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Synthesis and Characterization of Novel Amphiphilic Polymers as Drug Delivery Nano Carriers

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Polymer nano-particles have been widely investigated in the last decade due to a variety of potential applications. In particular, polymers which can self assemble into micellar nano-particles can be effectively used as vehicles for drug delivery. Considerable efforts are underway to develop better drug delivery nano carriers for high drug loading capacity for a wide variety of bioactive compounds. In this study, several new polymers were synthesized in bulk (solventless condition) by a chemo-enzymatic methodology using *Candida antarctica* lipase B (Novozyme 435) and molecular sieves (MS). The synthesized polymers demonstrated high drug loading capacity and the potential to encapsulate drugs which are poorly soluble in aqueous solvents.

Keywords: Encapsulation, self-assembly, amphiphilic polymer, nano-particles, drug delivery, chemo-enzymatic, Novozyme-435, *Candida antarctica* Lipase B, static light scattering, micelles

1. Introduction

Nanotechnology is one of the major emerging fields of science that promises to affect considerable changes in the way we view future health care. Improving the therapeutic index (1) of drugs is a major challenge for discovery in many therapeutic areas such as cancer, inflammatory and infectious diseases. The search for new drug-delivery approaches and new modes of action are the major driving forces in polymer therapeutics (2–5). A number of macromolecular delivery systems are under investigation to improve the efficacy of prospective drugs. Generally, these can be classified as nanoparticulate drug-delivery systems or as drug–polymer conjugates. Particulate delivery systems in which the drugs are physically incorporated into nanoparticles include emulsions, liposomes, and non-covalent polymeric carrier systems. However, in drug–polymer conjugates, a drug is covalently linked to polymers such as proteins, polysaccharides, or synthetic polymers. Herein, we have developed a flexible chemo-enzymatic method for the synthesis of amphiphilic pegylated copolymers (6–8). The main advantage of the synthetic method lies in the selectivity of the enzyme that leaves a functional group on the polymer chain. These functional groups can be explored for further modifications in the polymeric material. *Candida antarctica* B lipase has several advantages in organic synthesis such as superior catalytic power and selectivity under mild conditions with regard to temperature, pressure, and pH. Additional advantages include the catalyst's recyclability and use in bulk reaction media to avoid organic solvents. Herein, we report the condensation copolymerization of polyethylene glycol with dimethyl 5-hydroxy/amino isophthalate (**1a-b**) in bulk using *Candida antarctica* lipase B (CAL) (Scheme 1). These polymers were converted to amphiphilic analogues by tethering an alkyl chain to it through simple alkylation or acylation depending upon the linker used in the polymerization reaction. These amphiphilic polymers self assemble in aqueous solution to form nano-spheres. Aspirin was used as an active drug to measure encapsulation capacity of these nano-spheres. The nano-spheres were found to carry sufficient amount of drug. It is concluded that polymers with amidic alkyl chain can carry more aspirin than the polymers with the ether alkyl chain. *In vitro* studies have shown that these nano-carriers carry their loads into cells quite effectively. Additionally, *in vivo* studies have depicted the loaded nano-spheres to possess superior transdermal properties (8, 9).

