

Novel Hyperbranched Polyester Prepared via One Pot Polycondensation as Scaffold for Silver Nanoparticles

C. KAVITHA^{1,*}, K. PRIYA DASAN², K. VIDHYA¹ and V. THENDRAL¹

¹Department of Chemistry, Adhiyaman Arts & Science College for Women, Uthangarai, Krishnagiri-635 207, India

²Material Chemistry Division, Vellore Institute of Technology, Vellore-632 014, India

*Corresponding author: E-mail: kavithac1506@gmail.com

Received: 21 November 2017;

Accepted: 31 January 2018;

Published online: 28 February 2018;

AJC-18794

In this paper, novel water soluble hyperbranched polyester (HBPE) was synthesized by the reaction of 2,2',2''-nitrioltriethanol (B_3) and 2,3-dihydroxybutanedioic acid (A_2B_2) by the method polycondensation using *p*-toluenesulfonic acid as an acid catalyst. The reaction was carried out in different molar ratio for different generations such as G_1 , G_2 and G_5 , respectively. The water soluble HBPE were coupled with help of acrylic acid to enhance the end groups. Their structures were characterized by FT-IR spectroscopy, NMR (1H and ^{13}C) and molecular weights were measured by GPC and TGA. Silver nanoparticles were synthesized by a technique used reductive technique using HBPEs as matrix. Transmission electron microscope (TEM), UV-visible spectroscopy and X-ray diffraction (XRD) were used to characterize the structure of the particles, respectively. The thermal behaviour of pure polymer and polymer with silver nanoparticles has been studied by TGA. The antibacterial efficiency was evaluated against two common pathogenic bacteria *S. aureus* and *E. coli*.

Keywords: Soluble hyperbranched polyester, 2,2',2''-Nitrioltriethanol, Acrylic coating, Silver nanoparticles, Antibacterial activity.

INTRODUCTION

Hyperbranched polymers (HBPs) can be produced readily on an industrial scale a wide variety of raw materials, which means they are eco-friendly. Hyperbranched polymers are three dimensional structure. They have been studied widely over for past 20 years for their unusual properties such as low viscosity, high density of functional terminal groups, high solubility, good compatibility with other materials [1-7]. The reaction of HBPE led to various view for the upliftment of this materials in coating industries [8,9]. Hyperbranched polyester could be synthesized by easily affordable and low cost basic materials has impelled several research groups to study HBPEs in detail. Huge number of end groups offers different crosslinking method. The approximate circular structure of HBPEs makes the viscosity level low and high of molecular weight is high [10-13]. Hyperbranched polyester have attractive rheological properties and can be used in a wide variety of applications as biocides, additives, rheology modifiers, etc. [14-16]. Several HBPE with the help acrylate of methacrylate end groups have demonstrated in recent years [17-19]. The individual properties of HBPE allow extremely efficient for UV and EB curing applications, if they contain any one of the end groups (acrylate or, at least methacrylate end groups).

Now-a-days, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine. The use of nano-sized silver particles as antibacterial agents has develop into further common as scientifically advances compose their production more inexpensive [20-22]. Nowadays, silver ions (Ag^+) are used to avoid bacterial growth in a multiplicity of therapeutic applications, such as, catheters, dental work and the curing of burn wounds [23-25]. Silver cations show broad antibacterial action at very low concentrations and are previously being used for the remedial action of traumatic injuries and burn wounds. Silver ions also exhibit low levels of toxicity to humans being and are protected agents for the deduction of biofilms. Bactericidal actions of nanoparticles is accredited to the occurrence of electronic effects to are brought concerning as a result of changes in restricted electronic structures of the surfaces owing to smaller sizes. These property are measured to be contributing near development of reactivity of silver nanoparticles surfaces. The opposed charges of nanoparticles and bacteria are accredited to their union and bioactivity due to electrostatic forces. It is rational to state that compulsory of nanoparticles to the bacteria depends on the surface area accessible for communication.

