

Synthesis and Characterization of Nano Ag End Capped L-Cysteine Bridged Diblock Copolymer

M. Jeyapriya, B. Meenarathi, L. Kannammal, S. Palanikumar and R. Anbarasan*

Department of Polymer Technology, Kamaraj College of Engineering and Technology, Virudhunagar 626001, Tamilnadu, India

Abstract The target of the present investigation is synthesis and characterization of an amphiphilic diblock copolymer with antibacterial property. Ring opening polymerization (ROP) of ϵ -caprolactone (CL) and tetrahydrofuran (THF) was carried out under inert atmosphere by using L-cysteine as a bridging agent in the presence of stannous octoate (SO) as a catalyst. The nano silver end capped diblock copolymer was synthesized by *in situ* method. Thus obtained nano silver end capped L-cysteine bridged diblock copolymer was characterized by various analytical methods like Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, circular dichroism (CD), fluorescence spectroscopy, gel permeation chromatography (GPC), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), field emission scanning electron microscopy (FESEM), high resolution transmission electron microscopy (HRTEM) and zeta potential. The antimicrobial property of the nano silver end capped diblock copolymer against e-coli was tested.

Keywords: Ring opening polymerization; Amphiphilic diblock copolymer; Synthesis; XPS; HRTEM.

INTRODUCTION

Poly(ϵ -caprolactone) (PCL) is a linear polyester and can be synthesized by the ring opening polymerization (ROP) of CL through co-ordination insertion mechanism. PCL has more micro voids on its backbone and it is very much useful in the bio-medical field as a drug carrier. This urged us to do the research work on PCL. Various anionic^[1–3] and cationic^[4–5] initiators are being used for its synthesis. Meenarathi *et al.*^[6] reported about the near infrared (NIR)-dye functionalized multiwall carbon nano tube (MWCNT) as a cationic initiator for the ring opening polymerization of CL. Due to the bio-compatibility and bio-degradability, PCL is used in the bio-medical field as a drug carrier^[7, 8]. Unfortunately, its bio-medical applications are restricted to some extent due to the absence of functional groups for further structural modification, semi-crystallinity and its hydrophobicity. This problem can be outwitted by using the functionalized initiator^[9–11]. The hydroxyl end capped PCL is used for the synthesis of di or tri block copolymers. Currently, the synthesis of PCL based amphiphilic diblock copolymers is a fascinating field of research^[12–15] because of their dispersibility in both the organic and aqueous media. So far, the report on L-cysteine initiated ROP of PCL based amphiphilic diblock copolymer is not available in the literature.

Poly(tetrahydrofuran) (PTHF) is a linear polyether with excellent water solubility. PTHF is synthesized by the ROP of THF by the cationic mechanism^[16–18]. In the bio-medical field PTHF is used as an amphiphilic group and in the electrochemistry field it is used as a solid electrolyte^[19]. The diblock copolymer has wide bio-medical applications^[20–22]. In recent years, the bio-medical application of aliphatic amphiphilic polymers has been

* Corresponding author: R. Anbarasan, E-mail: anbu_may3@yahoo.co.in

Received March 18, 2015; Revised April 18, 2015; Accepted April 21, 2015

doi: 10.1007/s10118-015-1688-x

increased due to their bio-compatibility and bio-degradability. To the best of our knowledge, L-cysteine bridged amphiphilic copolymer consists of PCL and PTHF is not yet published^[23–25].

Polymeric materials with excellent bacterial resistance surface are of great importance in bio-medical field as an antibacterial material. This can be achieved by the conjugation or surface functionalization of nano Ag by the polymeric materials. The nano Ag can be synthesized by both the chemical^[26–28] as well as by the biological^[29, 30] route. The biological route offers longer time and with high polydispersity value. This drawback can be easily tackled by the chemical reduction method^[31, 32]. Very few reports are available for the synthesis of amphiphilic diblock copolymer with nano silver end capping by using L-cysteine as a bridging agent.

L-cysteine is an amino acid with a good hydrogen donor thiol group^[33–35]. A trifunctional L-cysteine is used as a raw material for the manufacture of proteins and led to the formation of fantastic helical structure^[36, 37]. This urged us to do the present investigation by using L-cysteine as a bridging agent for the assembling of diblock copolymer with antimicrobial property. The present study is aimed to synthesize L-cysteine bridged nano Ag end capped hydrophobic-hydrophilic diblock copolymeric recipient. The novelty of the present investigation is the synthesis of Ag nanoparticles with the help of amphiphilic diblock copolymer as a capping agent and it can increase the molecular weight of the polymer through its surface catalytic effect and acting as a cross linking centre in an eco-friendly manner.

EXPERIMENTAL

Materials

ϵ -Caprolactone (CL) was purchased from Aldrich, India. Tetrahydrofuran (THF), chloroform (CHCl_3), phthalic anhydride (PAH) and diethylether were purchased from S.D. Fine chemicals, India. Stannous octoate (SO, Merck, India) and L-cysteine (Ottochemi, India) were purchased and used as received. AgNO_3 was received from CDH, India. PET fabric was received as a gift sample from Rajapalayam, India. Double distilled (DD) water was used for solution making purpose.

Synthesis of nano silver end capped PCL-PTHF diblock copolymer includes three steps.

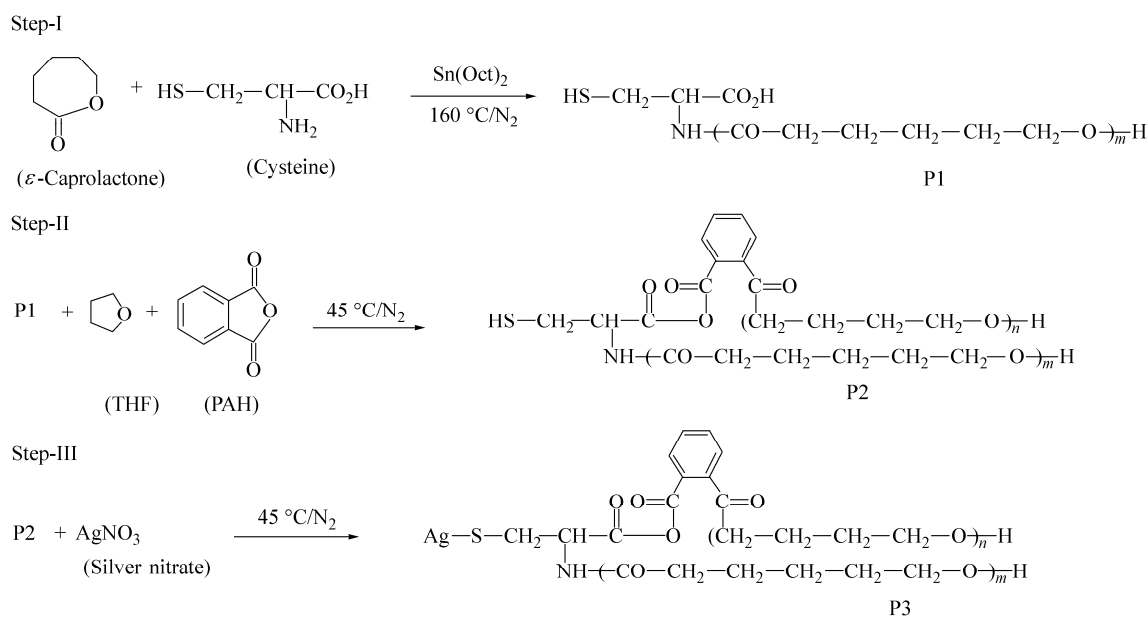
Synthesis of PCL

Ring opening polymerization of CL was done by bulk polymerization method. 2 g CL monomer was taken in a 25 mL capacity round bottomed flask (RBF). The catalyst, SO was added into the RBF. The monomer to catalyst (M/C) ratio must be maintained at 1000. This will help to increase the molecular weight of the polymer without any branching or crosslinking and also reduce the back-biting reaction. A required amount of L-cysteine (M/I = 100) was added and the RBF was purged with sulfur free nitrogen gas under mild stirring at room temperature for 10 min. Then, the sealed RBF was put into an oil bath at 160 °C. At higher temperatures only the ROP of CL is the favorable one under $[\text{M/C}] = 1000$. Excess utilization of catalyst will reduce the molecular weight of the polymer and it is hazardous to the human beings. Since PCL is a bio-medical polymer, excess use of catalyst is prohibited. For this purpose only 160 °C was preferred. The nitrogen purging was continued for another 2 h with mild stirring. During the course of the reaction, the medium becomes highly viscous^[6]. The amino groups present in the cysteine moiety were involved in the ROP of CL. At the end of the reaction, the viscous mass was removed from the oil bath, cooled, dissolved in 25 mL CHCl_3 and transferred to a 500 mL beaker. The PCL was re-precipitated by the addition of 250 mL diethylether. Thus obtained white precipitate was washed three times with water to remove the unreacted monomer/L-cysteine/catalyst. After thorough washing, the white precipitate was dried by freeze drying. The purified PCL sample was weighed and stored in a zip lock cover. The product is L-cysteine end capped PCL^[6] and named as P1. The reaction is mentioned in Scheme 1.