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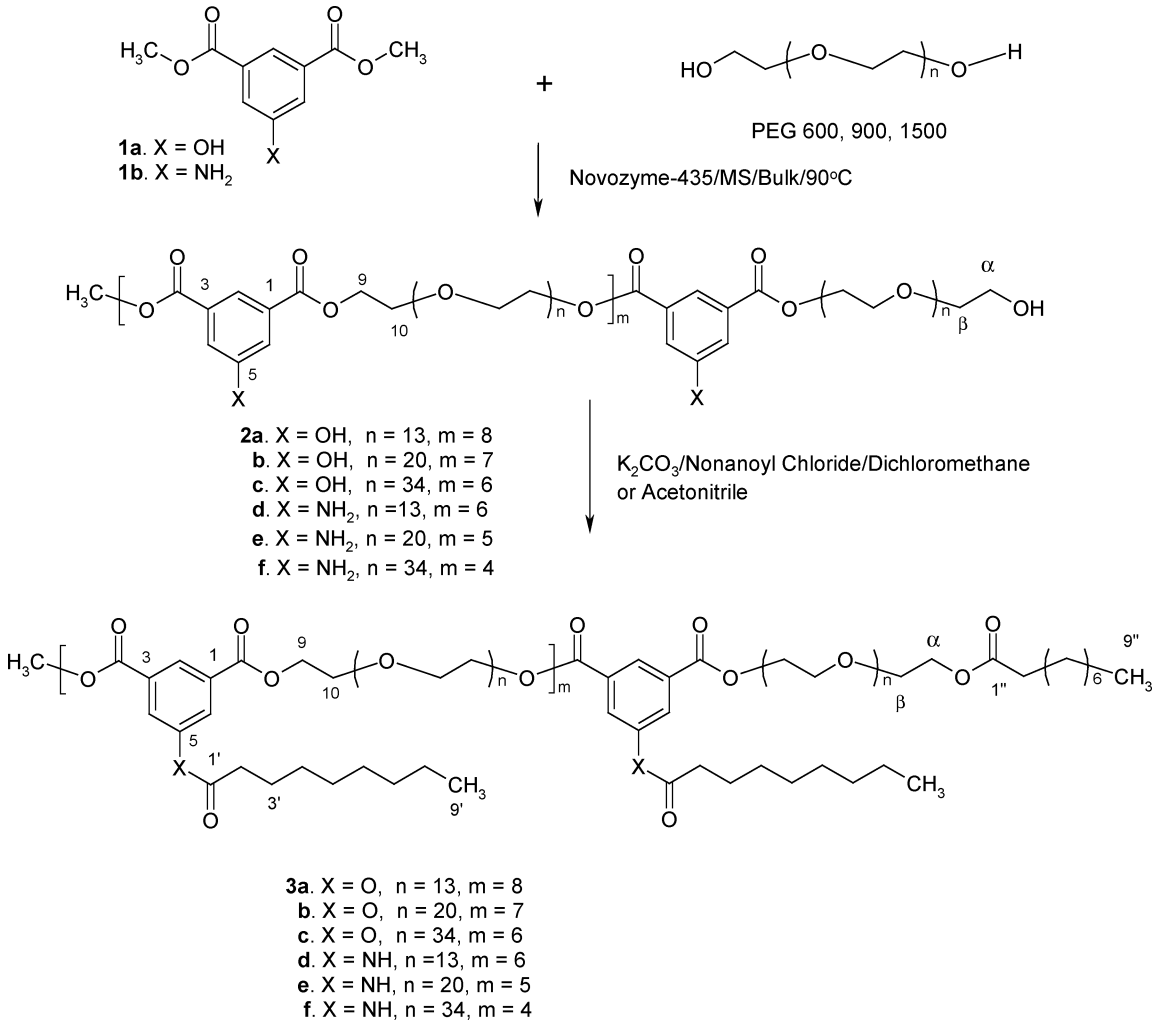

Sch. 1. Synthesis of amphiphilic pegylated copolymers (3a–f).

2. Experimental

2.1. *Materials*

Dimethyl 5-hydroxyisophthalate, dimethyl 5- aminoisophthalate, nonanoyl chloride, anhydrous potassium carbonate, acetonitrile, dichloromethane, molecular sieves (4Å, beads, 8–12 mesh) and polyethylene glycols (PEG 600, 900, 1500) were purchased from Aldrich (Milwaukee, WI). Novozyme 435, an immobilized enzyme, was a gift from the Novozymes, Inc., Denmark. Anhydrous potassium carbonate was fused overnight at 200°C before use, whereas polyethylene glycols were dried under vacuum at 60°C for 3 h prior to their use. Molecular sieves were washed with anhydrous acetone and activated at 200°C for 24 h and then cooled to room temperature under vacuum before use. All other chemicals and solvents were of analytical grade and used without further purification. Dialysis membranes of different molecular weight cut-offs were purchased from Spectrum Laboratories, Inc., CA.

2.2. *Characterization*

Gel permeation chromatography (GPC) was used to determine the molecular weight and molecular weight distribution, Mw/Mn of polymers using THF as a solvent and polystyrene as a standard. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DPX 500 spectrometer operating at 500 and 125 MHz, respectively, using TMS as an internal standard. Infrared spectra were recorded as neat samples on a Nicolet 4700 Fourier transform infrared (FT-IR) spectrometer by Thermo Electron Corporation. UV-visible spectra were recorded on a Agilent 8453 spectrophotometer. Static light scattering data were collected on a laser light scattering photometer (Wyatt Technology DAWN Model F) equipped with a 632 nm He-Ne laser as the light source.

2.3. *General procedure for polymer synthesis*

Dimethyl 5-hydroxyisophthalate/Dimethyl 5- aminoisophthalate (**1a–b**, 1.0 mmol) and PEG 600, 900 or 1500

(1.0 mmol) were placed in a round-bottom flask and stirred until homogeneous. This was followed by the addition of enzyme (Novozyme 435, 10% by weight *wrt* monomers) and 4Å molecular sieves (20% by weight *wrt* monomers) into the homogeneous mixture. The resultant reaction mixture was stirred at 90°C under vacuum (100 millitorr) for 48 h and quenched by adding chloroform. This was followed by removing the enzyme and molecular sieves by filtration. The obtained filtrate was concentrated to get the product. The product was redissolved in deionized water for dialysis using membrane (MWCO 6000). After the completion of dialysis, the product polymer **2a–f** was obtained as a semi-solid by freeze-drying. The spectroscopic characterization of polymers **2a–c** have already been reported (6).

