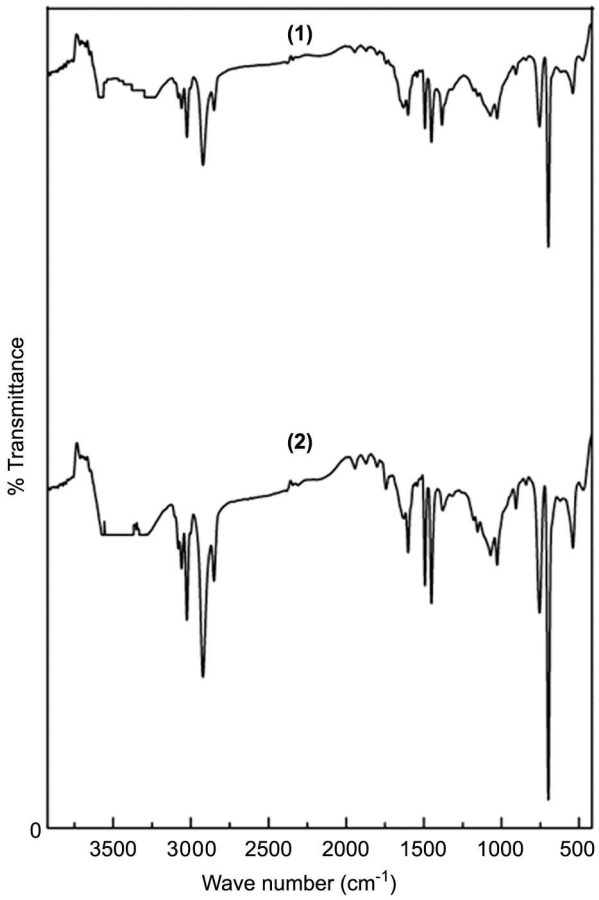


Fig. 1 — FTIR spectra of PS nanofiber (1) and Ag NPs doped PS composite nanofiber (2).

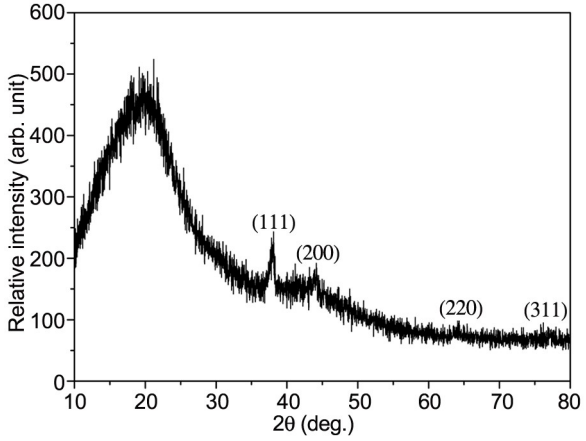


Fig. 2 — XRD pattern of Ag NPs embedded PS composite nanofiber.

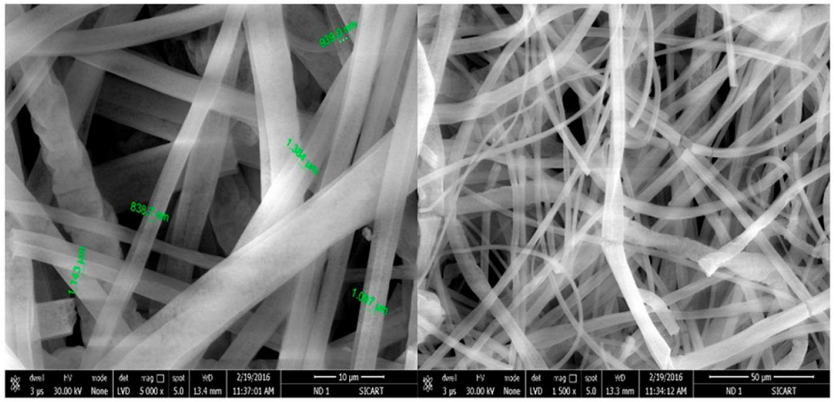


Fig. 3 — SEM images of PS nanofiber.

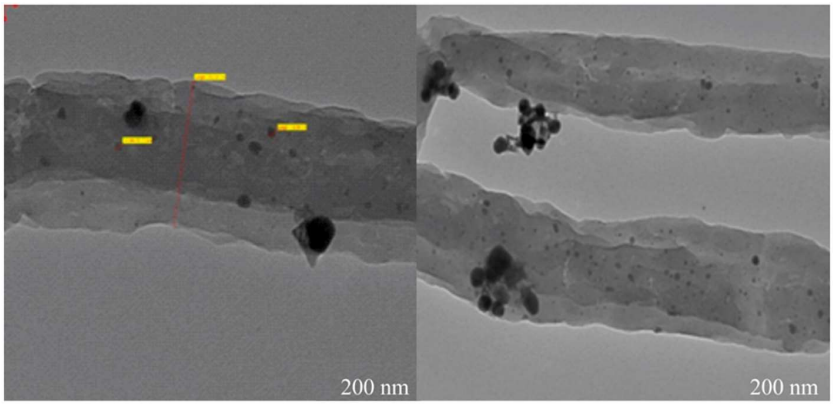


Fig. 4 — TEM images of Ag nano particles doped PS composite nanofibers.