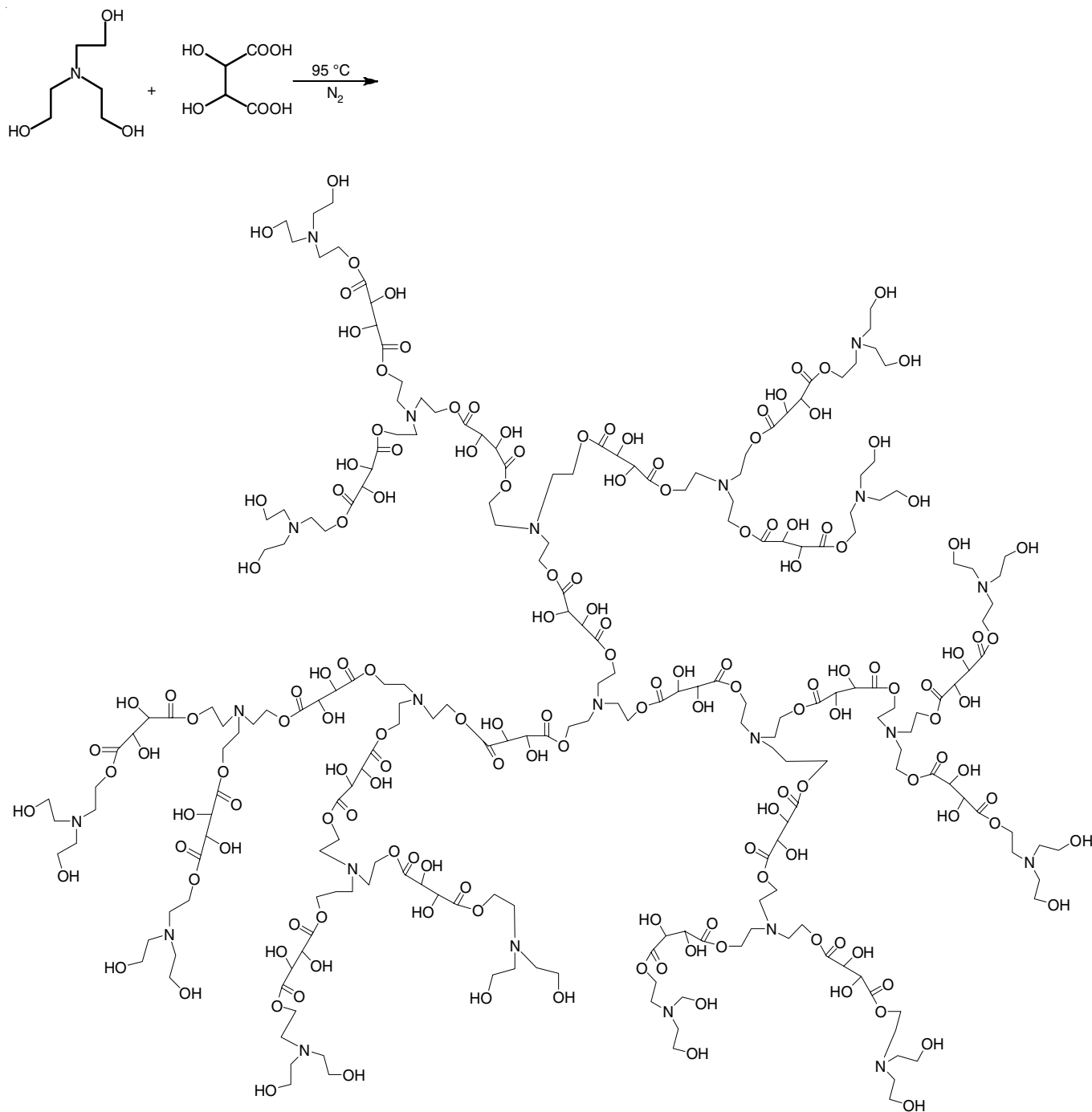
Berzelius [26] reported the method of preparation of HBP resin using tartaric acid as an A_2B_2 monomer and glycerol as a

B₃ monomer not done elsewhere. In the present study, the same method of (A₂B₂ + B₃) preparative approach is followed for the synthesis of HBPE using tartaric acid as an A₂B₂ monomer and 2,2',2''-nitrilotriethanol as an B₃ monomer. The HBPE produced was highly soluble in water paves the way for its usage widely in the field of coating technology. The resulting products in the present work were analyzed using FTIR, ¹H NMR, ¹³C NMR and GPC. The molecular weight and solubility of HBPE was explained in detail. Silver nanoparticles prepared in HBPE matrix through reductive technique. The nanosilver/HBPE were analyzed by XRD, UV-visible and TEM. The antimicrobial properties of nanosilver/HBPE were evaluated against *S. aureus* and *E. coli*.

EXPERIMENTAL

Tri ethanol amine (>99%) and 2,3-dihydroxy butanedioic acid had been purchased by the Sigma Aldrich. Dimethyl sulfoxide, acrylic acid and NaBH₄ was procured from SD Fine Chemicals. Silver nitrate (Merck, India) were used as received. *p*-Toluene sulfonic acid (*p*-TSA, >99%) was purchased by Rankam Chemicals. Nutrient agar and nutrient broth was from Difco Laboratories, Detroit, 82 MI, USA. Water were purified by applying the Milli-Q system (>18 MΩ cm, Millipore).

Characterization: FTIR spectra of macropolyester (HBPE) was taken in to account for a wave range of 4000-450 cm⁻¹ by applying JASCO 400 infrared spectrometer. ¹³C NMR and ¹H



Scheme-I: Synthesis of HBPE from 2,3-dihydroxybutanedioic acid and 2,2',2''-nitrilotriethanol

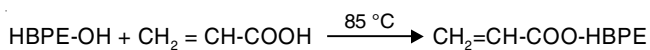
NMR spectra of HBPE were stored on a Bruker (400MHz) NMR spectrometer was taken as a sample at room temperature using DMSO as the solvent and trimethylsilane (TMS) as the internal standard. Molecular weight of HBPE was analyzed using GPC to make, Water-515 were analyzed with polyethylene as standards. Tetrahydrofuran (THF) were taken as the eluent and the rate of flow was kept at 1 mL/min. Thermogravimetric analysis of polymer were conducted in a temperature interval of 30-300 °C under nitrogen atmosphere with a flow rate of 10 mL/min using a Perkin Elmer thermogravimetric analyzer, Pyrisi TGA. The spectrum of nanosilver was obtained in ethanol medium applying UV visible spectroscope of HITACHI U-2800 spectrophotometer. The TEM analyses were carried out using JEOLJEM 2100 electron microscope at 80KV. The resulting samples were analyzed for the purity of the phase purity by powder XRD of make BRUKER (Germany, D8 Advance diffract meter).

Viscosity measurement: The viscosity of HBPE had been studied by using an Ostwald viscometer. Hyperbranched polyester of various concentrations were synthesized by the dilution of the samples. The capillary viscometer were packed with 20 mL of sample and were equilibrated in a water bath for 10 min at 30 ± 0.1 °C. The end samples were allowed to pour throughout the capillary and the flow times were noted and to analyse reduced viscosity. Three values were made for every sample.

Synthesis of HBPE: Hyperbranched polyester was prepared by melt polycondensation at 95 °C of tri ethanol amine and 2,3-dihydroxybutanedioic acid and *p*-toluene sulfonic acid (0.017 g) as an acid catalyst. The 1:1 ratio of the substance were taken in 500 mL four necked flask monomers equipped with N₂ inlet, using an a magnetic stirrer and a drying tube for 7 h. The reactant mixture were slowly heated and then maintained at 95 °C for 7 h to end the reaction. The schematic illustration of the reactions is specified in **Scheme-I**.