2.4. *Poly(poly(oxyethylene-600)-oxy-5-aminoiso phthaloyl] (2d)*

Synthesis of poly[(poly (oxyethylene-600)-oxy-5-aminoisophthaloyl] (**2d**) was achieved by following the general procedure via reaction of dimethyl 5-amino isophthalate (5.00 g, 23.92 mmol), and polyethylene glycol (PEG-600) (14.28 g, 23.80 mmol), using molecular sieves (3.84 g) and Novozyme-435 (1.92 g).

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 3.58–3.63 (brs, 52H, methylene PEG protons), 3.77 (t, 4H, *H*-10), 3.85 (s, 3H, OCH_3 end group), 4.17 (brs, 2H, NH_2, D_2O exchangable), 4.40 (t, 4H, *H*-9), 7.46 (brs, 2H, *H*-4 and *H*−6), 7.96 (s, 1H, *H*-2)

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 52.90 (−OCH_3 end group), 62.01 (CH_2OH PEG end group), 64.57 (OCH_2), 69.46 (OCH_2), 70.89–71.13 (methylene carbons of PEG main chain), 72.91 (CH_2CH_2OH, PEG end group), 120.05 (CH×2), 120.55 (CH), 131.66 (q×2), 147.79 (q), 166.38 (> CO), 166.81 (−COOMe end group).

IR ν max: 3357, 2866, 1716, 1630**,** 1603, 1452, 1345, 1227, 1090, 1034, 997, 947, 845, 758, 722, 674, 545 cm^{-1}

UV λ_{max}**(MeOH)**: 343, 268 nm.

$\mathbf{M}_n$(GPC) 5740 Da, PD = 1.4, isolated yield 50%

2.5. *Pol [(poly (oxyethylene-900)-oxy-5-aminoiso phthaloyl] (2e)*

Synthesis of poly[(poly (oxyethylene-900)-oxy-5-aminoisophthaloyl] (**2e**) was achieved via reaction of dimethyl 5-amino isophthalate (5.00 g, 23.92 mmol) with polyethylene glycol (PEG-900) (21.42 g, 23.80 mmol) in the presence of molecular sieves (5.28 g) and Novozyme-435 (2.64 g).

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 3.58–3.70 (brs, 80H, methylene PEG protons), 3.84 (t, 4H, *H*-10), 3.91 (s, 3H, OCH_3 end group), 4.17 (brs, 2H, NH_2, D_2O exchangable), 4.41 (t, 4H, *H*-9), 7.51 (brs, 2H, *H*-4 and *H*−6), 8.04 (s, 1H, *H*-2).

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 52.90 (−OCH_3 end group), 62.01 (CH_2OH PEG end group), 64.62 (OCH_2), 69.52 (OCH_2), 70.72–71.10 (methylene carbons of PEG main chain), 72.91 (CH_2CH_2OH, PEG end group), 120.12 (CH×2), 120.78 (CH), 131.78 (q×2), 147.64 (q), 166.39 (> CO), 166.81 (−COOMe end group).

IR ν max: 3357, 2879, 1716, 1630**,** 1603, 1465, 1342, 1279, 1231, 1097, 961, 841, 758, 722, 647, 527 cm^{-1}

UV λ_{max}**(MeOH)**: 345, 270 nm

$\mathbf{M}_n$ (GPC) 6720 Da, PD = 1.4, isolated yield 50%

2.6. *Poly[(poly (oxyethylene-1500)-oxy-5-aminoiso phthaloyl] (2f)*

Synthesis of poly [(poly (oxyethylene-1500)-oxy-5-aminoisophthaloyl] (**2f**) was achieved via reaction of dimethyl 5-aminoisophthalate (5.00 g, 23.92 mmol), and polyethylene glycol (PEG-1500) (35.71 g, 23.80 mmol) in the presence of molecular sieves (9.42 g) and Novozyme-435 (4.71 g).

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 3.54–3.67 (brs, 136H, methylene PEG protons), 3.78 (t, 4H, *H*-10), 3.86 (s, 3H, OCH_3 end group), 4.21 (brs, 2H, NH_2, D_2O exchangable), 4.42 (t, 4H, *H*-9), 7.46 (brs, 2H, *H*-4 and *H*−6), 7.98 (s, 1H, *H*-2).

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 52.59 (−OCH_3 end group), 62.01 (CH_2OH PEG end group), 64.57 (OCH_2), 69.83 (OCH_2), 70.69–71.06 (methylene carbons of PEG main chain), 72.48 (CH_2CH_2OH, PEG end group), 120.02 (CH×2), 120.58 (CH), 131.69 (q×2), 147.77 (q), 166.36 (> CO), 166.90 (−COOMe end group).