Synthesis of PCL-L-Cysteine-PTHF Diblock Copolymer

For the synthesis of PCL-PTHF diblock copolymer, 0.50 g of PCL obtained in the previous step was taken as a macro initiator. 20 mL of THF (as a co-monomer) was charged in a 50 mL RBF. 0.01 g PAH and 0.50 g macro initiator (PCL) were also added. The RBF containing the reaction mixture was introduced into an oil bath maintained at 45 °C under nitrogen purging with mild stirring. The PAH induces the ROP of THF. The carboxyl

group present in the cysteine might involve in the ROP of PAH and THF. Sometimes the OH group present at the PCL chain end might be involved in the ROP. After 6 h of ROP, the contents were poured into the 100 mL dil. HCl solution to arrest the ROP of THF^[16, 17]. The beaker was heated to 45 °C to evaporate the unreacted THF. Then the solution was dried by freeze drying method. After drying one can get a pale brown colored PCL-L-cysteine-PTHF diblock copolymer. Thus obtained polymer sample (P2) is weighed and stored under N₂ atmosphere. The reaction is mentioned in Scheme 1.



Scheme 1 Synthesis of PCL-Cys(Ag)-PTHF

Synthesis of Nano Silver End Capped PCL-L-Cysteine-PTHF Diblock Copolymer

In the third step, 1.0 g of L-cysteine bridged diblock copolymer was dissolved in 50 mL of THF taken in a 250 mL RBF fitted with a condenser. 0.50 g AgNO₃ was dissolved in 10 mL of DD water and mixed with the diblock copolymer solution. The contents were heated to 40 °C for 2 h by the ice-cold water circulation in the condenser. In such a way one can avoid the evaporation of THF solvent. During the mixing, a brown color precipitate appeared. At the end of the reaction, the condenser was removed and the heating was continued for another 10 min in a fuming cupboard. Water molecules retained in the RB flask was dried by freeze drying method. Finally, one can get a brown colored nano Ag end capped PCL-L-cysteine-PTHF diblock copolymer (P3). During the reaction, the Ag⁺ was reduced to Ag⁰. This was done through the polyol methodology^[30]. Sometimes the thiol or amino group present in the L-cysteine initiator might involve in the reduction reaction. The reaction is mentioned in Scheme 1.

Characterization

Circular dichroism (CD) was studied by using circular dichroism spectropolarimeter, Jasco J-715 model instrument. FTIR spectra for the samples were recorded with the help of a Shimadzu 8400 S, Japan instrument by the KBr pelletization method from 400 cm⁻¹ to 4000 cm⁻¹. Fluorescence spectral measurement was carried out with the help of an instrument Elico SL 172, India. The surface morphology and particle sizes of the samples were determined by FE-SEM, Hitachi S4800 Japan instrument. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were measured by using Universal V4.4A TA Instruments under nitrogen atmosphere at the heating rate of 10 K·min⁻¹ from room temperature to 373 K. The second heating scan of the sample was considered to delete the previous thermal history of the sample. Molecular weight determination of diblock copolymer samples was carried out by using gel permeation chromatography, Perkin Elmer Series 200 in

THF solvent and poly(styrene) as an internal standard. ^1H and ^{13}C nuclear magnetic resonance (NMR) (500 MHz) spectra were obtained using an NMR apparatus (Varian, Unity Inova-500 NMR) at room temperature in CDCl_3 solvent. A Zetasizer (Nano-ZS Malvern Instrument USA) was used to measure the zeta potential of dispersions. High resolution transmission electron microscopy (HRTEM) was measured for P1, P2 and P3 samples by using a JEM-200 CX, USA transmission electron microscope instrument.

Antimicrobial Study-Spread Plate Method

Suspend 51.53 g of agar medium in 100 mL DD water. Boil the medium until the solute dissolves completely and sterilize it by autoclaving at 0.10 MPa pressure and 121°C temperature for 15 min. Pour the agar medium to a Petri dish, mix it well before pouring. Spread out the 0.1 mL of broth culture of e-coli over the agar medium. Punch a well at the centre of the Petri dish for 3 mm. Take 25 mL of polymer sample and incubate it for 24 h and inject it into the punched well.

RESULTS AND DISCUSSION

FTIR Spectroscopy

The FTIR spectra (Fig. 1) confirm the functional groups present in the polymers. Spectrum a represents the FTIR spectrum of L-cysteine initiated ROP of CL. A peak at 3500 cm^{-1} is due to the $-\text{OH}$ stretching of PCL. The $-\text{NH}$ stretching of L-cysteine is observed at 3356 cm^{-1} . The C—H symmetric and anti symmetric stretching can be seen at 2872 cm^{-1} and 2943 cm^{-1} respectively. The C=O stretching of PCL appeared at 1731 cm^{-1} ^[38]. The ether C—O—C linkage appeared at 1194 cm^{-1} . The C—H out of plane bending vibration appeared at 735 cm^{-1} . Spectrum b represents the FTIR spectrum of P2. Here also the above said peaks corresponding to PCL are observed. The appearance of new peak confirmed the presence of THF units in diblock copolymer backbone. A twin peak at 2528 and 2653 cm^{-1} is due to the aromatic C—H symmetric and antisymmetric stretching respectively. During the ROP of THF little bit of PAH was added as a comonomer to accelerate the diblock copolymer formation. The sharp carbonyl stretching appeared as a doublet peak around 1750 cm^{-1} . The first sharp carbonyl is associated with the carbonyl stretching of PCL whereas the second broad carbonyl stretching is associated with the PAH units. A sharp peak at 1586 cm^{-1} is due to the tetrahydrofuranium cationic structure^[20]. The aliphatic ether linkage can be seen at 1202 and 1265 cm^{-1} as a twin peak. The appearance of cationic structure and ether linkage confirmed the presence of THF units in the diblock copolymer chain. The aromatic —CH bending can be seen around $675\text{--}800\text{ cm}^{-1}$ which confirmed the presence of PAH units in the diblock copolymer backbone. The appearance of peaks corresponding to C=O and aromatic C—H bending confirmed the presence of PAH units in the diblock copolymer chain. Spectrum c reveals the FTIR spectrum of

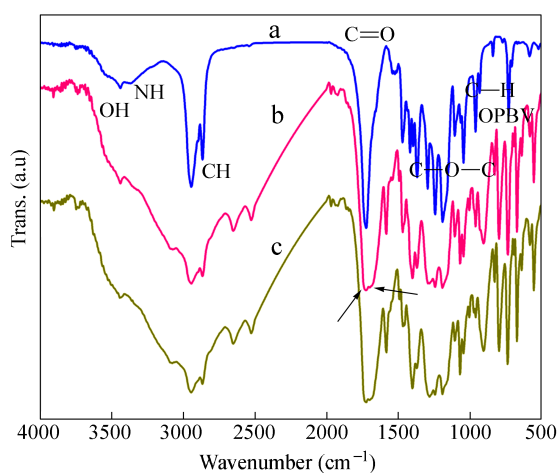


Fig. 1 FTIR spectra of (a) P1, (b) P2 and (c) P3

nano silver end capped PCL-L-cysteine-PTHF copolymer (P3). Here the peaks corresponding to CL and THF repeating units are observed. There is no separate new peak for the silver nanoparticles. Thus the FTIR spectra confirmed the presence of various functionalities in P1, P2 and P3 backbones.

NMR

The structure of L-cysteine initiated ROP CL was confirmed by NMR spectra. Figure 2(a) represents the ^1H -NMR spectrum of PCL. The $-\text{OCH}_2$ proton appeared at $\delta = 4.1$. The CH_2 next to $-\text{OCH}_2$ group appeared at $\delta = 2.4$. The methylene proton adjacent to carbonyl group appeared at $\delta = 1.44$. The middle protons of CL units appeared at $\delta = 1.71$. Peaks at $\delta = 0$ and 7.3 corresponds to the TMS and chloroform respectively. The end $-\text{OCH}_2$ protons are appearing at $\delta = 3.67^{[6, 38]}$. The thiol proton from L-cysteine unit is appearing at $\delta = 4.33$. The $-\text{NH}$ proton of L-cysteine at $\delta = 3.81$ confirmed the ROP of CL by L-cysteine as a chemical initiator. The appearance of thiol proton and decrease in peak intensity of $-\text{NH}$ proton confirmed that the amino group of L-cysteine was involved in the ROP of CL. It was noted that the thiol group remains as such throughout the ROP of CL. It meant that the amino group of L-cysteine was more actively involved in the ROP of CL than $-\text{COOH}$ and $-\text{SH}$ groups. Figure 2(b) confirmed the ^{13}C -NMR spectrum of L-cysteine initiated ROP of CL. The carbonyl carbon appeared at $\delta = 174$. A broad peak around $\delta = 80$ is due to the solvent. The $-\text{OCH}_2$ carbon signal appeared at $\delta = 65$. The methylene carbon next to the carbonyl carbon appeared at $\delta = 36$. The remaining carbon signals appeared as mentioned in the ^{13}C -NMR spectrum. Both ^1H and ^{13}C -NMR spectra confirmed the ROP of CL by L-cysteine. The methylene and carbonyl signals of L-cysteine units appeared at $\delta = 62$ and 195 respectively.

