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Novel PEGylated Amphiphilic Copolymers as Nanocarriers for Drug Delivery: Synthesis, Characterization and Curcumin Encapsulation

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Amphiphilic polymers can self assemble into micellar nano-particles and can be effectively used as nano carriers for drug delivery. A number of macromolecular delivery systems are under investigation to improve the efficacy of prospective drugs. In this study, seven new co-polymers were synthesized under mild reaction conditions in bulk (without solvent) by chemoenzymatic approach using *Candida antarctica* lipase (Novozyme 435) and molecular sieves, subsequently these polymers were treated with different long chain bromoalkanes and acid chlorides for attachment of the lipophilic moieties to the backbone polymer via an ether or an ester linkage, respectively in order to make them amphiphilic. These synthesized nano-particles demonstrated high drug loading capacity and have the potential to encapsulate hydrophobic drugs.

Keywords: EGylated amphiphilic copolymers, nanocarriers, drug delivery, curcumin encapsulation

1 Introduction

Amphiphilic copolymers are well established as building blocks for the preparation of micellar drug carriers (1). Over the past decade, the effectiveness of such self-assembled drug delivery devices has been demonstrated numerous times. Nano-particles based on polymers have been widely investigated for pharmaceutical applications in drug delivery systems (2, 3). The continuous development of new drug delivery systems is driven by the need to maximize therapeutic activity while minimizing negative side effects (4). Hydrophobic drugs can be physically entrapped in the core of copolymer micelles and transported at concentrations that can exceed their intrinsic water solubility.

PEGylation, which refers to the modification of biomaterials and biomolecules using poly(ethylene glycol) (PEG), has become increasingly important in the fields of pharmaceuticals and biomedical engineering (5–7). PEG is a nontoxic, hydrophilic polymer with low interfacial free energy in water and high-chain mobility inducing effective steric stabilization effects (8).

We have developed a flexible chemo-enzymatic approach for the synthesis of amphiphilic PEGylated copolymers (9–11). The advantage of this method is the selectivity of the enzyme that leaves a free functional group on the polymer backbone. These functional groups can be further modified by attaching hydrophobic chains to make these polymers as amphiphilic PEGylated copolymers. *Candida antarctica* lipase B (Novozym 435) has several advantages in organic synthesis over conventional chemical synthesis as it exhibits a high degree of selectivity and works under mild reaction conditions of temperature, pressure and pH. Additional advantages are the recyclability of the enzyme and use in bulk reaction media to avoid organic solvents.

In the present work, we report on the condensation copolymerization of poly(ethylene glycol) (Mw 900) with dimethyl 5-hydroxyisophthalate (**1**) in bulk using *Candida antarctica* lipase B (CAL) (Sch. 1). Long chain fatty acid chlorides and long chain bromoalkanes were used to attach long hydrophobic chains to these polymers via an ether or an ester linkage to make them amphiphilic. These amphiphilic copolymers self assemble in aqueous solution to form nano-particles. The synthesized co-polymers were

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well characterized by various spectroscopic techniques. Curcumin, a naturally occurring bioactive compound was used as an active drug to measure encapsulation capacity of these nano-particles. The nano-particles were found to carry a significant amount of the drug (9–21% of curcumin).

2 Experimental

2.1 Materials

Dimethyl 5-hydroxyisophthalate, acid chlorides, 1-bromoalkanes, potassium carbonate, acetonitrile, dichloromethane, molecular sieves (4Å beads, 8–12 mesh) and polyethylene glycol (PEG 900) were purchased from Aldrich (Milwaukee, WI). Novozyme 435, an immobilized enzyme, was a gift from Novozymes, Inc., Denmark. Potassium carbonate was fused overnight at 200°C before use, whereas polyethylene glycol was dried under vacuum at 60°C for 3 h prior to its 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 spectra were recorded on a Bruker DPX 500 spectrometer operating at 500 MHz and ^{13}C-NMR spectra were recorded on a Bruker DPX 200 spectrometer operating at 50 MHz 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 an Agilent 8453 spectrophotometer. Dynamic light scattering (DLS) data were collected on a 50 mW He-Ne Laser having photodiode detector (BI-APD), a digital time correlator (BI-9000) and software from BrookHaven Instruments Corporation.