IR ν max: 3356, 2881, 1717, 1630**,** 1604, 1466, 1359, 1341, 1278, 1234, 1145, 1096, 1059, 960, 840, 758, 722, 528 cm^{-1}

UV λ_{max}**(MeOH)**: 344, 268 nm

$\mathbf{M}_n$ (GPC) 8600 Da, PD = 1.4, isolated yield 50%

2.7. *General Procedure for Acylation of Polymers*

Polymer **2a–c** (**1.0 mmol**) was dissolved in anhydrous acetonitrile followed by the addition of anhydrous potassium carbonate (**3.0 mmol**) and nonanoyl chloride (**1.2 mmol**) under nitrogen. The reaction mixture was refluxed and progress of the reaction monitored by TLC using ethyl acetate in petroleum ether (30%). Polymer **2d–f** (**1.0 mmol**) was dissolved in anhydrous dichloromethane followed by the addition of anhydrous potassium carbonate (**3.0 mmol**) and nonanoyl chloride (**1.2 mmol**) under nitrogen. The reaction mixture was stirred at room temperature for 6 h. After completion, salt was removed by filtration and the solvent removed under vacuum to give the products **3a–f**.

2.8. *Poly[(polyoxyethylene-600)-oxy-5-nonanoyloxyiso phthaloyl] (3a)*

Synthesis of poly [(polyoxyethylene-600)-oxy-5- nonanoyloxyisophthaloyl] (**3a**) was achieved via refluxing of poly[(polyoxyethylene-600)-oxy-5-hydroxyisophthaloyl] (**2a**) (1.0 g, 1.21 mmol), anhydrous potassium carbonate

(0.50 g, 3.65 mmol) and nonanoyl chloride (0.258 g, 1.46 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.84–0.88 (t, 3H, *H*-9′ & 3H, *H*-9″ of end group), 1.24–1.42 [brs, 10H, (CH_2×5), *H*-4′-8′ and 10H, (CH_2×5), *H*-4″-8″] 1.60 (m, 2H, *H*-3″), 1.77 (m, 2H, *H*-3′), 2.30 (t, 2H, *H*-2″), 2.57(t, 2H, *H*-2′), 3.57–3.67 (brs, 52 H, methylene protons of PEG main chain), 3.83 (t, 4H, *H*-10), 3.92 (s, 3H, OCH_3 end group), 4.20 (t, 2H, $H-\alpha$), 4.48 (t, 4H, *H*-9), 7.93 (brs, 2H, *H*-4 and *H*−6), 8.55 (s, 1H, *H*-2).

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 14.49 (*C*H_3), 22.98 (*C*H_2), 25.26 (*C*H_2), 29.44 (*C*H_2 × 2), 29.54 (*C*H_2), 32.15 (*C*H_2), 34.58 (*C*H_2), 64.98 (O*C*H_2), 69.56 (O*C*H_2), 70.50–71.17 (methylene carbons of PEG main chain), 127.67 (*C*H × 2), 128.45 (*C*H), 132.35 (q × 2), 151.15 (q), 165.21 (-*C*OO), 172.13 (*C*O).

IR νmax: 2870, 1770, 1716, 1604, 1457, 1361, 1313, 1236, 1145, 1099, 1037, 908, 752, 669, 647, 617, 529 cm^{-1}

UV λ_{max}(MeOH): 293, 286 nm

2.9. *Poly[(polyoxyethylene-900)-oxy-5-nonanoyloxyisophthaloyl] (3b)*

Synthesis of poly [(polyoxyethylene-900)-oxy-5- nonanoyloxyisophthaloyl] (**3b**) was achieved via refluxing of poly [(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2b**) (1.0g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67mmol) and nonanoyl chloride (0.189 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.86–0.90 (t, 3H, *H*-9′ and 3H, *H*-9″ of end group), 1.29–1.43 [brs, 10H, (CH_2× 5), *H*-4′-8′ and 10H, (CH_2× 5), *H*-4″-8″], 1.60 (m, 2H, *H*-3″), 1.77 (m, 2H, *H*-3′) 2.32 (t, 2H, *H*-2″), 2.59 (t, 2H, *H*-2′), 3.59–3.68 (brs, 80 H, methylene protons of PEG main chain), 3.84 (t, 4H, *H*-10), 3.95 (s, 3H, OCH_3 end group), 4.22 (t, 2H, $H-\alpha$), 4.50 (t, 4H, *H*-9), 7.94 (brs, 2H, *H*-4 and *H*−6), 8.56 (s, 1H, *H*-2).

^{13}C-NMR (δc CDCl$_3$, 125 MHz) : 14.43 (*C*H_3), 22.98 (*C*H_2), 25.16 (*C*H_2), 29.45 (*C*H_2), 29.50 (*C*H_2), 29.64 (*C*H_2), 32.15 (*C*H_2), 34.59 (*C*H_2), 64.98 (O*C*H_2), 69.41 (O*C*H_2), 70.80–71.15 (methylene carbons of PEG main chain), 127.72 (*C*H×2), 128.48 (*C*H), 132.34 (q×2), 151.13 (q), 165.22 (-*C*OO), 172.12 (*C*O).