Figure 2(c) reveals the ^1H -NMR spectrum of PCL-L-cysteine-PTHF diblock copolymer system. In this system a little bit of PAH was added as a comonomer. The aromatic protons appeared at $\delta = 7.55$ to 7.91 . The $-\text{OCH}_2$ proton of THF appeared at $\delta = 4.2$. The same peak was mentioned for $-\text{OCH}_2$ protons of CL units. On peak integration, we can confirm the block copolymerization of THF units with PCL. The $-\text{SH}$ proton of L-cysteine appeared at $\delta = 4.33$ which included the thiol groups and remained as such during the block copolymerization with THF in the presence of PAH. Figure 2(d) enumerates the ^{13}C -NMR spectrum of PCL-L-cysteine-PTHF diblock copolymer. The carbon signals of corresponding PCL units appeared. Further, the aromatic carbon signals appeared at $\delta = 134$ as a small hump. The $-\text{OCH}_2$ carbon signal of THF units appeared at $\delta = 62$. The methylene carbon signals of THF unit can be seen at $\delta = 33$. Thus both ^1H and ^{13}C -NMR both confirmed the chemical structure of PCL-L-cysteine-PTHF diblock copolymer.

Figure 2(e) confirms the chemical structure of nano silver end capped PCL-L-cysteine-PTHF amphiphilic diblock copolymer. While adding silver nitrate solution with diblock copolymer in THF solution, there are three possible ways for the reduction of Ag^+ to Ag^0 . The first possibility is the thiol group of L-cysteine which can reduce the Ag^+ to Ag^0 . The second possibility is the imino group of L-cysteine which can reduce the Ag^+ to Ag^0 . The third possibility is the $-\text{OH}$ end group of diblock copolymer which can reduce the Ag^+ to Ag^0 through polyol methodology^[30]. Among these three possibilities, the third possibility is the most viable route. This can be confirmed by ^1H -NMR spectrum. The hydrogen signals of PCL and PTHF units are observed. Above all, the thiol signal of L-cysteine which appeared at $\delta = 4.3$. It can be inferred that during the reduction reaction, the thiol group was not fully involved, secondly the imino proton at $\delta = 3.81$ disappeared which confirmed that imino group is also a more surface liable group. Even then, the thiol group is a suitable candidate for the binding with metal nanoparticle. The ^1H -NMR declares that the $-\text{NH}$ or $-\text{OH}$ group might reduce the Ag^+ to Ag^0 . Figure 2(f) indicates the ^{13}C -NMR spectrum of nano silver end capped PCL-L-cysteine-PTHF diblock copolymer. Here the carbon signal due to PCL and PTHF segments are observed. The ^{13}C -NMR cannot reveal anything about the surface binding or reduction of Ag^+ to Ag^0 . From the NMR analysis one can come to a conclusion that the $-\text{OH}$ group is more active towards the surface binding rather than $-\text{NH}$ or $-\text{SH}$ groups particularly in the case of amphiphilic diblock copolymer.

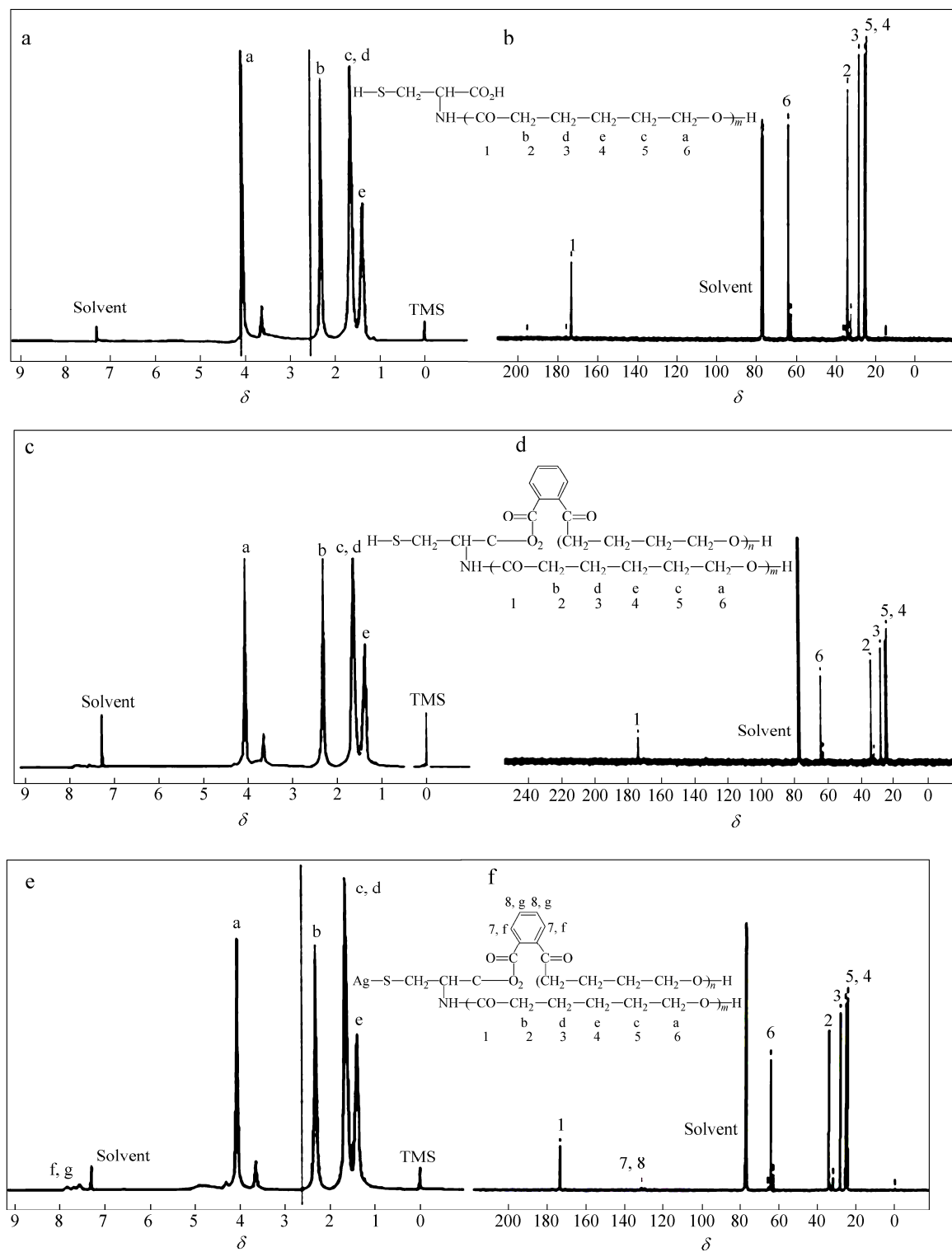


Fig. 2 $^1\text{H-NMR}$ spectra of (a) P1, (c) P2 and (e) P3; $^{13}\text{C-NMR}$ spectra of (b) P1, (d) P2 and (f) P3

CD

Figure 3 shows the CD spectrum of L-cysteine and its conjugates. The CD spectrum of pristine L-cysteine with peaks appeared at 254.8, 259.6, 266.3, 278.0 and 285.1 nm corresponding to the various possible intra and inter molecular hydrogen bondings. The circular dichroism of cysteine decorated silver nanoparticle is reported in the literature with all the possible inter and intra molecular hydrogen bondings^[35]. Peaks appeared in the negative region confirmed the presence of L-cysteine. Peaks at far UV region confirmed the $n \rightarrow \pi^*$ transition of L-cysteine. For the CD spectrum of L-cysteine mediated ROP of CL one can also observe the above said peaks but within the inverse region. It means that after the ROP of CL the conformation of L-cysteine was altered. This is due to the chemical conjugation of neutral PCL chain with the amino group of L-cysteine. It can be inferred that one end of L-cysteine was attached with the high molecular weight PCL through a sigma bond formation. As a result, some amount of hydrogen bonding disappeared and the same can be evidenced by the disappearance of a peak at 266.3 nm. It indirectly tells us that the amino group of L-cysteine involved in the ROP of CL. For the CD spectrum of PCL-L-cysteine-PTHF system one can see the peaks corresponding to the $-\text{NH}$ group of L-cysteine within negative region. It indicates that the other end of L-cysteine was chemically conjugated with PTHF. Moreover, hydrophilic PTHF offers more hydrogen bonding centre through the ether linkages. By referring the intensity of the peak, one can say that the amount of hydrogen bonding was suppressed after the ROP of CL and THF. For the CD spectrum of nano Ag end capped PCL-L-cysteine-PTHF diblock copolymer system, all the hydrogen bondings were completely exhausted. The amino group of L-cysteine was involved in the ROP of CL. The $-\text{CO}_2\text{H}$ group of L-cysteine was involved in the ROP of THF. The $-\text{SH}$ group of L-cysteine or $-\text{OH}$ group of PCL or PTHF was involved in the mild reduction reaction and surface binding with the Ag nano particle. In such a way all the functional groups were blocked. During the nano silver end capping process, the $-\text{OH}$ groups of PCL, PTHF, $-\text{SH}$ and $-\text{NH}$ group of L-cysteine might be involved. The $-\text{OH}$ group of PCL and PTHF may lead to the surface binding reaction through the polyol methodology. After the surface binding with Ag nanoparticle again the conformation of L-cysteine was changed. The CD report of L-cysteine conjugated Ag nano particle is available in the literature^[35]. Our report is entirely different from that report. This is because of chemical conjugation of two different polymers at their ends and side branching with Ag nanoparticle. Figure 1 indicates the FTIR spectra of the polymers with possible intermolecular hydrogen bonding even after end capping with Ag nanoparticle. This also suggested that the intramolecular hydrogen bondings were completely broken. Since the polyol methodology is a slow reaction and the thiol group of L-cysteine actively participate the mild reduction reaction and it is acting as a good metal surface binding group. Hence, the FTIR and CD data combinedly declared that the thiol group of L-cysteine is a suitable candidate to bind with the metal nanoparticle surface rather than the $-\text{OH}$ group of PCL or PTHF.