2.3 General Procedure For Polymer Synthesis

Dimethyl 5-hydroxyisophthalate (**1**, 1.0 mmol) and PEG 900 (1.0 mmol) were placed in a round-bottom flask and stirred until homogeneous, Novozyme 435 (10% by weight *wrt* monomers) and 4Å molecular sieves (10% by weight *wrt* PEG) were added. The resultant reaction mixture was stirred using a magnetic bead at 90 °C under vacuum (100 millitorr) for 48 h and for additional 12 h with overhead stirrer at 90 °C under vacuum and then quenched by adding chloroform. The enzyme and molecular sieves were removed by filtration and the filtrate was concentrated to get the product, which was redissolved in deionized water for dialysis using membrane (MWCO 6000). After the completion of dialysis, the product polymer **2** was obtained as a solid by freeze-drying. The spectroscopic characterization of polymer **2** has already been reported (9).

2.4 General Procedure for Alkylation of Polymer

Polymer **2** (0.89 mmol) was dissolved in anhydrous acetonitrile, anhydrous potassium carbonate (2.67 mmol) and bromoalkanes (1.07 mmol) were added. The reaction mixture was refluxed under nitrogen and the progress of the reaction monitored by TLC using ethyl acetate in petroleum ether (30 %). After completion of the reaction (6 h), salt was removed by filtration and the solvent removed from the filtrate under vacuum to give the products **3–6**.

2.5 Poly[(polyoxyethylene-900)-oxy-5-tetradecyloxyisophthaloyl] (3)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-tetradecyloxyisophthaloyl] (**3**) was achieved via refluxing poly[(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67 mmol) and 1-bromotetradecane (0.296 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.89 (t, 3H, *H*-14'), 1.26–1.38 (m, 22H, *H*-3'-13'), 1.80 (m, 2H, *H*-2'), 3.59-3.69 (brs, methylene protons of PEG main chain), 3.84 (t, 4H, H-10), 3.92 (s, 3H, $-$OCH_3 end group), 4.01 (t, 2H, *H*-1'), 4.48 (t, 4H, *H*-9), 7.72 (s, 2H, *H*-4 and *H*-6), 8.21 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 14.39 (CH_3), 22.86 (2 $\times C$H$_2$), 25.98 (CH_2), 29.41 (CH_2), 29.68 (CH_2), 30.01 (6 $\times C$H$_2$), 32.17 (CH_2), 63.89 (C-10, OCH_2), 68.51 (C-1', OCH_2), 69.32 (C-9, OCH_2), 70.32–71.08 (methylene carbons of PEG main chain), 119.83 ($CH \times 2$), 122.45 (CH), 131.92 (2 × q), 158.64 (q), 165.84 ($-COO$).

IRυmax: 3514, 2869, 1720, 1596, 1448, 1341, 1319, 1302, 1231, 1091, 1036, 947, 849, 759, 719, 511 cm^{-1}

UV λ_{max}(MeOH): 312 nm

2.6 Poly[(polyoxyethylene-900)-oxy-5-hexadecyloxyisophthaloyl] (4)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-hexadecyloxyisophthaloyl] (**4**) was achieved via refluxing poly[(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67 mmol) and 1-bromohexadecane (0.327 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.90 (t, 3H, *H*-16'), 1.28–1.40 (m, 26H, *H*-3'-15'), 1.82 (m, 2H, *H*-2'), 3.66-3.71 (brs, methylene protons of PEG main chain), 3.86 (t,

4H, H-10), 3.96 (s, 3H, -OCH_3 end group), 4.05 (t, 2H, *H*-1′), 4.50 (t, 4H, *H*-9), 7.76 (s, 2H, *H*-4 and *H*-6), 8.29 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 14.58 (*C*H$_3$), 23.07 (*C*H$_2$), 26.37 (*C*H$_2$), 29.48 (*C*H$_2$), 29.92 (2 × *C*H$_2$), 30.21 (8 × *C*H$_2$), 32.42 (*C*H$_2$), 64.93 (C-10, O*C*H$_2$), 69.24 (C-1', O*C*H$_2$), 69.51 (C-9, O*C*H$_2$), 70.93–71.38 (methylene carbons of PEG main chain), 120.38 (*C*H × 2), 123.42 (*C*H), 132.67 (2 × q), 159.63 (q), 166.24 (-*C*OO).