IR νmax: 2862, 1765, 1724, 1595, 1455, 1348, 1313, 1234, 1145, 1096, 1034, 947, 861, 757, 512 cm^{-1}

UV λ_{max}(MeOH): 293, 285 nm

2.10. *Poly[(polyoxyethylene-1500)-oxy-5-nonanoyloxyisophthaloyl] (3c)*

Synthesis of poly[(polyoxyethylene-1500)-oxy-5- nonanoyloxyisophthaloyl] (**3c**) was achieved via refluxing of poly[(polyoxyethylene-1500)-oxy-5-hydroxyisophthaloyl] (**2c**) (1.0 g, 0.581 mmol), anhydrous potassium carbonate (0.240 g, 1.74 mmol) and nonanoyl chloride (0.123 g, 0.69 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.84–0.88 (t, 3H, *H*-9′ and 3H, *H*-9″ of end group), 1.25–1.42 [brs, 10H, (CH_2× 5), *H*-4′-8′ and 10H, (CH_2× 5), *H*-4″-8″], 1.60 (m, 2H, *H*-3″), 1.76 (m, 2H, *H*-3′), 2.32 (t, 2H, *H*-2″), 2.59 (t, 2H, *H*-2′), 3.57–3.68 (brs, 136 H, methylene protons of PEG main chain), 3.83 (t, 4H, *H*-10), 3.92 (s, 3H, OCH_3 end group), 4.21 (t, 2H, $H-\alpha$), 4.49 (t, 4H, *H*-9), 7.93 (brs, 2H, *H*-4 and *H*−6), 8.55 (s, 1H, *H*-2).

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 14.51 (*C*H_3), 22.16 (*C*H_2), 25.27 (*C*H_2), 29.45 (*C*H_2x2), 29.55 (*C*H_2), 32.15 (*C*H_2), 34.59 (*C*H_2), 64.98 (O*C*H_2), 69.56 (O*C*H_2), 70.29–71.36 (methylene carbons of PEG main chain), 127.74 (*C*H×2), 128.45 (*C*H), 132.32 (qx2), 151.10 (q), 165.23 (-*C*OO), 172.15 (*C*O).

IR νmax: 2882, 1765, 1725, 1595, 1465, 1359, 1342, 1315, 1279, 1238, 1145, 1100, 1060, 960, 841, 757, 722, 529 cm^{-1}

UV λ_{max}(MeOH):, 293, 286 nm

2.11. *Poly[(polyoxyethylene-600)-oxy-5-(nonanoylamino)isophthaloyl] (3d)*

Synthesis of poly[(polyoxyethylene-600)-oxy-5- (nonanoylamino) isophthaloyl] (**3d**) was achieved via stirring of poly[(poly (oxyethylene-600)-oxy-5-aminoisophthaloyl] (**2d**) (1.00 g, 1.218 mmol), with nonanoyl chloride (0.25 g, 1.46 mmol) and anhydrous potassium carbonate (0.504 g, 3.65 mmol) at room temperature.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.85–0.88 (t, 3H, *H*-9′ and 3H, *H*-9″ of end group), 1.25–1.33 [brs, 10H, (CH_2× 5), *H*-4′-8′ and 10H, (CH_2 × 5), *H*-4″-8″], 1.63 (m, 2H, *H*-3″), 1.72 (m, 2H, *H*-3′), 2.34 (t, 2H, *H*-2″), 2.42 (t, 2H, *H*-2′), 3.62–3.70 (brs, 52 H, methylene protons of PEG main chain), 3.83 (t, 4H, *H*-10), 3.92 (s, 3H, OCH_3 end group), 4.23 (t, 2H, $H-\alpha$), 4.48 (t, 4H, *H*-9), 8.37 (s, 1H, *H*-2), 8.51 (brs, 2H, *H*-4 and *H*−6), 8.63 (brs, 1H, N*H*, D_2O exchangable)

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 14.46 (*C*H_3), 23.01 (*C*H_2), 26.03 (*C*H_2), 29.55 (*C*H_2), 29.77 (*C*H_2), 29.83 (*C*H_2), 32.21 (*C*H_2), 37.65 (*C*H_2), 64.77 (O*C*H_2), 69.43 (O*C*H_2), 70.82–71.08 (methylene carbons of PEG main chain), 123.11 (*C*Hx2), 125.27 (*C*H), 132.46 (q × 2), 139.79 (q), 165.93 (-*C*OO), 172.76 (NH*C*O).

IR νmax: 2859, 1721, 1692, 1602, 1556, 1451, 1347, 1310, 1231, 1096, 1036, 949, 846, 758, 719, 512 cm^{-1}