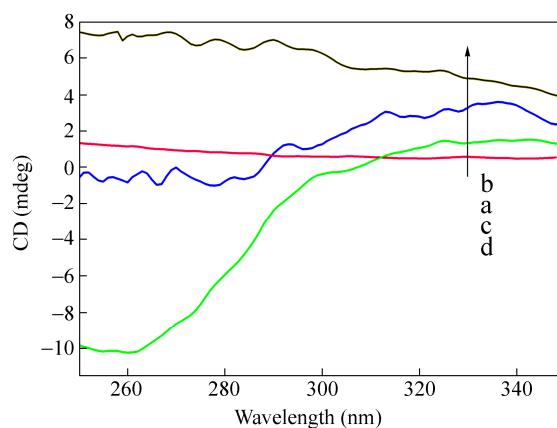


Fig. 3 CD spectra of (a) L-cysteine, (b) P1, (c) P2 and (d) P3

Fluorescence Emission

Amino acids exhibit fluorescence properties. The pristine L-cysteine exhibits one emission peak around 320 nm with the fluorescence emission intensity (FEI) of 75 cps (Spectrum a, Fig. 4). The FEI of L-cysteine end capped PCL is represented in Fig. 4 (Spectrum b) with the intensity value of 195 cps. The L-cysteine after the conjugation with PCL, the emission peak was red shifted with the increase in FEI. P1 exhibits an emission peak at 370 nm. Both increase in intensity and red shift in peak position confirmed the existence of chemical interaction between L-cysteine and PCL, *i.e.*, one end of the PCL was attached to L-cysteine whereas the other end of PCL was occupied by —OH group. L-cysteine contains an amino, one carbonyl with a thiol group. The emission spectrum of P2 is shown in Fig. 4 (Spectrum c). Here one can see some interesting points that after the ROP of THF in the presence of L-cysteine-PCL system, the emission peak was blue shifted. At the same time the emission intensity was raised to 650 cps. The blue shift in the emission peak (350 nm) urged us to come to a conclusion that during the conjugation of the hydrophobic and hydrophilic segments, the size of the diblock copolymer was reduced in the interface region. The increase in FEI can be explained as follows: i) After the conjugation with THF units the hydrophilic character of the resultant copolymer was raised. ii) Moreover, the interface region between the hydrophilic and hydrophobic polymer chains exhibited the formation of polymer nano particles. After the formation of polymer nano particles, the surface area to volume ratio was increased due to the spherical shape of the polymer nano particles. For the sake of comparison, the emission spectrum of P3 is shown in Fig. 4 (Spectrum d). After the silver end capping process, the thiol group or the amino group of L-cysteine was bound with the silver nano particle. The emission peak was blue shifted to 335 nm with the reduction in the FEI. Both the blue shift in the emission peak and the reduction in FEI are purely due to the reduction in size of diblock copolymer and also the silver nano particles with the zero valent oxidation state. This concluded that the silver nano particles can reduce the FEI of amino acid. In overall comparison, the diblock copolymer exhibited the highest FEI with the particle size reduction. It is a well known factor that either PCL or PTHF does not have fluorescence property. The base unit of protein like cysteine only has fluorescence property due to the presence of different chromophores. The fluorescence emission spectrum of protein base unit is well explained in the literature^[39]. The literature also supports the conjugation of chromophoric or fluorescence probes^[6] that is chemically attached with the PCL or PTHF backbone. Then only the polymer exhibits the fluorescence activity. Based on the literature, one can come to the conclusion that without the conjugation of fluoroprobes or chromophoric groups, the simple polymer cannot exhibit fluorescence property. The emission spectrum recommends that P2 is a suitable candidate for the MRI or bio-imaging process because of its bio-compatibility, biodegradability and high FEI value.

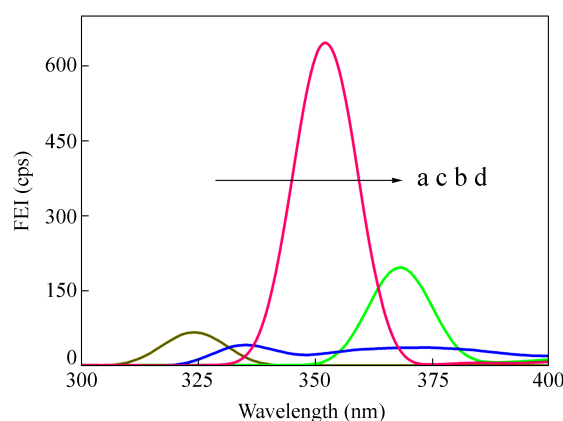


Fig. 4 Fluorescence spectra of (a) L-cysteine, (b) P1, (c) P2 and (d) P3

GPC Analysis

Polymer synthesized in the experimental part can be confirmed by GPC analysis (Fig. 5). The L-cysteine initiated ROP of CL yielded the M_w of 5.5×10^3 g/mol and M_n of 1.9×10^3 ^[40] with the polydispersity (PD) value of 2.8. The GPC results declared that the PCL synthesized by using L-cysteine as an initiator under nitrogen atmosphere for 2 h produced the oligomer at 160 °C. The PD value inferred that P1 (Fig. 5a) synthesized by using L-cysteine as a chemical initiator does not have a narrow molecular weight distribution. The GPC trace of diblock copolymer is given in Fig. 5(b). The appearance of a twin peak confirmed the presence of homo as well as block copolymer. During the diblock copolymerization of PCL in the presence of THF some of the PCL chains were extended by THF via PAH initiation. It is very difficult to isolate homo PCL from the diblock copolymer because both the polymer systems are freely soluble in CHCl_3 and THF. Moreover, the molecular weight difference between them is also very small. The first non shading peak corresponds to the homopolymer of PCL. The second peak corresponds to diblock copolymer with the M_w , M_n and PDI are determined as 5.7×10^3 g/mol, 3.5×10^3 g/mol and 1.62 respectively. After the diblock copolymerization, the polymer yielded a narrow molecular weight distribution. By the way of diblock copolymerization the following points were achieved. 1) By way of introducing the THF units to the PCL homopolymer the hydrophilicity was developed. 2) Both the M_w and M_n were increased and finally produced a polymer with narrow molecular weight. 3) As a result of diblock copolymerization between PCL and PTHF there is a chance for the increase of bio-compatibility, bio-degradability and high loading chance for drug carrier application. The GPC of P3 is mentioned in Fig. 5(c). The chromatogram exhibited three peaks corresponding to homo PCL, diblock copolymer and nano silver end capped diblock copolymer. During the *in situ* preparation of silver nanoparticles both the M_w and M_n weights were enormously increased. The M_w and M_n are 28.5×10^4 g/mol and 14.5×10^4 g/mol respectively. From the above M_w and M_n one can calculate the PDI value of 1.9. By the way of doing the *in situ* preparation of silver nanoparticles, some positive values were added. Both the M_w and M_n were abnormally increased from the oligomeric form of a diblock copolymer. The increase in molecular weight of diblock copolymer can be explained as follows: i) The crosslinking of silver nanoparticles with diblock copolymer, *i.e.* the surface of the silver nanoparticles were chemically binded by the diblock units. As a result, the chain length of the diblock copolymer was increased with the possible Ag nanoparticle as a crosslinking junction. ii) The peculiar behavior of nano material is the surface area to volume ratio. The zero valent Ag nanoparticles were activated on the sphere surface of the Ag nano particles. The specialty of Ag nanoparticle is the antimicrobial property. The antimicrobial property of P3 against e-coli is discussed in the forthcoming antimicrobial testing portion. By way of doing the Ag end capping of a diblock copolymer, one can get various advantages including the gain in molecular weight and the increase in hydrophilic nature that would be very much useful for the drug loading application. In general, the molecular weight of the biomedical polymer like PCL has the molecular weight of less than 10000 g/mol. The recent investigation on Near IR dye functionalized carbon nanotube initiated ROP of CL exhibited the M_w varied between 1.6×10^3 g/mol and 12.7×10^3 g/mol while varying the $[M/I]$ value between 10 and 400^[6]. In the present investigation also both P1 and P2 coincide with the literature report, but the synthesis of silver nanoparticles by *in situ* method led to the formation of very high molecular weight polymer. This is due to the surface catalytic effect of the nano material. The previous literature indicates the preparation of PVA/metal oxide or metal hydroxide nano composites. The added nano material accelerated the formation of keto group from the secondary alcoholic group^[40, 41]. In addition, they also influenced the formation of $\text{C}=\text{C}$ on the PVA backbone and the same was quantitatively estimated^[40, 41]. Surprisingly, the silver nano particles actively involved in the surface binding reaction and hence led to a very high molecular weight polymer. This proves that each nano material has its own surface catalytic effect and catalytic activity towards various chemical reactions. The present result declares that silver nano particle prepared by *in situ* method has the capability of surface binding reaction and led to a very high molecular weight polymer. The GPC results informed that one can synthesize high molecular weight polymer during the *in situ* synthesis with silver nano particle.