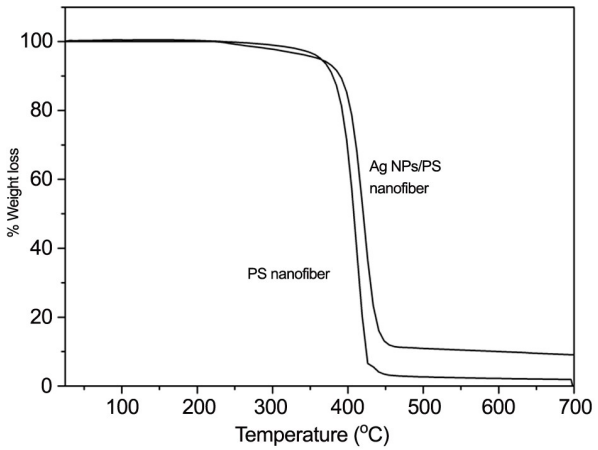


Fig. 5 — TGA traces of PS nanofiber and Ag NPs embedded PS composite nanofiber.

particle. Similar results from literature are presented in Table S3.

The antibacterial activity of PS nanofiber and nano Ag embedded PS composite nanofiber was investigated against *E. coli* and *B. subtilis* employing visible cell count method also. The bacterial solution was exposed to the nanofibers for 15 min, 30 min, 45 min, 60 min and 24 h. Number of bacterial colonies is shown in Supplementary Data, Figs S2-S5. Maximum colonies were seen in petri plates containing PS nanofiber. The numbers of colonies are tabulated in Table S4. It can be seen that after 24 h, there were 0 CFU/mL colonies when nano Ag doped polymer composite nanofiber was used. In the case of neat PS nanofiber, the inhibition to the growth of microorganism was quite less after 15 min, 30 min, 45 min and even after 24 h and it was difficult to count the colonies.

Results from literature based on water disinfectant studies are shown in Table S5. Comparison of antibacterial activity of PS nanofiber and nano Ag embedded PS composite nanofiber demonstrated that Ag NPs/PS composite nanofiber was much more effective than PS nanofiber, this is due to Ag nanoparticles which are very potent antibacterial agents. The Ag particles get attached to the cell walls and disturb cell wall permeability and cellular respiration. The antibacterial activity of nanofiber of nano Ag embedded PS composite is enhanced. Due to electrostatic interaction between Ag and Gram

negative bacteria, the nano Ag doped PS composite nanofiber was more effective against Gram negative bacteria. The number of bacterial colonies (Table S4) in Ag embedded PS composite nanofiber after 24 h is zero, suggesting possible use of the polymer composite nanofiber as water sanitizer.

In summary, nanofibers of PS and Ag NPs/PS were prepared by electrospinning technique and characterized by FTIR spectroscopy. The presence of Ag nanoparticles in PS composite nanofiber was confirmed by XRD study. It was found from TGA data that PS nanofiber is more thermally stable than Ag NPs/PS composite nanofiber. Antibacterial activity of the nanofibers of PS and Ag NPs/PS composite against *E. coli* and *B. subtillis* was investigated by two methods viz., well diffusion and viable cell count method. Because of the presence of Ag nanoparticles, PS composite nanofiber was found to possess superior antibacterial activity compared to PS nanofiber. The results of this study suggest that nanofibers of polymer composite containing Ag nanoparticles may find applications in different areas such as wound dressing, coating of biomedical materials and purification of water. The above technique will be highly useful in providing a microbe free environment which will be helpful in preventing infections particularly in hospitals where the possibility of acquiring infections are the highest.

Acknowledgement

The authors thank the Department of Advanced Organic Chemistry, P. D. Patel Institute of Applied Sciences, Charotar University of Science & Technology (CHARUSAT), India, for providing laboratory facilities.

Supplementary data

Supplementary data associated with this article are available in the electronic form at http:// www.niscair.res.in/jinfo/ijca/IJCA_58A(02)288-293_SupplData.pdf.