UV λ_{max}(MeOH): 398, 356, 310 nm

2.12. *Poly[(polyoxyethylene-900)-oxy-5-(nonanoylamino) isophthaloyl] (3e)*

Synthesis of poly[(polyoxyethylene-900)-oxy-5- (nonanoylamino)isophthaloyl] (**3e**) was achieved via stirring of poly[(poly(oxyethylene-900)-oxy-5-aminoisophthaloyl] (**2e**) (1.0 g, 0.892 mmol) with nonanoyl chloride (0.189 g, 1.07 mmol) and anhydrous potassium carbonate (0.369 g, 2.67 mmol) at room temperature.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.88–0.90 (t, 3H, *H*-9′ and 3H, *H*-9″ of end group), 1.28–1.38 [brs, 10H, (C$H_2\times$ 5) *H*-4′-8′ and 10H, (C$H_2\times$ 5), *H*-4″-8″], 1.65 (m, 2H, *H*-3″), 1.73 (m, 2H, *H*-3′), 2.33 (t, 2H, *H*-2″), 2.42 (t, 2H, *H*-2′), 3.63–3.71 (brs, 80 H, methylene protons of PEG main chain), 3.84 (t, 4H, *H*-10), 3.93 (s, 3H, OCH_3 end group), 4.23 (t, 2H, $H-\alpha$), 4.49 (t, 4H, *H*-9), 8.40 (s, 1H, *H*-2), 8.50 (brs, 2H, *H*-4 and *H*−6), 8.63 (brs, 1H, N*H*,D_2O exchangable).

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 14.46 (*C*H$_3$), 23.01 (*C*H$_2$), 26.03 (*C*H$_2$), 29.54 (*C*H$_2$), 29.77 (*C*H$_2$), 29.83 (*C*H$_2$), 32.21 (*C*H$_2$), 37.68 (*C*H$_2$), 64.77 (O*C*H$_2$), 69.43 (O*C*H$_2$), 70.51–71.08 (methylene carbons of PEG main chain), 125.29 (*C*H×2), 126.06 (*C*H), 131.37 (q×2), 139.88 (q), 165.36 (-*C*OO), 172.82 (NH*C*O).

IR νmax: 2861, 1723, 1693, 1603, 1556, 1452, 1347, 1302, 1233, 1095, 1036, 948, 847, 759, 720, 509 cm^{-1}

UV λ_{max}(MeOH): 398, 356, 310 nm

2.13. *Poly[(polyoxyethylene-1500)-oxy-5-(nonanoylamino) isophthaloyl] 3f)*

Synthesis of poly[(polyoxyethylene-1500)-oxy-5-(nonanoylamino)isophthaloyl] (3f) was achieved via stirring of poly[(poly (oxyethylene-1500)-oxy-5-aminoisophthaloyl] (2f) (1.0 g, 0.5810 mmol), with nonanoyl chloride (0.123 g, 0.69 mmol) and anhydrous potassium carbonate (0.240 g, 1.74 mmol) at room temperature.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.86–0.89 (t, 3H, *H*-9′ and 3H, *H*-9″ of end group), 1.23–1.37 [brs, 10H, (CH_2 × 5) *H*-4′-8′ and 10H, (C$H_2\times$5), *H*-4″-8″], 1.62 (m, 2H, *H*-3″), 1.73 (m, 2H, *H*-3′), 2.33 (t, 2H, *H*-2″), 2.41(t, 2H, *H*-2′), 3.56–3.71 (brs, 136H, methylene protons of PEG main chain), 3.92 (t, 4H, *H*-10), 3.93 (s, 3H, OCH_3 end group), 4.22 (t, 2H, $H-\alpha$), 4.49 (t, 4H, *H*-9), 8.39 (brs, 1H, *H*-2), 8.50 (brs, 2H, *H*-4 and *H*−6), 8.63 (brs, 1H, N*H*,D_2O exchangable)

^{13}C-NMR (δc CDCl$_3$, 125 MHz): 14.42 (*C*H$_3$), 23.01 (*C*H$_2$), 25.79 (*C*H$_2$), 29.55 (*C*H$_2$), 29.77 (*C*H$_2$), 29.83 (*C*H$_2$), 32.21 (*C*H$_2$), 37.75 (*C*H$_2$), 64.80 (O*C*H$_2$), 69.45 (O*C*H$_2$), 70.70–71.60 (methylene carbons of PEG main chain), 125.16 (*C*H×2), 126.21 (*C*H), 131.51 (q×2), 139.60 (q), 165.86 (-*C*OO), 172.48 (NH*C*O).

IR νmax: 2883, 1725, 1693, 1556, 1465, 1359, 1341, 1279, 1239, 1146, 1103, 1060, 958, 841, 757, 723, 528 cm^{-1}

UV λ_{max}(MeOH): 392, 356, 310 nm

2.14. *Method for encapsulation of hydrophobic drugs*

The copolymers **3d–f** and the hydrophobic drug aspirin were dissolved in methanol to obtain 1:2 drug/polymer w/w ratios and stirred for 15 min. Organic solvent was removed under vacuum. The resulting viscous mixture of drug and polymer was dissolved in water with vigorous stirring to form nanoparticles. Non-incorporated aspirin was separated by filtration of the nanoparticle suspension through a 0.2 μm filter (aspirin crystals cannot pass through the filter unless the drug is solubilized by nanoparticles). The aspirin concentration in the filtrate was estimated by UV spectroscopy using a calibration curve for aspirin in methanol. The % encapsulation of aspirin was found to be in the range of 10–17% in ether chain polymers, whereas it was 21–26% in amidic chain polymers.