IRυmax: 3517, 2867, 1728, 1593, 1457, 1349, 1327, 1301, 1230, 1098, 1041, 941, 843, 752, 716, 511 cm^{-1}

UV λ_{max}(MeOH): 317 nm

2.7 Poly[(polyoxyethylene-900)-oxy-5-octadecyloxyisophthaloyl] (5)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-octadecyloxyisophthaloyl] (**5**) was achieved via refluxing poly[(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67mmol) and 1-bromooctadecane (0.36 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.88 (t, 3H, *H*-18'), 1.25–1.38 (m, 30H, *H*-3'-17'), 1.81 (m, 2H, *H*-2'), 3.60-3.72 (brs, methylene protons of PEG main chain), 3.79 (t, 4H, H-10), 3.95 (s, 3H, -OCH_3 end group), 4.04 (t, 2H, *H*-1′), 4.49 (t, 4H, *H*-9), 7.73 (s, 2H, *H*-4 and *H*-6), 8.21 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 14.39 (*C*H$_3$), 23.22 (2 × *C*H$_2$), 26.41 (*C*H$_2$), 29.14 (*C*H$_2$), 29.81 (2 × *C*H$_2$), 30.14 (8 × *C*H$_2$), 32.46 (2 × *C*H$_2$), 64.79 (C-10, O*C*H$_2$), 69.12 (C-1', O*C*H$_2$), 69.86 (C-9, O*C*H$_2$), 70.92–71.41 (methylene carbons of PEG main chain), 120.53 (*C*H × 2), 123.42 (*C*H), 132.11 (2 × q), 159.35 (q), 166.28 (-*C*OO).

IRυmax: 3518, 2859, 1718, 1590, 1451, 1342, 1319, 1301, 1231, 1094, 1033, 947, 839, 748, 718, 502 cm^{-1}

UV λ_{max}(MeOH): 317 nm

2.8 Poly[(polyoxyethylene-900)-oxy-5-decadecyloxyisophthaloyl](6)

Synthesis of poly [(polyoxyethylene-900)-oxy-5-decadecyloxyisophthaloyl] (**6**) was achieved via refluxing poly [(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67 mmol) and 1-bromoeicosane (0.39 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.90 (t, 3H, *H*-20'), 1.28–1.40 (m, 34H, *H*-3'-19'), 1.82 (m, 2H, *H*-2'), 3.66-3.71 (brs, methylene protons of PEG main chain), 3.86 (t, 4H, H-10), 3.96 (s, 3H, -OCH_3 end group), 4.05 (t, 2H, *H*-1′), 4.50 (t, 4H, *H*-9), 7.76 (s, 2H, *H*-4 and *H*-6), 8.29 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 14.51 (*C*H$_3$), 23.07 (2 × *C*H$_2$), 26.38 (*C*H$_2$), 29.52 (*C*H$_2$), 29.74 (2 × *C*H$_2$), 30.08 (10 × *C*H$_2$), 32.30 (2 × *C*H$_2$), 64.81 (C-10, O*C*H$_2$),69.04 (C-1', O*C*H$_2$), 69.49 (C-9, O*C*H$_2$), 70.91–71.30 (methylene carbons of PEG main chain), 120.34 (*C*H × 2), 123.35 (*C*H), 132.04 (2 × q), 159.56 (q), 166.08 (-*C*OO).

IRυmax: 3515, 2863, 1721, 1595, 1455, 1348, 1322, 1308, 1233, 1095, 1039, 948, 846, 758, 720, 509 cm^{-1}

UV λ_{max}(MeOH): 314 nm

2.9 General Procedure for Acylation of Polymer

Polymer **2** (0.89 mmol) was dissolved in anhydrous acetonitrile, anhydrous potassium carbonate (2.67 mmol) and acid chlorides (1.07 mmol) were added under nitrogen. The reaction mixture was refluxed and progress of the reaction monitored by TLC using ethyl acetate in petroleum ether (30%). After completion of the reaction (6 h), salt was removed by filtration and the solvent removed under vacuum to give the products **7–9**.