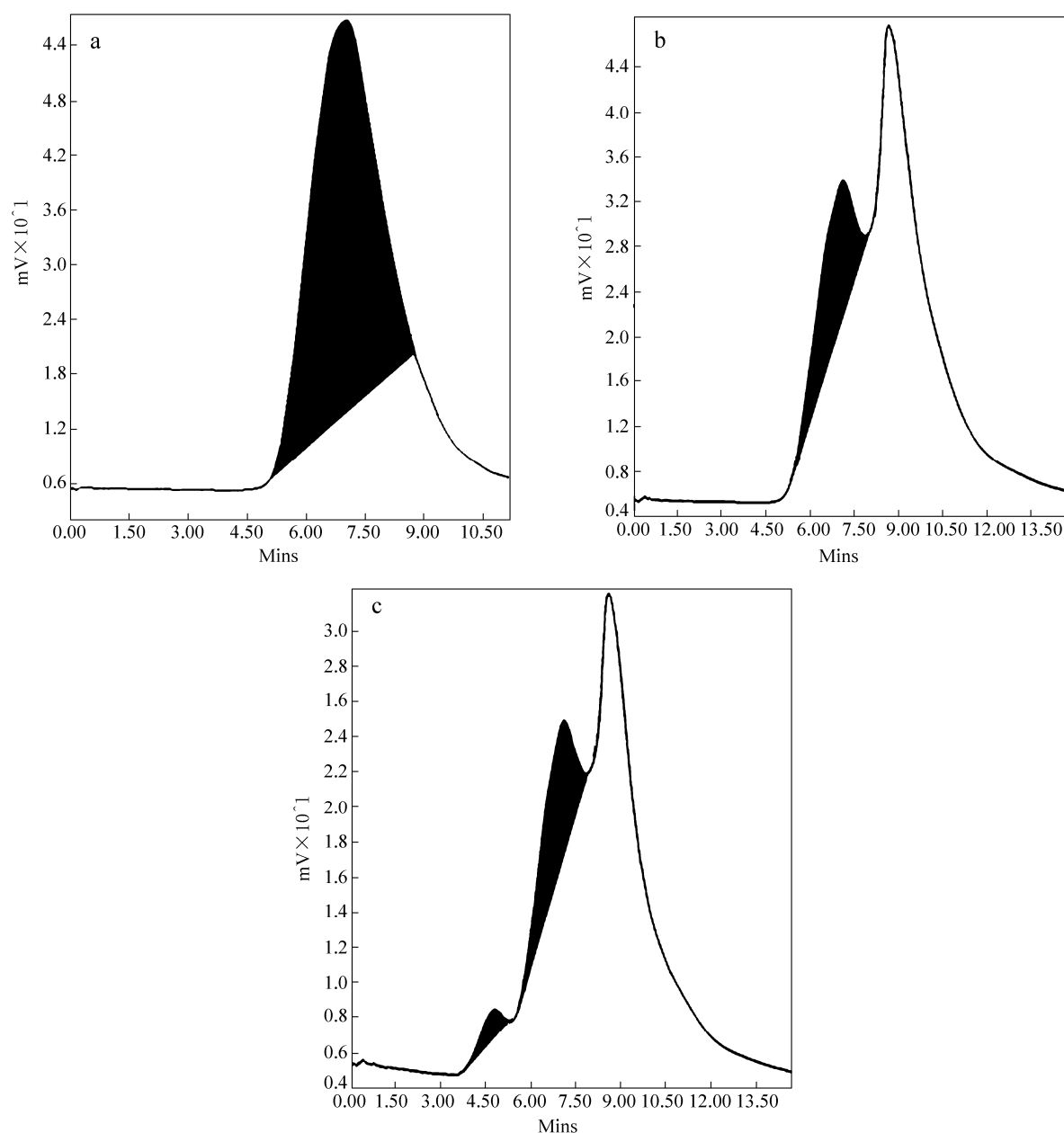


Fig. 5 GPC results of (a) P1, (b) P2 and (c) P3

FESEM

The surface morphology of L-cysteine initiated ROP of CL is shown in Fig. 6. The surface morphology of PCL contains some micro voids with dried sky like morphology (Fig. 6a). This is a peculiar surface morphology of PCL irrespective of initiators. The presence of micro voids (indicated by arrow mark) on the surface of PCL is very much useful in the drug delivery application. During the drug loading process the drug which can simply inserted in the voids without any physical or chemical interaction with the backbone polymer. While changing the temperature or pressure or pH the drug was forced to release from the void. Moreover, the neutral ester group can also interact with the different functional groups of drug molecules through the secondary forces of attraction. These two combined factors accelerate the drug loading of PCL. Figure 6(b) indicates the surface morphology of diblock copolymer *i.e.* PCL-L-cysteine-PTHF. Here also one can see the micro voids with the

dried sky like morphology. Apart from these two, some nano sized particles (indicated by circle mark) are also seen. Appearance of such a nano particle is due to the interface region between the hydrophobic PCL and hydrophilic PTHF. Hence, the combination of hydrophilic and hydrophobic segments leads to the formation of nano particles *i.e.* polymer nano particles formation. The advantage of polymer nano particles formation is the uploading of more and more drugs on its surface. This is due to the increase in surface area to volume ratio. This polymer nano particle is more active than its bulk. Due to the high surface activity, the polymer nano particles can interact with the drug in a fast manner. In the present investigation, due to the presence of micro voids and polymer nano particles the drug loading efficiency might be increased. Above all, due to the presence of PTHF units, the aqueous solubility of the system was increased. In final, the drug loading or drug delivery activity in the present system would be raised. Figure 6(c) represents the surface morphology of P3 system. Now the surface morphology becomes entirely different from that of P1 and P2. Here one can see a nano particle, micro voids and some micro worms. The important point here is the dried sky like morphology of PCL changed into a worm like morphology with the length of 3–4 μm . The agglomerated silver nano particles will impart the antimicrobial property to the polymer system. As a result, one can get an antimicrobial polymer. The presence of micro voids, polymer nano particles, silver nano particles (antimicrobial property)^[42] and micro worms added more value to the material, particularly in the drug carrying activity. The Ag nano particle exhibits the surface catalytic effect due its nano size. Silver nano particles can also accelerate the drug carrying activity with improved antimicrobial property. Hence the present investigation yielded a new material for the drug carrying or delivery application.

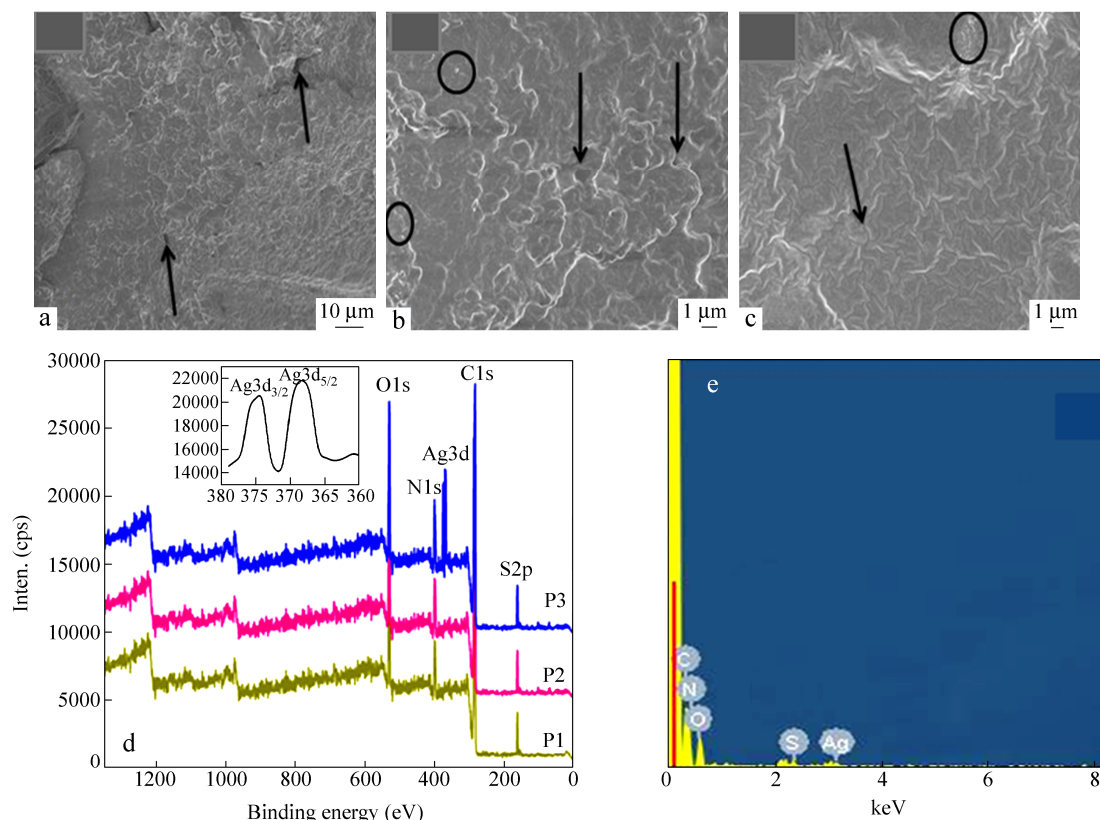


Fig. 6 FESEM images of (a) P1, (b) P2 and (c) P3; (d) XPS spectra of P1, P2 and P3; (e) EDX spectrum of P3