3. Results and discussion

The Novozyme-435 (immobilized *Candida antarctica* lipase B) catalyzed condensation of **1a-b** and PEG (M_n600, 900, 1500) under solventless conditions using molecular sieves gave the polymers **2a-f** in 50% isolated yield (Scheme 1)**.** The structures of the polymers **2a-f** were assigned using ^{1}H and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum of **2d-f** showed a triplet between δ 4.40–4.42 (4H, for *H*-9 protons) indicating the formation of ester bonds in the polymer. This was further confirmed by the disappearance of the signal due to the methoxyl protons of dimethyl 5-hydroxyisophthalate at δ 3.85 ppm. The methoxyl protons were now present as an end group. This observation was further supported by its ^{13}C-NMR spectrum which showed a peak at δ 65.57 due to the formation of ester bond. The lipase-catalyzed reaction was highly *chemo*-selective (6,7) as there was no reaction with phenolic hydroxyl group. The ^{1}H and ^{13}C-NMR spectra of the products **2a-f** did not indicate any trace of trans-esterification between the phenolic hydroxyl and main chain ester units. The number average molecular weight of the polymers **2a-f** were in the range of ∼6000–12000 Da (PD 1.4), as determined by GPC. The degrees of polymerization and molecular weights were also determined by end group analysis as indicated in Table 1. The calculation of molecular weight by ^{1}H-NMR was based on the integration of the methoxyl end group at δ 3.86 and integration of the triplet from the formed ester bond at δ 4.40–4.42.

The polymers **2a–f** were further functionalized by acylation with nonanoyl chloride using anhydrous potassium carbonate in acetonitrile/dichloromethane depending upon the linkers used in polymerization. These reactions gave the acylated copolymers **3a–f** in 80% isolated yields. The structures of these polymers were established by ^{1}H-NMR and ^{13}C-NMR spectra. Acylation of amino polymers were confirmed by the down field shifts in the aromatic protons at δ 7.46–7.51 (brs, 2H, *H*-4 and *H*−6) and 7.96–8.03 (s, 1H, *H*-2) before acylation to 8.37–8.39 (brs, 2H, *H*-2) and 8.50–8.51 (s, 2H, *H*-4 and *H*−6) after acylation. The methylene protons (H-9) of the ester bond shifted from δ 4.40–4.42 to 4.48–4.49 in amino polymers (**3d–f**). Similar shifts were also observed in hydroxyl polymers (**3a–c**). Furthermore, carbon NMR also supported the acylation reaction. In the course of acylation reactions, end group acylation was also observed. This can be visualized by the

Table 1. Molecular wt, percentage of encapsulation of aspirin, particle size before and after encapsulation of the polymers.

SN	*Linker*	*Peg size*	*Alkyl chain*	*Molecular Wt by GPC (Dalton)*	*Molecular Wt by NMR (Dalton)*	*Radius of gyration (Rg,) before encapsulation (nm)*	*Radius of Gyration (Rg) after encapsulation (nm)*	*Percentage of encapsulation*
1	5-hydoxyisophthalate	600	Nonanoyl	9.0×10^3	8.6×10^3	NA	NA	NA
2	5-hydoxyisophthalate	900	Nonanoyl	11.0×10^3	10.0×10^3	NA	NA	NA
3	5-hydoxyisophthalate	1500	Nonanoyl	14.0×10^3	13.0×10^3	NA	NA	NA
4	5-aminoisophthalate	600	Nonanoyl	8.0×10^3	6.7×10^3	59.4 ± 8.4	53.6 ± 8.8	26.0%
5	5-aminoisophthalate	900	Nonanoyl	8.0×10^3	7.5×10^3	62.4 ± 6.5	69.9 ± 6.1	21.0%
6	5-aminoisophthalate	1500	Nonanoyl	8.0×10^3	9.3×10^3	64.0 ± 12.6	70.8 ± 1.9	21.0%
7	5-hydoxyisophthalate	600	Decyl	*	*	29.0 ± 8.6	51.0 ± 1.2	16.6%
8	5-hydoxyisophthalate	900	Decyl	*	*	35.1 ± 12.8	52.8 ± 11.8	14.6%
9	5-hydoxyisophthalate	1500	Decyl	*	*	43.7 ± 16.0	49.9 ± 11.5	10.0%

*Ref. 6.

presence of a peak at δ 4.23 due to the formation of ester bond between nonanoyl chloride and end group hydroxyl of PEG chain. The degree of functionalization was determined by comparing the intensity of the signal at aromatic protons before and after acylation and was found to be >95%. In the amino polymers, the results were confirmed by shifts observed due to amidation from δ 4.17 (brs, 2H, NH_2, D_2O exchangable) to 8.63 (brs, 1H, CON*H*,D_2O exchangable). UV absorbance was used to further support the formation of acylated product which showed maxima at 343, 268 nm before acylation and at 398, 356, 310 nm after acylation in the amino polymers. The NMR spectra shown in Figure 1 show the downfield shift in the aromatic protons, as well as the changes in the aliphatic region upon acylation.