References

1 Maul J, Frushour B, Kontoff J, Eichenauer H, Ott K H & Schade C, *Ullmann's Encyclopedia of Industrial Chemistry*, 29 (2007) 475.
2 Youssef A M & Abdel Aziz M S, *Polym Plast Technol Eng*, 52 (2013) 607.
3 Murphy P G, Macdonald D A & Lickly T D, *Food Chem Toxicol*, 30 (1992) 225.
4 Prime D & Paul S, *IEEE Trans Dielectr Insul*, 15 (2008) 905.
5 Modjarrad K & Ebnesajjad S, *Handbook of Polymer Applications in Medicine and Medical Devices*, (Elsevier) 2014, p. 9.
6 Thomas S, Rafiei S, Maghsoodlou S & Afzali A, *Notes on Nanotechnology Foundations of Foundations of Nanotechnology*, (CRC Press, Taylor and Francis group) 2015, p. 198.
7 Paul P, *An Introduction to Electrospinning Process, Man-Made Textiles in India* (CRC Press, Taylor and Francis group), 2005, p. 198.
8 Bajakova J, Chaloupek J, Lacarin M & Lukáš D, *Nanocon*, 9 (2011) 21.
9 Hiremath N & Bhat G, *Nanosci Technol*, 2 (2015) 1.
10 Watanabe K, Kim B S & Kim I S, *Polym Rev*, 51 (2011) 288.
11 Park J C, Ito T & Kim K O, *Polym J*, 4 (2010) 273.
12 Ma J, Zhang Q & Mayo A, *Nanoscale*, 7 (2015) 16899.
13 He J & Wan Y, *Fiber polym*, 9 (2008) 140.
14 Tsuji H, Nakano M, Hashimoto M & Takashima K, *Biomacromole*, 7 (2006) 3316.
15 Karakaş H, Saraç A, Polat T & Budak E, *World Acad Sci Eng Technol*, 7 (2013) 177.
16 Sun K & Li Z H, *Express Polym Lett*, 5 (2011) 342.
17 Tungprapa S, Puangparn T & Weerasombut M, *Cellulose*, 14 (2007) 563.
18 Zhang H & Qian X M, *Adv Mater Res*, 148 (2011) 1138.
19 Nasreen S A A N, Sundarrajan S, Nizar S A S, Balamurugan R & Ramakrishna S, *Membranes*, 3 (2013) 266.
20 Khan N, Misra P M, Koch T & Mohanty A, *studies by undergraduate researchers at Guelph*, 5 (2012) 63.
21 Shekh M I, Patel D M, Patel K P & Patel R M, *Fiber Polym*, 17 (2016) 358.
22 Faccini M, Vaquero C & Amantia D, *J Nanomater*, 2012 (2012) 1.
23 Pham Q P, Sharma U & Mikos A G, *Tissue eng*, 12 (2006) 1197.
24 Z Rożek, W Kaczorowski, D Lukáš, P Louda & S Mitura, *J Achieve Mater Manufact Eng*, 27 (2008) 35.
25 Liu L, Liu Z, Bai H & Sun D D, *Water Res*, 46 (2012) 1101.
26 Amna T, Hassan M S & Barakat N A M, *Appl Microbiol Biotechnol*, 93 (2012) 743.
27 Saeed K, Sajjad H, Tae Jin O & Park S Y, *J Membr Sci*, 322 (2008) 400.
28 Wu M C, Sapi A & Avila A, *Nano Res*, 4 (2011) 360.
29 Yousef A, Barakat N A M & Amna T, *Macromol Res*, 20 (2012) 1243.
30 Cheon J Y, Kang Y O & Park W H, *Fiber Polym*, 16 (2015) 840.
31 Song M Y, Kim D K, Ihn K J, Jo S M & Kim D Y, *Nanotechnol*, 15 (2004) 1861.
32 Song W, Wang Y & Zhao B, *J Phys Chem*, 111 (2007) 12786.
33 Abdo H S, Khalil K A, Al Deyab S S, Altaleb H & Sherif E S M, *Fibers Polym*, 14 (2013) 1985.
34 Shekh M I, Patel N N, Patel K P, Patel R M & Ray A, *Env Sci Poll Res*, 24 (2017) 5701.
35 Shalaby T, Mahmoud O & Al-oufy A, *Int J Mater Sci Appl*, 4 (2015) 293.
36 Malhotra B, Keshwani A & Kharkwal H, *Front Microbial*, 6 (2015) 1.
37 Simoncic B & Tomsic B, *Text Res J*, 80 (2010) 1721.
38 Shah P M, Schafer V & Knothe H, *Vet Micr*, 35 (1993) 269.

39 Chaudhary A, Gupta A, Mathur R B & Dhakate S R, *Adv Mater Lett*, 5 (2014) 562-568.
40 Abdelgawad A M, Hudson S M & Rojas O J, *Carb Polym*, 100 (2014) 166.
41 Tung L H, Hu A T, McKinley S V & Paul A M, *J Polym Sci*, 19 (1981) 2027.
42 Mahapatra A, Garg N, Nayak B P, Mishra B G & Hota G, *J Appl Polym Sci*, 124 (2012) 1178.
43 Broido A, *J Poly Sci Part A-2: Polym Phys*, 7 (1969) 1761.
44 Shi Q, Vitchuli N, Nowak J, Noar J, Caldwell J M, Breidt F, Bourham M, McCord M & Zhang X, *J Mater Chem*, 21 (2011) 10330.