Hyperbranched polyester with acrylic acid

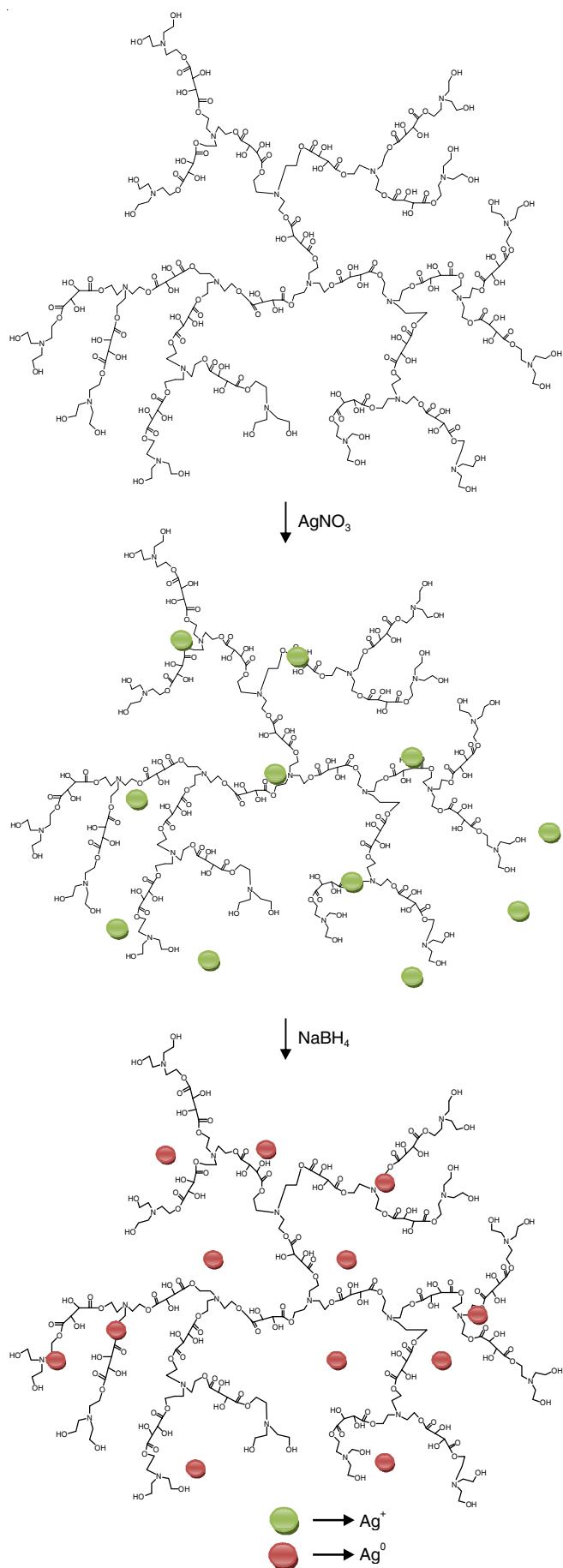
Modification of HBPE: 5 g of HBPE was dissolved in 10 mL of water. It was reacted with acrylic acid in a three necked round flask which was equipped with a magnetic stirrer and a nitrogen gas inlet by applying *p*-toluene sulfonic acid (0.017 g) as a catalyst. The reactions were carried out for 5 h at 85 °C. The reaction is given in **Scheme-II**.



Scheme-II: Synthesis of acrylic modified hyperbranched polyester

Preparation of silver nanoparticles: 1 g of acrylic modified hyperbranched polyester (AHBPE) with 4 mL water had been taken in a round bottom flask with regular stirring. 2 mL solution of 0.1 g of AgNO₃ in water was added drop-wise by using vigorous stirring with room temperature. After completion of the addition, the reaction mixture was stirred for another 20 min. Then 0.1 g of aqueous solution of NaBH₄ was added drop-wise into mixture. The colour of resultant solution of the polymer turned to deep brown (**Scheme-III**).

Preparation of culture media: The media of culture were prepared by dissolving broth powder (1.3 g) in 100 mL water. It were sterilized at 15 lbs pressure by autoclaving at 121 °C



Scheme-III: Synthesis of silver nanoparticles with in HBPE templates

for 15 min. it were cooled in a laminar hood, which were sterilized before hand by washing thoroughly with absolute alcohol and followed by UV irradiation for 20 min. A sterilized wire loop size stock microorganism were transferred in to the cold medium below laminar flow and the conical flask were replugged and the solution kept in an incubator oven at 37 °C for 24 h.

Antimicrobial activity: The nutrient agar powder (3.7 g) was dissolved in 100 mL water. The solution were sterilized and keep in to petridishes and cooled for adequate time to coagulate the agar medium. The above culture broth (2 mL) was equally spread in to entity plates applying sterile L-rod. The 5 mm diameter of well were prepared on agar plates applying sterilized crock borer. Different samples of Three kinds such as silver nitrate/HBPE, nanosilver/HBPE and pure HBPE were taken for analysis. Silver nitrate/HBPE was formed as said in the process of nanosilver. Nanosilver/HBPE is used for analysis by reducing the solution. The polyester is dissolved in DMSO. Applying sterile micropipette, the solutions of samples was poured on to every wells in all the plates. The plates were observed for incubation for 24 h at 37 °C.

RESULTS AND DISCUSSION

The results were obtained by reacting 2,2',2"-nitrilotriethanol and 2,3-dihydroxybutanedioic acid *via* A₂B₂ + B₃ method. Triethanolamine has a low vapour pressure and correspondingly low odour, but evaporation of triethanolamine from coatings is extremely slow. Tartaric acid is used in the tanning of leather. The reaction was maintained at moderately minimum temperature of 150 °C, with an time reaction which is optimized to minimize unless side reactions and to overcome gelation.