These amphiphilic polymers self assemble in aqueous solutions to form nano-spheres. Aspirin was used as a model to measure encapsulation capacity of the nano spheres. Aqueous solubility was not sufficient to perform encapsulation studies in case of polymers **3a–c.** The polymers **3d–f** were used to perform encapsulation studies as they were quite soluble in water. The percentage of encapsulation was estimated by UV spectroscopy using a calibration curve for aspirin in methanol. The observed percentage of encapsulation was compared with previously prepared polymers having an ether alkyl chain. The UV spectrum (Figure 2) clearly shows the encapsulation of aspirin in the nano-spheres. It was found that polymers with the amidic alkyl chain (**3d–f**) could encapsulate larger amounts of the aspirin than polymers with the ether chain (Table 1).

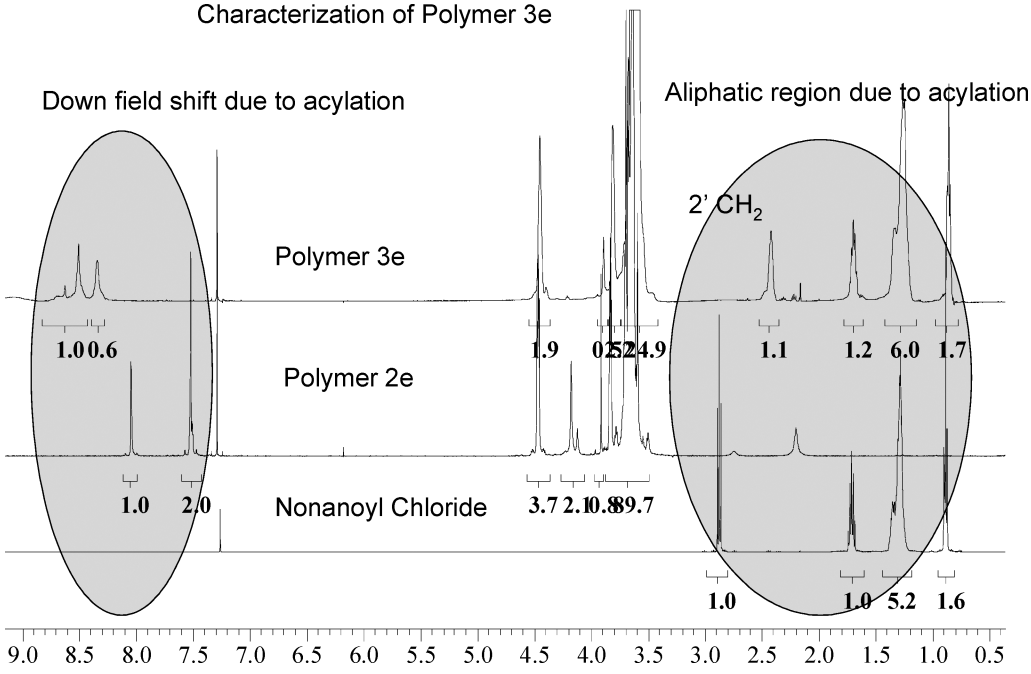

Fig. 1. Characterization of polymer **3e** by ^{1}H-NMR.

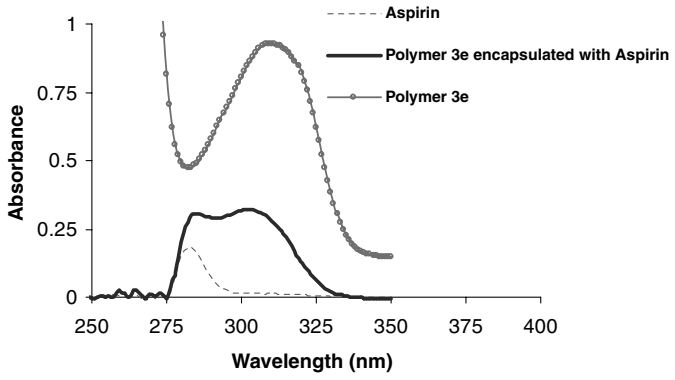

Fig. 2. UV spectra of encapsulated aspirin in polymer **3e**.

Particle size studies were performed by light scattering experiments at 10°C. The radius of gyration, before and after encapsulation with each polymer, were measured and listed in Table 1. It would appear that the radius of gyration of the amidic polymers does not significantly change with encapsulation. The alkyl ether polymers change significantly in size with the PEG 600 and 900 while with 1500 peg not much change was observed. This may be related to the amount of aspirin encapsulated.

4. Conclusions

In summary, six novel amphiphilic copolymers **(3a–f)** were synthesized and characterized. The ability of these polymers to form self assembled structures in solution provides enormous potential in drug delivery system. The polymers **3d–f** were evaluated for their drug encapsulation capacity using hydrophobic drug aspirin and compared with the previously known polymers. Synthesized polymers possessing amidic alkyl chain demonstrated better drug loading capacity than the previously reported polymers with an ether alkyl chain (6).

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