2.10 Poly[(polyoxyethylene-900)-oxy-5-tetradecanoyloxyisophthaloyl] (7)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-tetradecanoyloxyisophthaloyl] (**7**) was achieved via refluxing poly[(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67 mmol) and tetradecanoyl chloride (0.264 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.88-0.91 (t, 3H, *H*-14"and 3H, *H*-14"' of end group), 1.29–1.45 [brs, 20H, (CH$_2$ × 10), *H*-4"-13" and 20H (CH$_2$× 10), *H*-4"'-13"'], 1.64 (m, 2H, *H*-3"'), 1.77 (m, 2H, *H*-3"), 2.34 (t, 2H, *H*-2"'), 2.60 (t, 2H, *H*-2"), 3.66-3.71 (brs, methylene protons of PEG main chain), 3.85 (t, 4H, *H*-10), 3.96 (s, 3H, -OCH_3 end group), 4.24 (t, 2H, $H-\alpha$), 4.51 (t, 4H, *H*-9), 7.96 (s, 2H, *H*-4 and *H*-6), 8.58 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 14.49 (*C*H$_3$), 23.03 (*C*H$_2$), 25.14 (*C*H$_2$), 29.46 (*C*H$_2$), 29.61 (*C*H$_2$), 29.69 (*C*H$_2$), 29.80 (*C*H$_2$), 29.99 (3 × *C*H$_2$), 32.26 (*C*H$_2$), 34.25 (*C*H$_2$), 34.57 (*C*H$_2$), 64.98 (C-10, O*C*H$_2$), 69.38 (C-9, O*C*H$_2$), 70.53-71.20 (methylene carbons of PEG main chain), 72.98 (O*C*H$_2$, $C-\alpha$, end group), 127.77 (*C*H × 2), 128.48 (*C*H), 132.28 (q×2), 151.09 (q), 166.19 (2 × *C*OO), 170.83 (O*C*O end group), 172.21 (O*C*O)

IRυmax: 2864, 1765, 1723, 1597, 1452, 1348, 1300, 1233, 1094, 1035, 948, 846, 759, 722, 512 cm^{-1}

UV λ_{max}(MeOH): 316, 293, 284 nm

2.11 Poly[(polyoxyethylene-900)-oxy-5-hexadecanoyloxyisophthaloyl] (8)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-hexa-decan oyoxyisophthaloyl] (**8**) was achieved via refluxing poly[(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67 mmol) and hexadecanoyl chloride (0.294 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.86-0.90 (t, 3H, *H*-16"and 3H, *H*-16"' of end group), 1.27–1.43 [brs, 24H, ($CH_2\times$ 12), *H*-4"-15" and 24H ($CH_2\times$12), *H*-4"'-15"'], 1.61 (m, 2H, *H*-3"'), 1.73 (m, 2H, *H*-3"), 2.31 (t, 2H, *H*-2"'), 2.58 (t, 2H, *H*-2"), 3.64-3.70 (brs, methylene protons of PEG main chain), 3.82 (t, 4H, *H*-10), 3.93 (s, 3H, -OCH_3 end group), 4.21 (t, 2H, $H-\alpha$), 4.48 (t, 4H, *H*-9), 7.93 (s, 2H, *H*-4 and *H*-6), 8.52 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 14.43 (CH_3), 23.00 (CH_2), 25.11 (CH_2), 28.46 (CH_2), 29.11 (CH_2), 29.60 (CH_2), 29.71 (CH_2), 29.82 (3 $\times CH_2$), 32.10 (2 $\times CH_2$), 33.12 (CH_2), 34.11 (CH_2), 34.87 (CH_2), 65.98 (C-10, OCH_2), 68.38 (C-9, OCH_2), 70.13-71.28 (methylene carbons of PEG main chain), 73.18 (OCH_2, $C-\alpha$, end group), 126.67 ($CH\times 2$), 127.38 (CH), 133.29 (q×2), 150.28 (q), 167.10 (2 $\times$COO), 171.10 (O*C*O end group), 173.22 (O*C*O)

IR υmax: 2861, 1759, 1711, 1592, 1445, 1342, 1300, 1233, 1091, 1032, 947, 845, 752, 723, 515 cm^{-1}