The molecular weights of HBPEs have been determined using GPC using THF as solvent. The value of number average molecular weight (M_n), the weight average molecular weight and polydispersity index of HBPE are 950 g/mol, 969 g/mol and 1.016, respectively (Fig. 1). The intrinsic viscosity of HBPE was found to be 1.52 dL/g. The hydroxy values were found to be 64. The HBPE prepared was found to be water soluble and were soluble in polar solvents. Results of the elemental analysis for HBPE are shown in Table-1.

FTIR studies: The FTIR spectra of monomers and HBPE are shown in Fig. 2. The FTIR spectrum of triethanol amine shows an absorption band of 3342 cm⁻¹ due to hydroxy group and sharp peak at 2881 cm⁻¹ due to CH₂ stretching. The peak at 1040 cm⁻¹ is found to C-N stretching. The FTIR spectrum of chain extender (2,3-dihydroxybutanedioic acid shows an absorptions band of 3343 cm⁻¹ due to the hydroxyl groups and the peak at 1724 cm⁻¹ due to the C=O group. The absorption peak of FTIR spectrum at 3425 cm⁻¹ is assigned to -OH stretch vibration and the peaks at 1723 and 1165 cm⁻¹ are attributed to the ester and C-O-R groups. These data indicate that

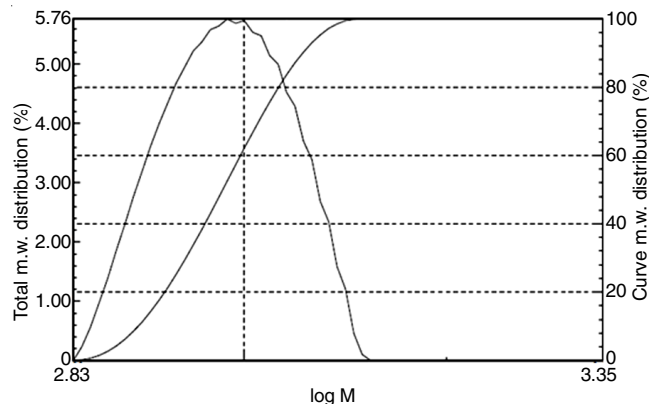


Fig. 1. GPC profile of HBPE from 2,3-dihydroxybutanedioic acid and 2,2',2"-nitrilotriethanol

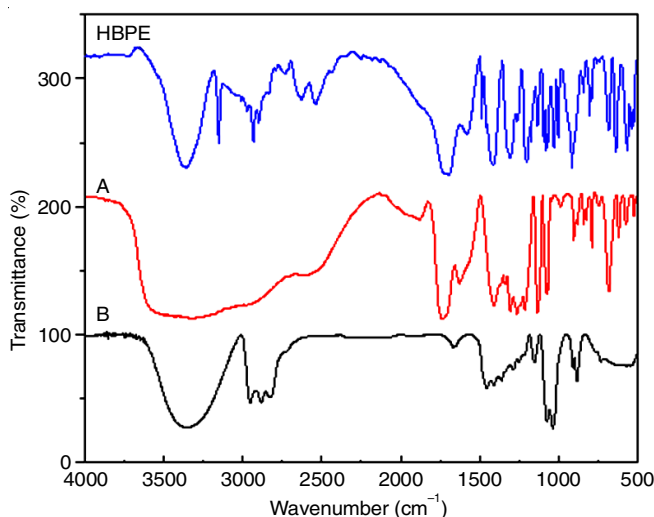


Fig. 2. FTIR spectra of HBPE from 2,3-dihydroxybutanedioic acid (A) and 2,2',2"-nitrilotriethanol (B)

the polymers contain hydroxy groups, ester bonds, carboxylic OH and benzene groups, which were in agreement with objective polymers. The peak strength of hydroxyl groups decreased in the case of acrylic modified HBPE. A peak at 1647 cm⁻¹ related to C=C bond presence of the introduction of acrylic group into HBPE.

NMR studies: The structures of synthesized polymers were identified by ¹H NMR and ¹³C NMR spectroscopic techniques. The ¹H NMR spectra of HBPE are given in Fig. 3. The signals and their integral areas appear around 2.2-3.4 can be attributed to methylene proton and the peak at 2.5 ppm is due to the solvent DMSO-*d*₆. The signals appearing around 5.5 can be attributed to the hydroxyl proton of the 2,3-dihydroxy butanedioic acid and The peak in between 4.2-4.34 ppm is taken in to account of the methylene protons attached to the ester units. The ¹³C NMR spectra of HBPE is shown in Fig. 4. The peaks at 160-172 ppm could be attributed to the carbonyl carbon of ester and 55-60 ppm is regarding to methylene carbon of ester

TABLE-1
ELEMENTAL ANALYSIS OF HBPE

Polymer	Theoretical value (%)				Experimental value (%)			
	C	H	N	O	C	H	N	O
HBPE	46.07	6.371	5.37	41.54	47.14	5.54	5.15	42.17

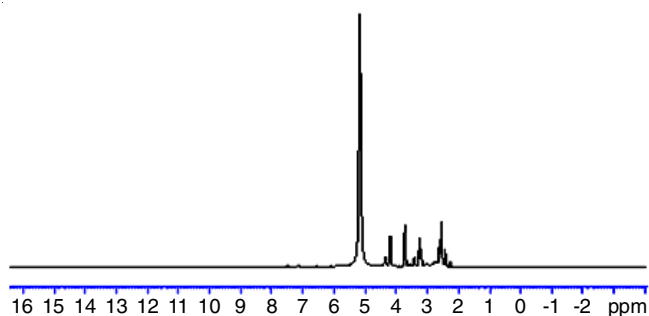


Fig. 3. ^1H NMR spectrum of HBPE from 2,3-dihydroxybutanedioic acid and 2,2',2''-nitrioltriethanol