XPS Profile

The outermost energy level of an element can be determined by XPS. Figure 6(d) indicates the XPS spectra of P1, P2 and P3. The C1S, N1S, O1S and S2P levels of L-cysteine conjugated PCL are observed at 283.1, 397.6,

530.1 and 162.2 eV respectively. The XPS of PCL-L-cysteine-PTHF system shows the C1S, N1S, O1S and S2P at the same energy levels. The binding energy level of N1S, O1S, S2P and C1S are in accordance with the already reported value^[6]. Here the important point is the intensity of C1S and O1S increased due to the block copolymerization of THF units. As a result of increase in both carbon and oxygen content the intensity of respective peaks was increased. For the XPS of PCL-L-cysteine-PTHF-Ag system again one can note the C1S, N1S, O1S and S2P levels. In addition to these, a new peak is observed corresponding to the Ag3d energy level^[43]. On enlarging the 3d energy level peak (attached as an insert) it exhibits two peaks at 368.8 eV due to the Ag3d_{5/2} and the second peak at 374.1 eV is responsible for the Ag3d_{3/2}. The appearance of Ag3d_{5/2} peak at 368.8 eV confirmed the formation of Ag(0). The Ag3d peak is derived from silver nano particles. Thus XPS proved that the Ag(+1) state of AgNO₃ was reduced by the NH₂/SH group of L-cysteine. The formation of silver nanoparticles can be confirmed by FESEM analysis. The GPC results declared that after the formation of silver nano particles the molecular weight of diblock copolymer is abnormally increased. This can be explained as follows: i) The reduction of Ag(+1) state to Ag(0) state was attained by the reduction reaction through the involvement of —SH or —OH or —NH₂ group. ii) The silver nano particles were formed by the polyol methodology. If reduction reaction occurred through the —SH or —NH₂ group during the washing or drying process the surface of the silver nanoparticles was detached from the —NH₂ or —SH group of L-cysteine. Moreover, this will not increase the molecular weight of the diblock copolymer. The other possibility is the polyol methodology, *i.e.* the —OH group of PCL or PTHF might be involved in the reduction reaction in an aqueous medium. The —OH end capped PCL or PTHF offered a chain extension reaction through the surface interaction with silver nano particle. As a result of chain extension reaction through surface binding with silver nano particle the molecular weight of the diblock copolymer was increased abnormally. The XPS also confirms the formation of silver nanoparticle.

EDAX

Figure 6(e) indicates the EDAX spectrum of P3. Peaks corresponding to C, N and O appeared below 1 keV. L-cysteine contains one thiol group which can interact with the silver nitrate solution to form silver nano particles. During this reaction the Ag⁺ ions were reduced to Ag⁰. The sulphur atom and silver nanoparticle appeared around 2.4 and 3.2 keV. The presence of S and N confirmed the L-cysteine initiated ROP of CL and THF. Hence the EDAX spectrum confirmed the contents of P3^[44]. The P3 gives the 1.02% content of Ag nanoparticle with 0.6% N, 0.7% S, 22.6% O and 75.08% C. This inferred that 1.02% of Ag nanoparticle was loaded with the diblock copolymer backbone.

HRTEM

Figure 7 indicates the formation of copolymer nano particles due to the conjugation of hydrophobic PCL and hydrophilic PTHF segments. Figure 7(a) confirms the copolymer nano particle with different shape and size varying between 80 and 120 nm. Figure 7(b) indicates the irregular shape of the copolymer nano particles. Figure 7(c) confirms the formation of copolymer nano particles in different shapes varying from nano sphere to nano square. Figures 7(d)–7(f) confirms the formation of silver nano particles with different shape and size. Figure 7(d) shows the particle size of 20–150 nm. Here one can see the nano sphere, distorted nano sphere and nano square forms of silver nano particles. Figure 7(e) confirms the unidirectional crystal growth of silver nano particles with different sizes and shapes^[45]. Figure 7(f) authenticates the SAED patterns and confirms the presence of 111 and 200 crystal planes and inferred the crystalline silver nano particles with FCC structure. The HRTEM study concludes that the size of the silver nano particle is very small with unidirectional crystal growth and confirms the FCC structure.

DSC Report

The phase transition in the diblock copolymer can be analyzed by DSC method (Fig. 8A). The L-Cys-PCL (P1) system exhibits one endothermic peak at 62.4 °C corresponding to the melt transition of PCL. The DSC thermogram of PCL-L-cysteine-PTHF (P2) system shows the T_m value of 60.86 °C. After the incorporation of THF units the hydrophilicity of the block copolymer was increased and hence decreased the T_m . After the

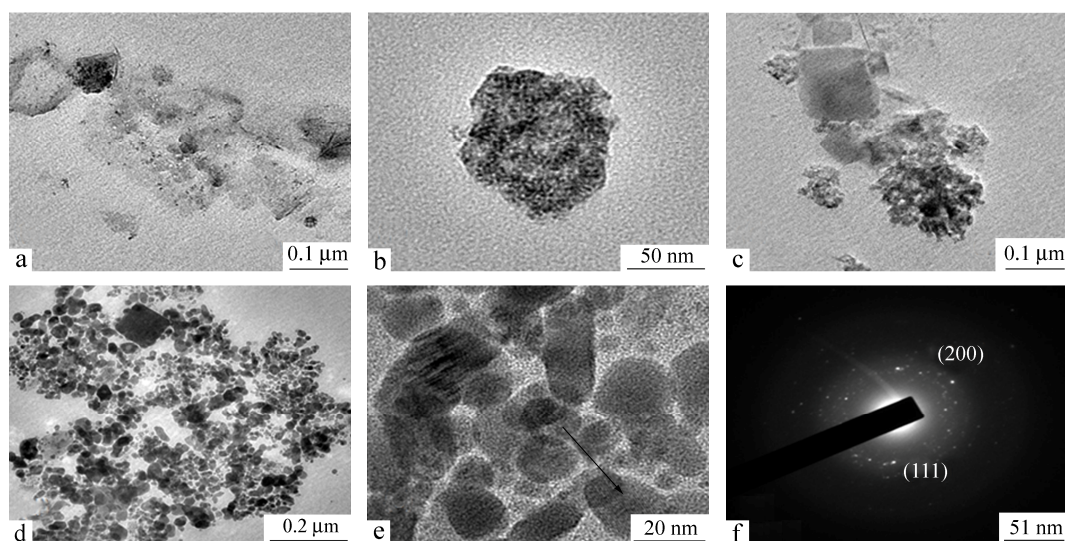


Fig. 7 HRTEM images of P2 (a, b, c) and P3 (d, e, f)

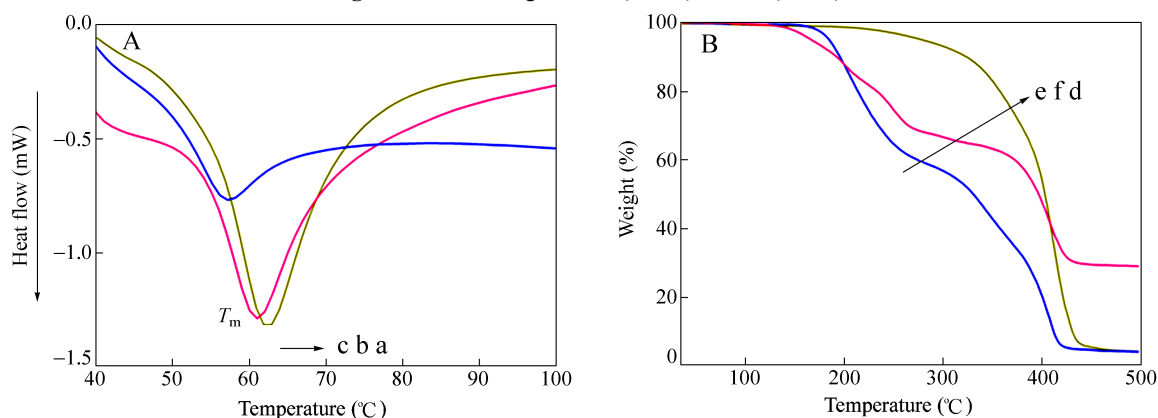


Fig. 8 (A) DSC curves of (a) P1, (b) P2 and (c) P3; (B) TGA curves of (d) P1, (e) P2 and (f) P3

diblock copolymer formation, the molecular weight of the system was increased. Even then the P2 system exhibits lower T_m due to the hydrophilicity. After the nano silver end capping process, the T_m of PCL-L-cysteine-PTHF-Ag (P3) system was still reduced to 57.1 °C. The important point here is that, after the nano silver end capping process, the melt transition peak was broadened. The broadening of the melt transition peak is due to the following reasons. 1) P2 on treatment with silver nitrate solution leads to the formation of silver nano particles at the same time the surface binding reaction occurs. Thus obtained surface binded P3 lead to the Ag NPs bridged cross linking system. 2) Due to the cross linking reaction, the molecular weight of the diblock copolymer was increased. 3) Due to the increase in the molecular weight of P3, the coil forms were increased. Due to the increase in coil structure the amorphous character is also linearly increased. 4) Due to the increase in surface area to volume ratio, the surface of the Ag nano particle was activated and simultaneously activated the neighboring —OH groups or —NH₂ or —SH groups. Due to these coupled effects of surface catalytic effect and cross linking, the molecular weight of the polymer was increased with the simultaneous increase of hydrophilicity, and hence the melting temperature of P2 was suppressed. The advantage of this methodology is the molecular weight of the polymer can be abnormally raised by the *in situ* Ag nano particle preparation. The T_m of P3 inferred that during the *in situ* preparation of Ag nano particles the T_m and the crystallinity were reduced whereas the hydrophilicity was raised. The recent literature^[46] indicates that the melting temperature of PCL varied between 40 and 60 °C while varying the [M/I] ratio. In the present investigation P1 and P2 are

matching with the literature value. Due to the presence of both hydrophobic and hydrophilic segments linked through the silver nanoparticles the system P3 behaved entirely different, the possible explanations are given above.