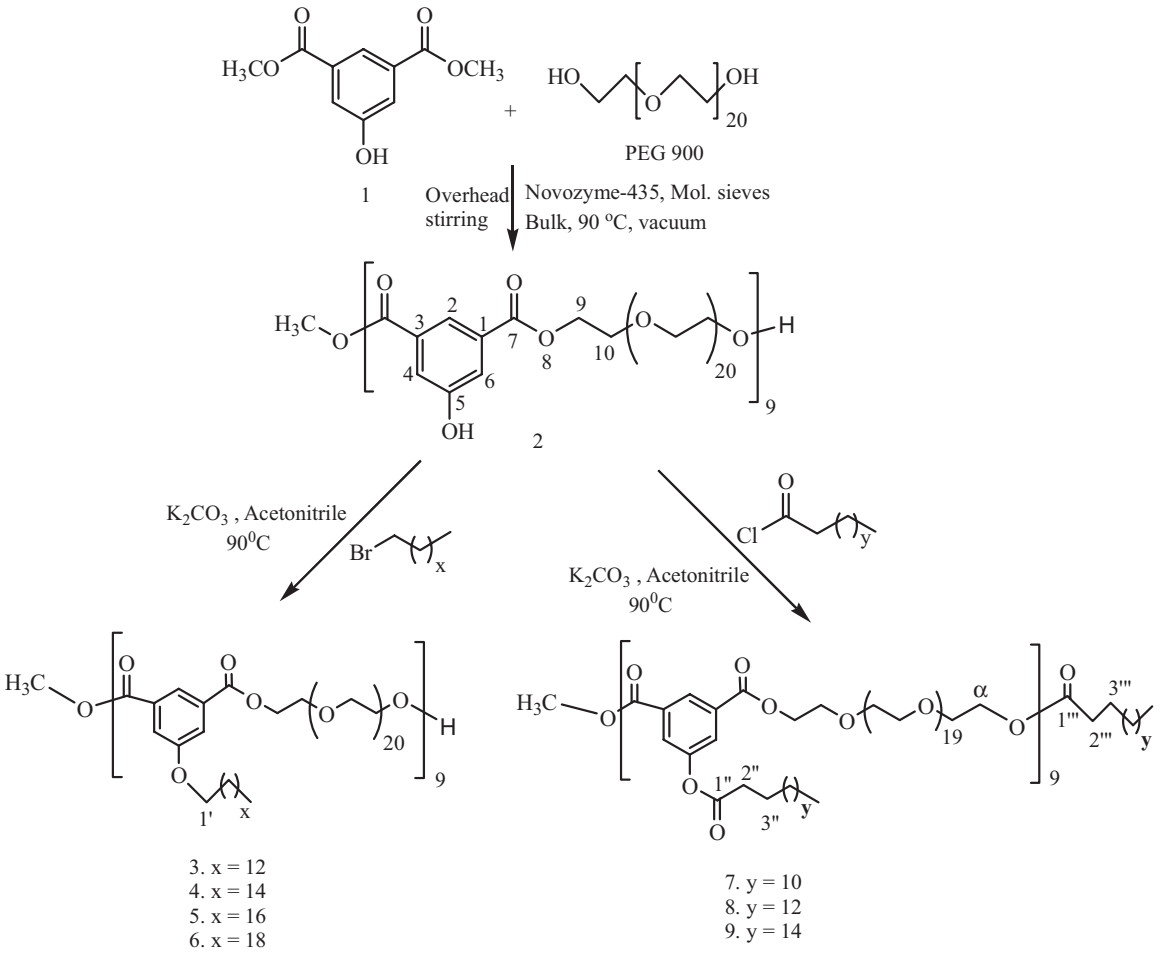
UV λ_{max}(MeOH): 311, 295, 281 nm

2.12 Poly[(polyoxyethylene-900)-oxy-5-octadecanoyloxyisophthaloyl] (9)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-octadecanoyloxyisophthaloyl] (**9**) was achieved via refluxing poly-[(polyoxyethylene-900)-oxy-5-hydroxyisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.369 g, 2.67 mmol) and octadecanoyl chloride (0.324 g, 1.07 mmol) at 80°C.

^{1}H-NMR (δ_HCDCl$_3$, 500 MHz): 0.81-0.89 (t, 3H, *H*-18"and 3H, *H*-18"' of end group), 1.21–1.40 [brs, 28H, ($CH_2\times$ 14), *H*-4"-17" and 28H ($CH_2\times$ 14), *H*-4"'-17"'], 1.60 (m, 2H, *H*-3"'), 1.72 (m, 2H, *H*-3"), 2.30 (t, 2H, *H*-2"'), 2.57 (t, 2H, *H*-2"), 3.59-3.69 (brs, methylene protons of PEG main chain), 3.80 (t, 4H, *H*-10), 3.91 (s, 3H, —OCH_3 end group), 4.21 (t, 2H, $H-\alpha$), 4.50 (t, 4H, *H*-9), 7.91 (s, 2H, *H*-4 and *H*-6), 8.52 (s, 1H, *H*-2).