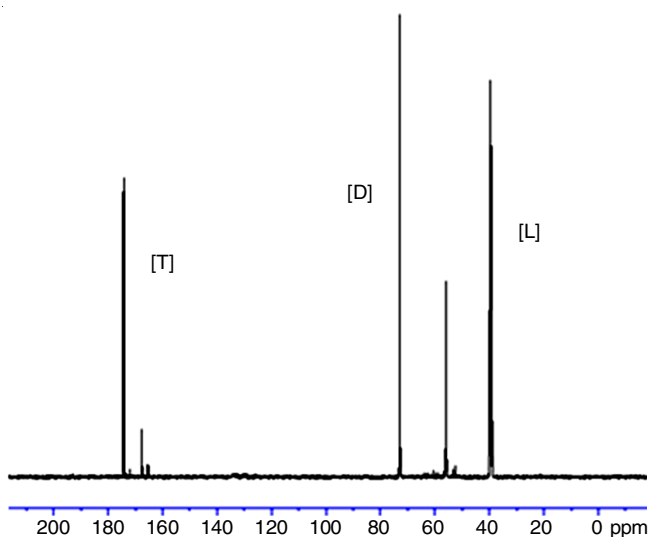


Fig. 4. ^{13}C NMR spectrum of HBPE from 2,3-dihydroxybutanedioic acid and 2,2',2''-nitrioltriethanol

units. The numbering of atoms and the assignments of the NMR signals from different units (D, L and T) of HBPE were done according to the reported method [27]. The ^{13}C NMR spectra of HBPE (Fig. 4) showed that the resonances from the carbons in the D, L and T units had different shifts due to the difference in their chemical environments. The percentage of T, L and D units present in HBPEs, are calculated from the integral area ratios of methylene and quaternary carbon zone of ^{13}C NMR spectra and the value was found to be 0.5.

Characterization of acrylic modified HBPE: The FTIR spectra of acrylic modified HBPE is shown in Fig. 5. The band width of hydroxyl groups lowered in the case of acrylic modified HBPE indicating that the coupling reaction has proceeded. A band at 1641 cm^{-1} analogous to $\text{C}=\text{C}$ bond implies the preamble of acrylic group into HBPE. The ^1H NMR spectra of HBPE is given in Fig. 6. ^1H NMR (400 MHz, $\text{DMSO-}d_6$, δ (ppm): 5.5 (OH proton); 3.7- 4.1 (CH_2OCO). The hydroxyl peak has sharply decreased after reaction of acrylic acid indicating the removal of OH group (Fig. 6). ^{13}C NMR spectrum of HBPE are shown in Fig. 7. ^{13}C NMR [170-172 ($-\text{CH}_2\text{COO}-$); 57-59 ppm (CH_2-OH)] showed that the hydroxy peak intensity has been reduced after adding acrylic acid (Fig. 7).

Characterization of silvernano/HBPE: The characteristic absorbance peak of this composite is determined with UV-visible spectrum. The UV-visible absorbance spectra of the nanoparticles is presented in Fig. 8. The final product formed

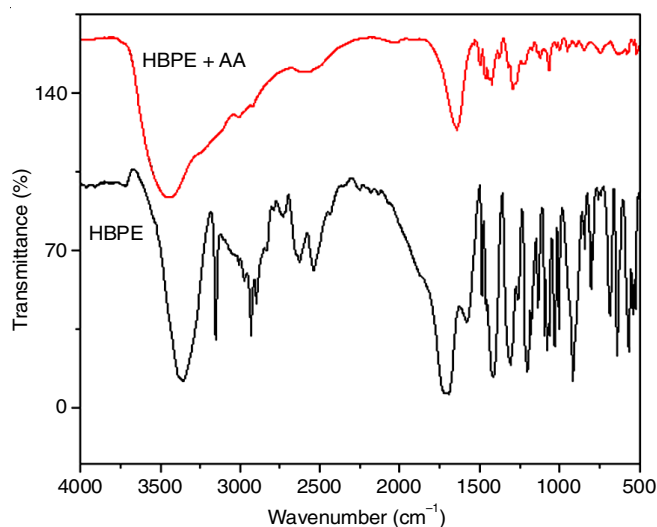


Fig. 5. FTIR spectra of HBPE and acrylic modified HBPE

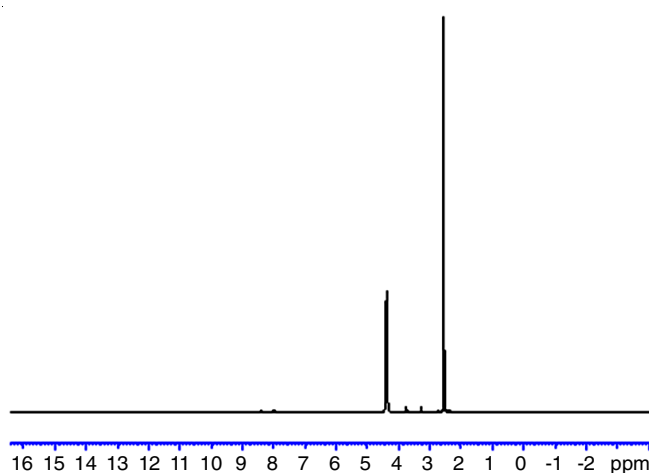


Fig. 6. ^1H NMR spectrum of acrylic modified HBPE

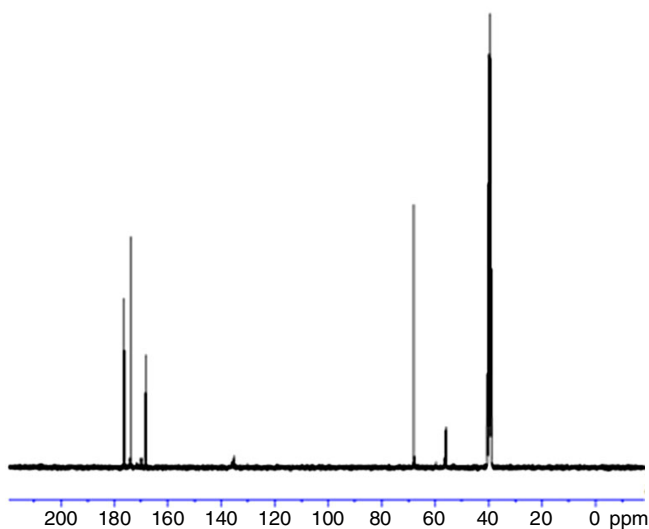


Fig. 7. ^{13}C NMR spectrum of acrylic modified HBPE

are uniform in dimension and diffusion at the used ratio in present study is supported by UV-visible and TEM studies. Hence some of the challenging issues of nanoparticles formation were achieved to certain extent through this technique. The reducing agent were used to ensure absolute reduction of