TGA History

The thermal stability of the polymers can be analyzed through TGA method (Fig. 8B). The thermogram of P1 exhibits a two step degradation process with the initial degradation temperature (T_{id}) of 203.7 °C. The first minor weight loss around 280 °C corresponds to the breaking of linkage between L-cysteine and PCL. The second major weight loss around 395 °C is ascribed to the breaking of ester linkage of PCL. After the complete degradation process the system exhibited 4.3% weight residue which remained above 450 °C. This is due to the formation of carbonaceous matter. The TGA thermogram of P2 exhibits a three step degradation process with the T_{id} of 163.2 °C due to the degradation of PTHF segments. The second major weight loss step around 356 °C is responsible for the degradation of PCL segments conjugated with the hydrophilic PTHF segments, *i.e.* the interface region between the hydrophobic and hydrophilic segments. The third minor weight loss is associated with the degradation of hydrophobic PCL chains present at the end of the diblock copolymer backbone. After the complete degradation the system exhibited 4.0% weight residue which remained above 450 °C. On comparison between P1 and P2 the following factors are tabulated. i) In the diblock copolymer composed of both hydrophilic and hydrophobic segments, the hydrophilic one undergoes rapid degradation process than the hydrophobic segments. Based on this concept the P2 shows lower thermal stability than the P1. ii) The T_{id} was also depressed. The TGA thermogram of P3 exhibits a two step degradation process. 135.5 °C was noted as a T_{id} for the P3 system. The first major weight loss around 220 °C is accounted by the degradation of hydrophilic THF units. The second major weight loss around 400 °C can be explained on the basis of PCL chain degradation process. The 29.2% residual weight remained above 450 °C was due to the presence of Ag nanoparticles and carbonaceous matters, *i.e.* polymer chains very nearer to the Ag nanoparticles. This indicates the increase in thermal stability of diblock copolymer. P3 on comparison with P2 exhibited somewhat good results. On overall comparison, the P3 exhibited the lowest T_{id} followed by the P2 and P1. The TGA thermograms of P1 and P2 are normal and matching with the literature. In the case of P3, the weight residue remained above 450 °C was increased. This is due to the loading of the silver nanoparticles. Our results can be compared with the literature values. The loading of nano sized Sb_2O_3 on the poly(aniline) copolymer^[47] increased the degradation temperature as well as the weight residue above 750 °C. Hence in the present investigation due to the loading of silver nanoparticles the weight residue above 450 °C was increased. The deviation of P3 from P2 is due to the increase in molecular weight of the polymer and the surface catalytic effect of Ag nano particles.

Zeta Potential

Zeta potential gives an idea about the surface charge of a material. The P1 exhibited two peaks (Fig. 9a) at +40 and -32 mV. This indicates that one end of the polymer chain was surrounded by positively charged species whereas the other end is equipped with negatively charged species. A material with both positive and negative surface charges can interact with any drug carrying positive or negative charges. The pH measured here is 6.8. Figure 9(b) indicates the zeta potential distribution of P2. A peak at +20 mV was observed for P2 which confirmed that during the diblock copolymerization there will be a formation of tetrahydrofuronium cation. Again this reduces the pH to 5.5. This can be further confirmed by FTIR spectroscopy. After the diblock copolymerization, a drug with negative charge only can approach the P2. Moreover, due to the lower surface potential any material with negative charge can easily interact with the P2. Figure 9(c) reveals the zeta potential distribution of P3 with two peaks at +25 and -25 mV. After the nano silver end capping process again one can observe the surface charges with two ends. Again this increases the pH strongly. Generally, a polymer with (± 30) mV is a right choice for the drug carrier application. Hence the zeta potential measurement recommends our nano silver end capped diblock copolymer which is a right choice for the drug carrying or drug delivery application. The zeta potential of chitosan grafted PCL and PCL nanofibrous scaffolds were reported from -30 mV to +50 mV. The present systems are also exhibiting the zeta potential values between +40 mV to -25 mV. This surface charge recommended that any drug can be loaded on the P1 or P2 or P3 systems^[48].

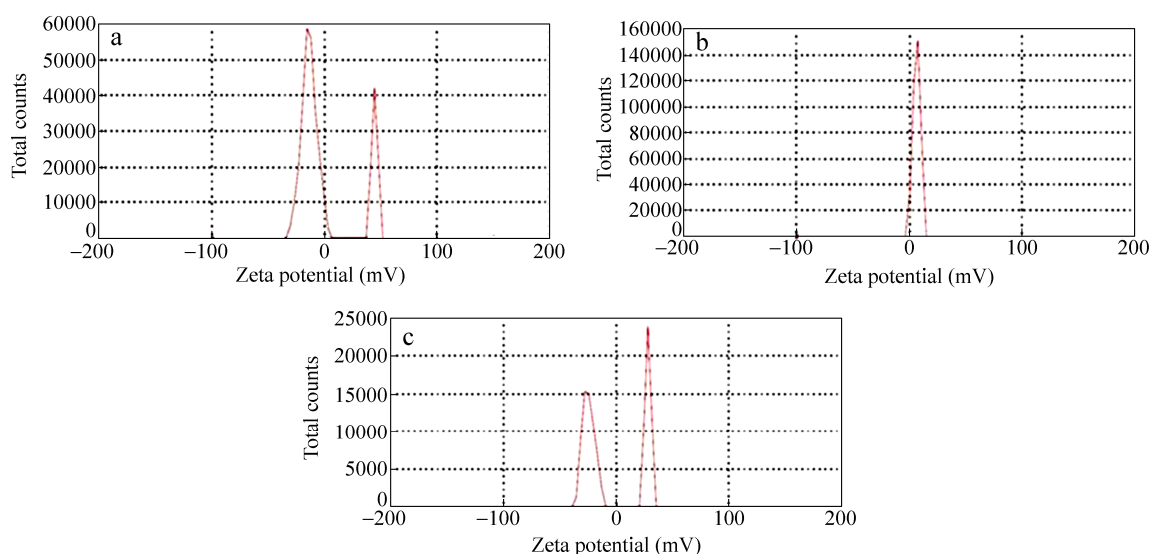


Fig. 9 Zeta potential of (a) P1, (b) P2 and (c) P3

Antimicrobial Property

The antimicrobial property of P2 is shown in Fig. 10(a) at different time intervals against e-coli. While increasing the incubation time the antimicrobial property of the system was increased due to the presence of tetrahydrofuronium cations. After 96 h of incubation, the materials were contaminated and hence suppression of antimicrobial property. Figure 10(b) indicates the antimicrobial activity of P3 at different time intervals. Here one can note that the diameter of the zone, antimicrobial property was increased with increase of incubation time. The antimicrobial property is associated with the activity of silver nano particles as well as the tetrahydrofuronium cations. The antimicrobial activity study confirmed the presence of antimicrobial property in P2 and P3. Due to the presence of silver nanoparticle, the antimicrobial property was imparted in the polymer systems^[49, 50].