^{13}C-NMR (δ_CCDCl$_3$, 125 MHz): 13.51 (CH_3), 24.02 (CH_2), 26.13 (CH_2), 28.54 (CH_2), 28.91 (CH_2), 29.19 (CH_2), 29.43 (CH_2), 29.87 (4 $\times CH_2$), 32.01 (3 $\times CH_2$),



Sch. 1. Synthesis of amphiphilic PEGylated copolymers **3-9**.

33.53 (CH_2), 34.01 (CH_2), 35.04 (CH_2), 65.92 (C-10, OCH_2), 69.43 (C-9, OCH_2), 70.01-71.19 (methylene carbons of PEG main chain), 72.02 (OCH_2, $C-\alpha$, end group), 128.17 ($CH \times 2$), 128.91 (CH), 133.17 (q × 2), 150.23 (q), 167.12 (2 × COO), 169.94 (OCO end group), 173.10 (-OCO)

IRυmax: 2892, 1753, 1791, 1601, 1498, 1368, 1301, 1267, 1087, 1035, 956, 895, 787, 702, 524 cm^{-1}

UV λ_{max}(MeOH): 319, 298, 289 nm

2.13 Method For Encapsulation of Curcumin

The copolymers **3–5** and **7–9** and curcumin were dissolved in methanol to obtain 1:2 drug/polymer (w/w ratio) and the contents were stirred for 15 min. Organic solvent was removed at room temperature under vacuum. The resulting viscous mixture of curcumin and polymer was dissolved in water with vigorous stirring to form nanoparticles for 2 h. Non-incorporated curcumin was separated by filtration of the nanoparticle suspension through a 0.2 μm filter (curcumin crystals cannot pass through the filter unless the curcumin is solubilized by nanoparticles). The curcumin concentration in the filtrate was estimated by UV spectroscopy using a calibration curve for curcumin in methanol. The % encapsulation of curcumin was found to be in the range of 15–22 % in the ether chain polymers **3–5**, whereas it was 9–15% in the ester chain polymers **7–9**.

3 Results and Discussion

Dimethyl 5-hydroxyisophthalate and PEG 900 were reacted under solventless conditions using molecular sieves and Novozyme 435 to give polymer **2** (Sch. 1). The detailed synthesis and characterization of polymer **2** has been published by us earlier (9). The number average molecular weight of the polymer **2** was in the range of 10,000–12,000 Da (PD 1.4), as determined by GPC.

Here, in an attempt, to synthesize amphiphilic polymers, we have functionalized polymer **2** by reacting it with long chain fatty acid chlorides (C-14 to C-18) or long chain alkyl bromides (C-14 to C-20), anhydrous potassium carbonate in acetonitrile. These reactions gave the acylated/alkylated copolymers **3-9** in 85% isolated yields. The structures of these polymers were established from their ^{1}H-NMR and ^{13}C-NMR spectra.

Alkylation of hydroxy polymer **2** was confirmed by the appearance of a new peak at δ 4.05 in the ^{1}H-NMR spectrum of the alkylated polymer **6** due to OCH_2 group as a result of ether linkage as shown in Figure 1. Peaks for other protons of the alkyl chain were also observed in the aliphatic region of the polymers **3–6**. Its ^{13}C-NMR spectrum also showed the presence of aliphatic carbons of the alkyl chain attached to the polymer ranging from δ 14.51-32.30, as well as the OCH_2 group around δ 69 (C-1'). However, there was not much shift in the δ values of the aromatic protons after alkylation, we did not observe any end group alkylation as well.

The formation of ester linkage with acid chlorides was supported by the down field shifts of the aromatic protons at δ 7.72 (s, 2H, *H*-4 and *H*-6) and δ 8.21 (s, 1H, *H*-2) before acylation (in the ^{1}H-NMR spectrum of **2**) to δ 7.86 (s, 2H, *H*-4 and *H*-6) and δ 8.52 (s, 1H, *H*-2) after acylation (in the ^{1}H NMR spectrum of **7**) (Fig. 2). Further, the ^{13}C NMR spectrum displayed signals at δ 170.11 for C-1' indicating the attachment of acyl side chain through ester linkage. In