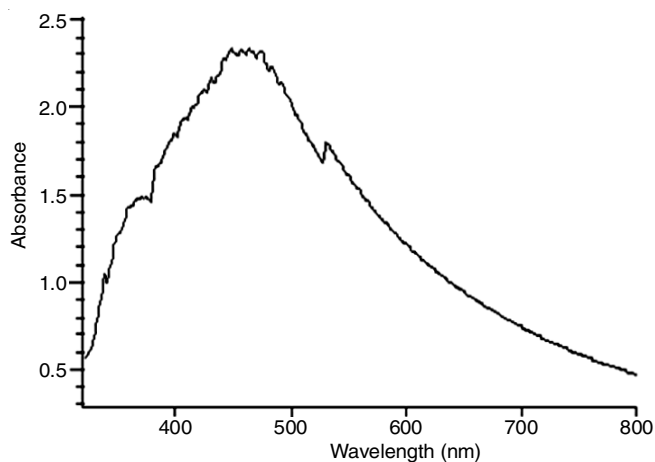


Fig. 8. UV spectrum of silver nanoparticles/HBPE

Ag^+ to Ag^0 , which is confirmed by the change reaction solution to brown and subsequent characterization of silver nanoparticles by different spectroscopic techniques. X-ray diffraction (XRD) is a very important method to characterize the structure of crystalline material. XRD pattern of silver nanoparticles is shown in Fig. 9. Here, four peaks can be seen at $2\theta = 38.4$, 44.1 , 64.3 and 77.4 , which are characteristic diffraction peak of metallic silver. These peaks correspond to the four d-spacing (111), (200), (220) and (311), respectively. All diffraction peaks mentioned to the characteristic FCC silver lines [28].

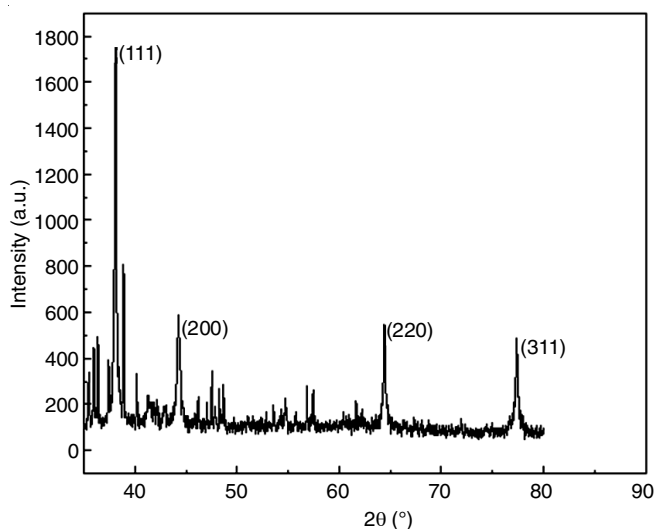


Fig. 9. XRD pattern of silver nanoparticles/HBPE

From Debye-Scherrer equation, the average size of silver nanoparticle synthesized was found to be 14.7 nm. To confirm the composition of silver nanoparticles, FTIR spectra of HBPE and silver nanoparticles encapsulated with HBPE were measured. Fig. 10 shows the FTIR spectra for HBPE and silver nanoparticles encapsulated with HBPE. The analysis showed that the spectral features of pure HBPE and silver nanoparticles encapsulated with HBPE are not so different. Some differences are observed only in the intensity of their peaks. The absorption peak of C=O stretching frequency of the carboxylic ester groups of the polyester observed at 1720 cm^{-1} shifts to 1698 cm^{-1} , which suggests that silver nanoparticles are protected by HBPE through the interaction between Ag and carbonyl group of HBPE [29].

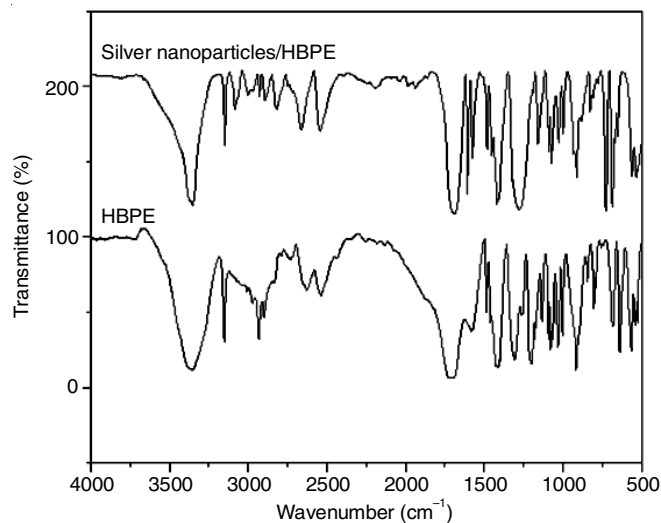


Fig. 10. FTIR spectra of (a) HBPE and (b) silver nanoparticles/HBPE

A TEM image of the prepared silver nanoparticles is shown in Fig. 11. The silver nanoparticles are spherical in shape with a smooth surface morphology. The diameter of the nanoparticles is found to be approximately 16 nm. TEM image also shows that the produced nanoparticles are more or less uniform in size and shape. The silver nanoparticles are circular in shape with a soft surface morphology. The nanoparticle diameter is found to be approximately 15.4 nm. TEM pattern also shows that the formed nanoparticles are huge or less uniform in shape and size.