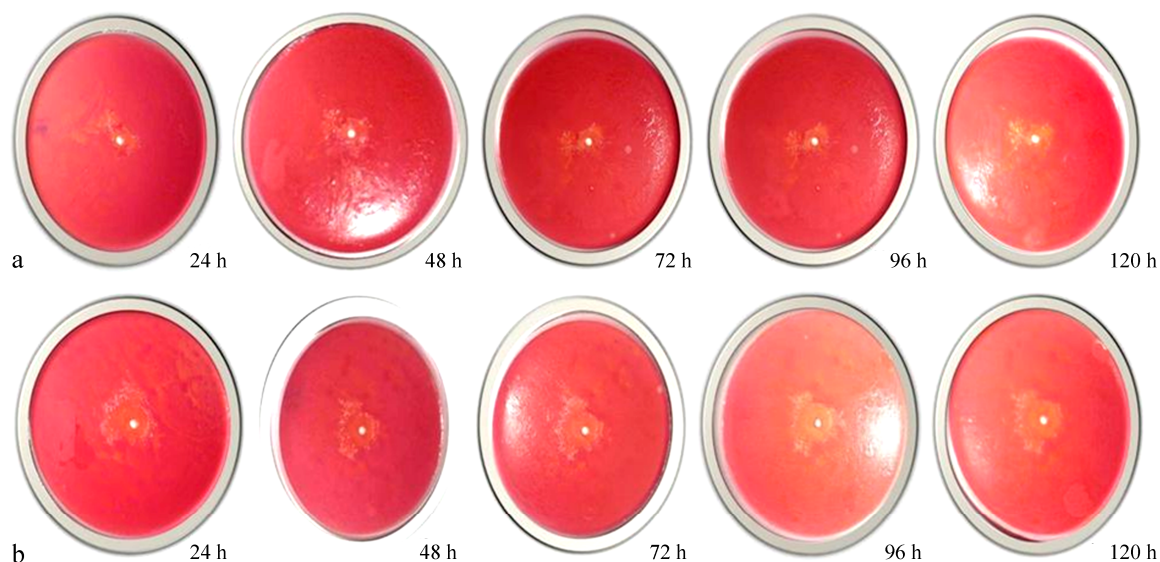


Fig. 10 Antimicrobial activity of (a) P2 and (b) P3 at different time intervals

CONCLUSIONS

From the above study the important points are presented here as conclusions. The FTIR spectra confirmed the functional groups present in the homopolymer and diblock copolymer. The ^{13}C -NMR spectrum confirmed the chemical structure of the polymer (a carbonyl carbon signal at $\delta = 173$). The L-cysteine functionalized PCL exhibited the highest T_m due to the hydrophobic nature. The TGA results declared that the weight residue remained above 450 °C is highest for the nano silver end capped diblock copolymer due to the encapsulation effect. The XPS results confirmed the presence of Ag $3d_{5/2}$ and Ag $3d_{3/2}$ energy levels. The CD spectral data confirmed the disappearance of an intramolecular hydrogen bonding after the nano silver end capping process. The nano silver end capped diblock copolymer exhibited the highest fluorescence emission intensity. The EDAX result confirmed the presence of 1.02% nanosized Ag loading. The GPC results exhibited the highest M_w for the nanosized Ag end capped diblock copolymer. The HRTEM report of P2 confirmed the formation of copolymer nanoparticle with different sizes and shapes. P3 exhibited the unidirectional crystal growth with different sizes and shapes. The nano silver end capped diblock copolymer exhibited the better antimicrobial property against e-coli.

ACKNOWLEDGEMENTS Mrs.G.Vijayalakshmi, Assistant Professor, Department of English is gratefully acknowledged for her valuable help during this manuscript preparation work.

REFERENCES

- 1 Liu, L.J., Cai, S.J., Tan, Y., Du, J.J., Dang, H.O., Wu, X.J., Wu, M.Y. and Liao, L.Q., J. Polym. Sci., Part A: Polym. Chem., 2009, 47: 6214
- 2 Oshimura, M. and Takasu, A., Macromolecules., 2010, 43: 2283
- 3 Li, J., Xie, W., Cheng, H.N., Nickol, R.G. and Wang, P.G., Macromolecules, 1999, 32: 2789
- 4 Ireland, B.J., Wheaton, C.A. and Hayes, P.G., Organomet., 2010, 29: 1079
- 5 Stevels, W.M., Ankone, M.J.K., Dijkstra, P.J. and Feijen, J., Macromolecules, 1996, 29: 8296
- 6 Meenarathi, B., Chen, H.H., Chen, P.H. and Anbarasan, R., J. Polym. Res., 2013, 20: 118
- 7 Kim, T.G. and Park, T.G., Biotechnol. Prog., 2006, 22: 1108
- 8 Jiang, G. and Xu, H., J. Appl. Polym. Sci., 2010, 118: 1372
- 9 Sugih, A.K., Picchioni, F., Janssen, M. and Heeres, H., J. Carbohydr. Polym., 2009, 77: 267
- 10 Riva, R., Lenoir, S., Jerome, R. and Lecomte, P.H., Polymer, 2005, 46: 8511
- 11 Sobczak, M. and Kolodziejewski, W., Molecules, 2009, 14: 621
- 12 Ji, S., Zhu, Z., Hoye, T.R. and Macosko, C.W., Macromol. Chem. Phys., 2009, 210: 823
- 13 Zhao, Y., Liang, H.J., Wang, S.G. and Wu, C.J., Phys. Chem. B, 2001, 105: 848
- 14 Zhang, Y.L., Dou, X.W. and Jin, T., Exp. Polym. Lett., 2010, 4: 599
- 15 Lee, S.C., Kang, S.W., Kim, C., Kwon, I.C. and Jeong, S.Y., Polymer, 2000, 41: 7091
- 16 Aouissi, A., Deyab, S.S.A. and Shahri, H.A., Molecules, 2010, 15: 1398
- 17 Aouissi, A., Deyab, S.S.A. and Shehri, H.A., Chinese J. Polym. Sci., 2010, 28(3): 305
- 18 Chiper, M., Hoogenboom, R. and Schubert, U.S., Eur. Polym. J., 2010, 46: 260
- 19 Kang, Y., Lee, M.H. and Rhee, S.B., Synth. Met., 1992, 47: 157
- 20 Filimoshkin, A.G., Kuchevskaya, A.S., Berezina, E.M. and Ogorodnikov, V.D., Exp. Polym. Lett., 2009, 3: 13
- 21 Jayabalan, M., Int. J. Biomater., 2009, 486710: 1
- 22 Wang, Y., Liu, X.Q., Sun, T.M., Xiong, M.H. and Wang, J.J., Control. Rel., 2008, 128: 32
- 23 Nomura, R. and Endo, T., Polym. Bull., 1995, 35: 683
- 24 Belbachir, M., Bouchama, A. and Ferrahi, M.I., Chem. Sci. J., 2012, 40: 1
- 25 Nomura, R. and Endo, T., Macromolecules, 1995, 28: 5372

- 26 Wing, C.G., Hernandez, R.P., Galicia, G.M., Sanchez, G.V., Alatorre, J.A. and Anaya, D.M., *Solid State Sci.*, 2009, 11: 1722
- 27 Da Silva Paula, M., Da Costa, C.S., Baldin, M.C., Scaini, G., Rezin, G.T., Segala, K., De Andrade, V.M., Franco, C.V. and Streck, E.L., *J. Braz. Chem. Soc.*, 2009, 20: 1556
- 28 Panacek, A., Kolar, M., Vecerova, R., Pucek, R., Soukupova, J., Krystof, V., Hamal, P., Zboril, R. and Kvitek, L., *Biomaterials*, 2009, 30: 6333
- 29 Bothun, G.D., *J. Nanobiotech.*, 2008, 6: 1
- 30 Bala, M. and Arya, V., *Int. J. Nanomater. Biostruc.*, 2013, 3: 37
- 31 Liang, H., Li, Z., Wang, W., Wu, Y. and Xu, H., *Adv. Mater.*, 2009, 21: 1
- 32 Shan, W.Q., Jian, J., Hong, F.J. and Cong, S.J., *Sci. China Ser. B: Chem.*, 2007, 50: 418
- 33 Song, F., Briseno, A.L. and Zhou, F., *Langmuir*, 2001, 17: 4081
- 34 Ramachandran, S., Bhadresh, R., Rami, R. and Udgaonkar, J.B., *J. Mol. Biol.*, 2000, 297: 733
- 35 Li, T., Park, H.G., Lee, H.S. and Choi, S.H., *Nanotechnology*, 2004, 15: S660
- 36 Kelly, S.M. and Price, N.C., *Curr. Protein Pep. Sci.*, 2000, 1: 349
- 37 Mc Mahon, H.A., Alfieri, K.N., Clark, K.A.A. and Londergan, C.H., *J. Phys. Chem. Lett.*, 2010, 1: 850
- 38 Chen, H.H., Anbarasan, R., Kuo, L.S. and Chen, P.H., *Mater. Chem. Phys.*, 2011, 126: 584
- 39 Zapun, A., Bardwell, A. and Creighton, T.E., *Biochem.*, 1993, 32: 5083
- 40 Gandhi, S., Harihara subramani, R., Ramakrishnan, T., Sivalabalan, A., Dhanalakshmi, V. and Anbarasan, R., *J. Mater. Sci.*, 2010, 45: 1688
- 41 Fathima Parveen, M., Umapathy, S., Dhanalakshmi, V. and Anbarasan, R., *J. Mater. Sci.*, 2009, 44: 5852
- 42 Arasu, V.T., Prabhu, D. and Soniya, M., *J. Biosci. Res.*, 2010, 1: 259
- 43 Tamata, K. and Park, C., *Chem. Mater.*, 2009, 21: 4248
- 44 Aguzzi, C., Sandri, G., Bonferoni, C., Cerezo, P., Rossi, S., Ferrari, F., Caramella, C. and Viseras, C., *Colloids Sur. B: Biointer.*, 2014, 113: 152
- 45 Shrivastava, S., Bera, T., Singh, S.K., Singh, G., Ramachandrarao, P. and Dash, D., *ACS Nano*, 2009, 3: 1357
- 46 Chen, H.H., Anbarasan, R., Kuo, L.S. and Chen, P.H., *J. Mater. Sci.*, 2011, 46: 1796
- 47 Jeyakumari, J.L., Yelilarasi, A., Dhanalakshmi, V. and Anbarasan, R., *Micro Nano Lett.*, 2010, 5: 241
- 48 Chen, H., Fan, X., Xia, J., Chen, P., Zhou, X., Huang, J., Yu, J. and Gu, P., *Int. J. Nanomedicine*, 2011, 6: 453
- 49 Forough, M. and Farhadi, K., *Turkish J. Eng. Env. Sci.*, 2010, 34: 281
- 50 Singh, P. and Raja, R.B., *Asian J. Exp. Biol. Sci.*, 2011, 2: 600