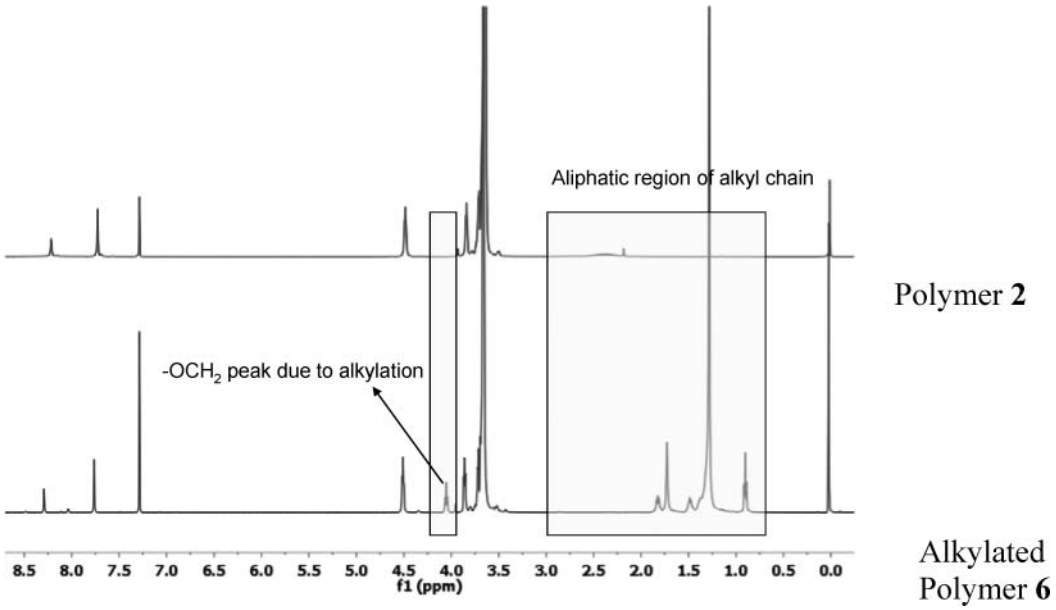


Fig. 1. The ^{1}H-NMR spectra of polymer **2** before and after alkylation.

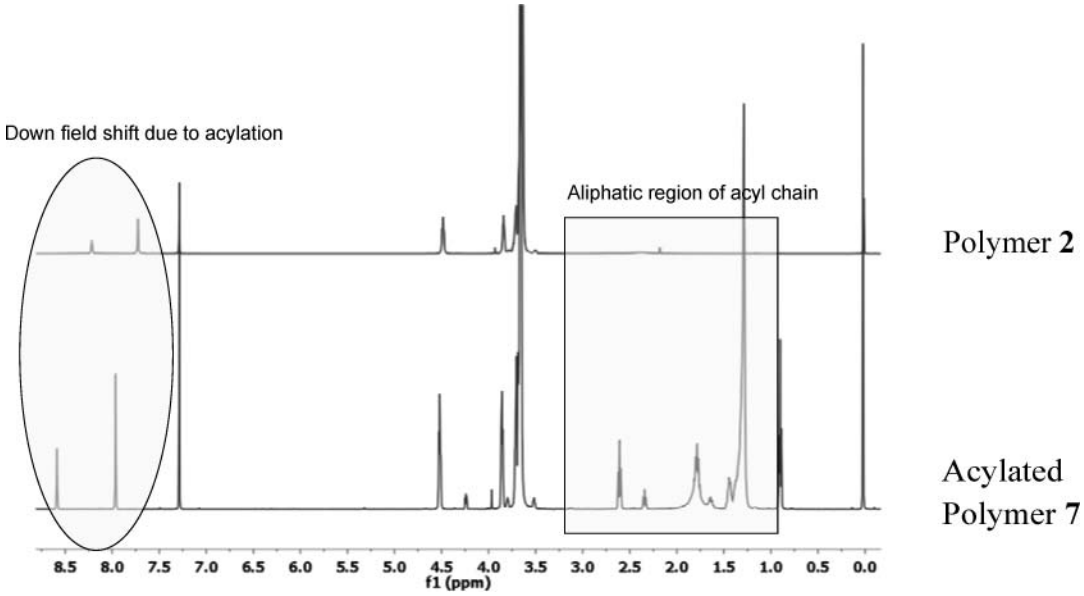


Fig. 2. The ^{1}H-NMR spectra of polymer **2** before and after acylation.

the course of acylation reactions, the presence of a peak at ***δ*** 4.24 indicated the end group acylation. This was confirmed by the signal at δ 170.83 for C-1" and signal at δ 72.98 for OCH_2 end group (C-α) in the ^{13}C-NMR spectrum of **7** as well. The ^{1}H-NMR spectrum of **7** (Fig. 2) also shows the expected changes in the aliphatic region upon acylation.