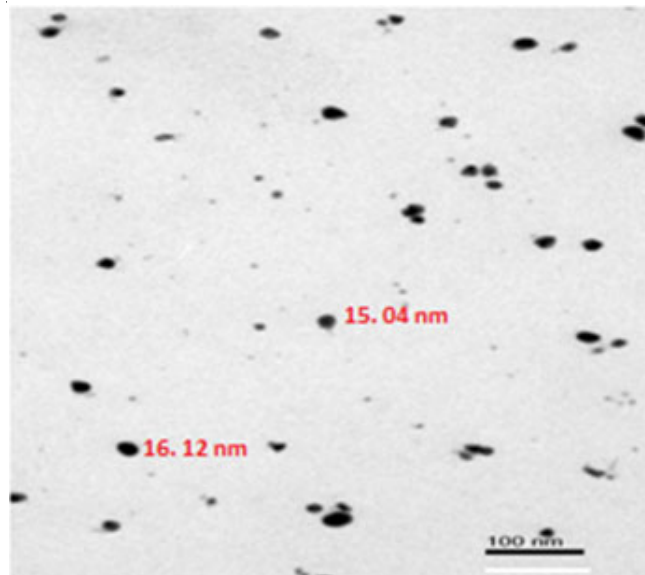


Fig. 11. TEM image of silver nanoparticles/HBPE

The thermogram of pure HBPE and nano-silver/HBPE is shown in Fig. 12. Evidently, the decomposition onset for nanosilver/HBPE is shifted to the higher temperature compared to the pure HBPE. The onset degradation temperature of HBPE is around $98.7\text{ }^\circ\text{C}$ meanwhile it is $99.5\text{ }^\circ\text{C}$ higher for nanosilver/HBPE. The overall thermal stability of HBPE is enhanced with nanosilver incorporation. The thermal properties of the systems were found to be almost similar at lower temperatures. However, the thermal stability of nanosilver/HBPE is found to be very

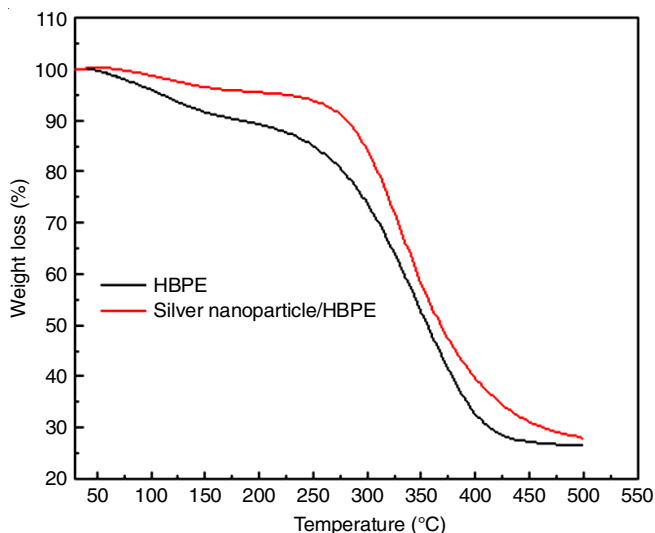


Fig. 12. TGA curves of HBPE and silver nanoparticles/HBPE

high at higher temperature compared to pure HBPE. This may be due to the nucleating effect of nanosilver. The silver nanoparticles in HBPE matrix act as the hardening (virtual cross linking) point in HBPE and thereby helping the soft segments to arrange in a regular pattern and thus improves the crystalline behaviour [30]. Therefore, by incorporating the silver nanoparticles within polymer, the thermal stability of the nanosilver/HBPE can be enhanced.

Antimicrobial activity: The antibacterial efficiency of the silvernano/HBPE against microbes was analyzed on zone of inhibition tests. After 24 h of incubation, the zone of inhibition of the silvernano/HBPE against Gram-negative and Gram-positive bacteria were found to be much superior than the zone of inhibition of AgNO₃/HBPE.

Conclusion

Hyperbranched polyester (HBPE) was prepared in this work via A₂B₂ + B₃ method as an acid-catalyzed esterification process by melt polycondensation from the 2,2',2''-nitriol-triethanol and 2,3-dihydroxybutanedioic acid. The different generation of the samples was obtained by varying the molar monomer/core ratio. The final products are combined with acrylic acid to changing the end groups. In this article, stable silver nanoparticles, protected by HBPE, were synthesized in water by NaBH₄ reducing agent. It can be concluded that HBPE is as a result of a template like dendrimers. From UV-visible spectrum and TEM micrographs of the silver nanoparticles, it can be found that the nanoparticles formed were spherical in structure. FTIR spectra analyzed that there was interaction between silver nanoparticles and HBPE played a crucial role on the stability of silver nanoparticles. Antimicrobial properties of silver nanoparticles are accredited to their whole surface area, as a enlarge surface to volume ratio of nanoparticles which provides much effect to the better antibacterial activity.

REFERENCES

- M. Johansson, E. Malmstrom and A. Hult, *Trends Polym. Sci.*, **4**, 398 (1996).
- A. Hult, M. Johansson and E. Malmstrom, ed.: J. Roovers, Hyperbranched Polymers, In: *Branched Polymers II, Advances in Polymer Science Book Series*, Springer, Berlin, Heidelberg, pp. 1-34 (1999).
- B. Pettersson, *Pigm. Resin Technol.*, **25**, 4 (1996); <https://doi.org/10.1108/eb043185>.
- B. Voit, *J. Polym. Sci. A Polym. Chem.*, **43**, 2679 (2005); <https://doi.org/10.1002/pola.20821>.
- P.N. Mehta, *Surface Coat. Int. Part B: Coatings Transac.*, **89**, 333 (2006); <https://doi.org/10.1007/BF02765586>.
- D. Yan, C. Gao and H. Frey, *Hyperbranched Polymers: Synthesis, Properties and Applications*, John Wiley & Sons, New York, pp. 5924-5973 (2009).
- B. Voit and A. Lederer, *Chem. Rev.*, **109**, 5924 (2009); <https://doi.org/10.1021/cr900068q>.
- X. Zhang, *Prog. Org. Coat.*, **69**, 295 (2010); <https://doi.org/10.1016/j.porgcoat.2010.08.007>.
- A. Asif and W. Shi, *Eur. Polym.*, **39**, 933 (2003); [https://doi.org/10.1016/S0014-3057\(02\)00311-7](https://doi.org/10.1016/S0014-3057(02)00311-7).
- M. Scholl, Z. Kadlecova and H.A. Klok, *Prog. Polym. Sci.*, **34**, 24 (2009); <https://doi.org/10.1016/j.progpolymsci.2008.09.001>.
- Y.H. Kim and O.W. Webster, eds.: M.K. Mishra and S. Kobayashi, *Hyperbranched Polymers, Star and Hyperbranched Polymers*, Marcel Dekker Inc., New York, pp. 201-38 (1999).
- K. Inoue, *Prog. Polym. Sci.*, **25**, 453 (2000); [https://doi.org/10.1016/S0079-6700\(00\)00011-3](https://doi.org/10.1016/S0079-6700(00)00011-3).
- H. Galina, J.B. Lechowicz and M. Walczak, *Macromolecules*, **35**, 3261 (2002); <https://doi.org/10.1021/ma011722t>.
- T. Emrick, H.-T. Chang and J.M.J. Frechet, *J. Polym. Sci. A Polym. Chem.*, **38**, 4850 (2000); [https://doi.org/10.1002/1099-0518\(200012\)38:1+<4850::AID-POLA230>3.0.CO;2-G](https://doi.org/10.1002/1099-0518(200012)38:1+<4850::AID-POLA230>3.0.CO;2-G).
- G.D. Figuly, *Hyperbranched Polyesters*, US Patent 5270402A, (1993).
- D.A. Tomalia and J.M.J. Frechet, *J. Polym. Sci. A Polym. Chem.*, **40**, 2719 (2002); <https://doi.org/10.1002/pola.10301>.
- B. Voit, D. Beyerlein, K.-J. Eichhorn, K. Grundke, D. Schmaljohann and T. Loontjens, *Chem. Eng. Technol.*, **25**, 704 (2002); [https://doi.org/10.1002/1521-4125\(20020709\)25:7<704::AID-CEAT704>3.0.CO;2-2](https://doi.org/10.1002/1521-4125(20020709)25:7<704::AID-CEAT704>3.0.CO;2-2).
- R.B. Frings and M. Wend: *New Hyperbranched Polyesters for UV Curing*, DIC Technical review, **9**, pp. 43-51 (2003).
- M. Johansson, T. Glauser, G. Rospo and A. Hult, *J. Appl. Polym. Sci.*, **75**, 612 (2000); [https://doi.org/10.1002/\(SICI\)1097-4628\(20000131\)75:5<612::AID-APP3>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1097-4628(20000131)75:5<612::AID-APP3>3.0.CO;2-1).
- A. Melaiye, Z. Sun, K. Hindi, A. Milsted, D. Ely, D.H. Reneker, C.A. Tessier and W.J. Youngs, *J. Am. Chem. Soc.*, **127**, 2285 (2005); <https://doi.org/10.1021/ja040226s>.
- I. Sondi and B. Salopek-Sondi, *J. Colloid Interface Sci.*, **275**, 177 (2004); <https://doi.org/10.1016/j.jcis.2004.02.012>.
- V. Alt, T. Bechert, P. Steinrucke, M. Wagener, P. Seidel, E. Dingeldein, E. Domann and R. Schnettler, *Biomaterials*, **25**, 4383 (2004); <https://doi.org/10.1016/j.biomaterials.2003.10.078>.
- H.J. Klases, *Burns*, **26**, 117 (2000); [https://doi.org/10.1016/S0305-4179\(99\)00108-4](https://doi.org/10.1016/S0305-4179(99)00108-4).
- S. Silver and L.T. Phung, *Annu. Rev. Microbiol.*, **50**, 753 (1996); <https://doi.org/10.1146/annurev.micro.50.1.753>.
- R.M. Slawson, M.I. Van Dyke, H. Lee and J.T. Trevors, *Plasmid*, **27**, 72 (1992); [https://doi.org/10.1016/0147-619X\(92\)90008-X](https://doi.org/10.1016/0147-619X(92)90008-X).
- R.H. Kienle and A.G. Hovey, *J. Am. Chem. Soc.*, **51**, 509 (1929); <https://doi.org/10.1021/ja01377a021>.
- J. Hao, M. Jikei and M. Kakimoto, *Macromolecules*, **36**, 3519 (2003); <https://doi.org/10.1021/ma021328a>.
- O.L.A. Monti, J.T. Fourkas and D.J. Nesbitt, *J. Phys. Chem. B*, **108**, 1604 (2004); <https://doi.org/10.1021/jp030492c>.
- R. Sun, H. Zhao, Y. Luo and Y. Liu, *J. Nanopart. Res.*, **13**, 1133 (2011); <https://doi.org/10.1007/s11051-010-0105-1>.
- H. Deka, N. Karak, R.D. Kalita and A.K. Buragohain, *Polym. Degrad. Stab.*, **95**, 1509 (2010); <https://doi.org/10.1016/j.polymdegradstab.2010.06.017>.