These amphiphilic polymers self assemble in their aqueous solution to form nano-particles. Curcumin was used as a model drug to measure encapsulation capacity of the nano-particles. Aqueous solubility was not sufficient to perform encapsulation studies in the case of polymer **6.** The polymers **3–5** and **7–9** were used to perform encapsulation studies. The percentage of encapsulation was estimated by UV spectroscopy using a calibration curve for curcumin in methanol. The UV spectrum (Fig. 3) clearly shows the encapsulation of curcumin in the nano-particles. It was found that polymers with ether alkyl chain **3-5** encapsulated 11-21% of curcumin however, percentage encapsulation was less in polymers with acyl chain (**7-9**) and varied from 9-15% (Table 1).

Particle size studies were performed by light scattering experiments at 25°C. The hydrodynamic radius, before and after encapsulation with each polymer, were measured and are listed in Table 1. It would appear that the hydrodynamic radius of these polymers did not significantly change with encapsulation.

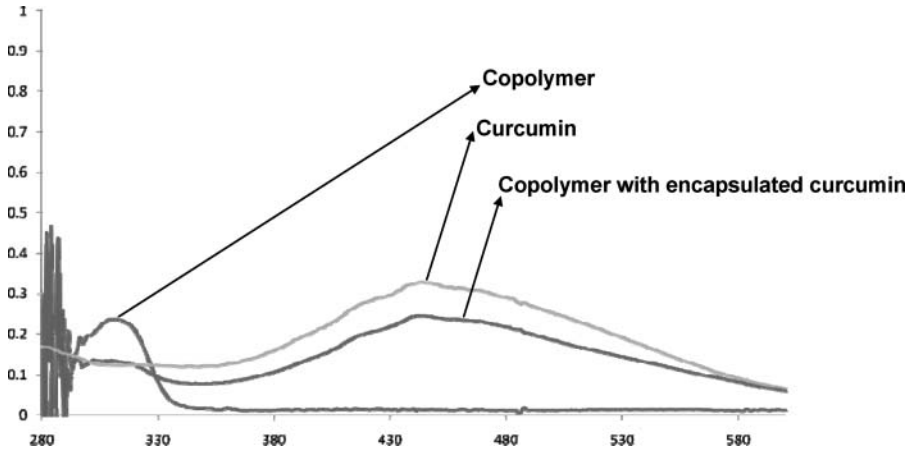


Fig. 3. UV spectra of curcumin, polymer **3** and the encapsulated curcumin.

Table 1. Percentage of encapsulation of curcumin and particle size of the polymer before and after encapsulation

Polymer	*Hydrophobic chain*	*Hydrodynamic radius (R_h) before encapsulation (nm)*	*Hydrodynamic radius (R_h) after encapsulation (nm)*	*Percentage of encapsulation of curcumin*
3	Myristyl (C-14)	14.8 ± 9.6	23.5 ± 6.8	21.47 %
4	Palmityl (C-16)	33.6 ± 8.8	32.3 ± 7.5	11.79 %
5	Stearyl (C-18)	45.1 ± 0.2	47.2 ± 4.3	15.41 %
6	Eicosanyl (C-20)	NA	NA	NA
7	Myristoyl (C-14)	37.9 ± 1.4	38.3 ± 2.4	14.46 %
8	Palmitoyl (C-16)	88.0 ± 2.8	84.1 ± 3.7	12.08 %
9	Stearoyl (C-18)	48.2 ± 0.2	49.3 ± 2.4	9.26 %

4 Conclusions

In summary, seven novel amphiphilic copolymers **3–9** have been synthesized and well characterized by various spectroscopic techniques. The ability of these polymers to form self assembled structures in solution provides enormous potential in developing drug delivery systems. The polymers were evaluated for their drug encapsulation capacity using curcumin, a bio-active naturally occurring compound. Synthesized polymers having long alkyl chains attached via an ether linkage demonstrated better curcumin loading capacity than the polymers having a long acyl chain attached via an ester linkage. Particle size was found to be in the range of 20-50 nm for polymers **3–7** and **9**, whereas polymer **8** showed particle size of ca 88 nm. There was no significant change in the particle size of these amphiphilic polymers after encapsulation of curcumin, except for polymer **3**.

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