

Amphiphilic Copolymers having Saturated and Unsaturated Aliphatic Side Chains as Nano Carriers for Drug Delivery Applications

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Nano-particles with controllable particle size and shape are of great interest in biomedical applications (1). From the standpoint of drug carrier design with wide applicability to a variety of hydrophobic drugs, an effective strategy would be to prepare a simple copolymer having the property to form stable polymeric micelles which can entrap hydrophobic drugs in the core (2). In this study, we have attached saturated and unsaturated long chain acid chlorides to the copolymer of PEG and dimethyl-5-aminoisophthalate via an amide linkage in order to make the copolymers amphiphilic. Further, bioactive compounds like β -carotene, which has considerable double bond unsaturation and curcumin, which has considerable aromatic unsaturation, were encapsulated in these amphiphilic copolymers which successfully demonstrated their drug loading ability.

Keywords: Nanocarriers, amphiphilic copolymers, encapsulation, drug delivery, bio-active, micelles and Novozyme-435

1 Introduction

Drug delivery systems play an important role by increasing the bioavailability of hydrophobic drugs *in vivo*. An elegant approach in achieving this goal is to use natural or synthetic amphiphilic polymers which self assemble into micelles. These amphiphilic polymers can be designed for the encapsulation of particular drugs through judicious design. The amphiphilic polymer system can be chosen such that the delivery vehicle (i.e., the amphiphilic polymers) will slowly release the drugs as they are degraded in the body by hydrolysis caused by the enzymes such as lipases or proteases present *in vivo*.

The architecture of our polymer platform allows encapsulating pharmaceutical actives and bioactive compounds

with a wide range of release profiles to create tailored drug loaded nanoparticles. In our group, we have already established the synthesis of such a copolymer under mild reaction conditions in bulk (without solvent) by a chemoenzymatic approach using *Candida antarctica* lipase (Novozyme-435) and molecular sieves (3–5). In this study, we will present the attachment of saturated long chain acid chlorides to the copolymer of PEG and dimethyl-5-aminoisophthalate via an amide linkage in order to make the copolymers amphiphilic. We have also designed and synthesized novel amphiphilic copolymers having unsaturated side chains in order to compare their drug loading capacity with the polymers having saturated side chains.

These amphiphilic copolymeric systems self assemble in aqueous media to form polymeric nanomicelles which are highly efficient drug delivery agents. Drug encapsulating nanoparticles offer extensive control over drug delivery location, drug dosage and drug release characteristics.

β -Carotene, a red-orange pigment, found in plants and fruits, is a precursor of vitamin-A and possesses antioxidant properties. Curcumin, the most active curcuminoid found in turmeric, has been shown to possess a multitude of

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beneficial effects in the treatment of cancers, cardiovascular diseases, inflammation, and possibly for the treatment of Alzheimer's diseases. With this knowledge in hand, bioactive compounds like β -carotene, which has considerable double bond unsaturation and curcumin and considerable aromatic unsaturation, have been encapsulated in amphiphilic copolymers under study in this paper, and successfully demonstrated their drug loading ability.

2 Experimental

2.1 Materials

Dimethyl-5-aminoisophthalate, acid chlorides, potassium carbonate, acetonitrile, 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 Novozyme, 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 ¹H-NMR spectra were recorded on a Bruker DPX 500 spectrometer operating at 500 MHz and ¹³C-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.

2.3 General Procedure for Polymer Synthesis

Dimethyl-5-aminoisophthalate (**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 re-

moved 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 (6).

2.4 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 stirred at room temperature 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 **3–6**.

2.5 Poly[(polyoxyethylene-900)-oxy-5-(nonanoylamino)isophthaloyl] (**3**)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-(nonanoylamino)isophthaloyl] (**3**) was achieved via stirring of poly[(polyoxyethylene-900)-oxy-5-aminoisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.37 g, 2.67 mmol) and nonanoyl chloride (0.19 g, 1.07 mmol) at room temperature.

¹H-NMR (δ_H CDCl₃, 500 MHz): 0.88–0.91 (t, 3H, *H*-9'' and 3H, *H*-9''' of end group), 1.26–1.36 [brs, 10H, (CH₂ × 5), *H*-4''-8'' and 10H, (CH₂ × 5), *H*-4'''-8'''], 1.64 (m, 2H, *H*-3'''), 1.73 (m, 2H, *H*-3''), 2.33 (t, 2H, *H*-2''), 2.42 (t, 2H, *H*-2'), 3.64–3.70 (brs, methylene protons of PEG main chain), 3.85 (t, 4H, *H*-10), 3.93 (s, 3H, -OCH₃ end group), 4.23 (t, 2H, *H*- α), 4.49 (t, 4H, *H*-9), 8.40 (s, 1H, *H*-2), 8.50 (s, 2H, *H*-4 and *H*-6), 8.63 (brs, 1H, -NHCO).

¹³C-NMR (δ_C CDCl₃, 125 MHz): 14.48 (CH₃), 23.02 (CH₂), 26.01 (CH₂), 29.81 (CH₂), 29.87 (CH₂), 30.83 (CH₂), 32.21 (CH₂), 38.57 (CH₂), 64.62 (C-9, OCH₂), 69.78 (C-10, OCH₂), 70.52–71.08 (methylene carbons of PEG main chain), 125.28 (CH₂), 126.04 (CH), 131.36 (qx2), 139.88 (q), 164.28 (-COO), 173.72 (-NHCO).

IR ν_{\max} : 2860, 1723, 1693, 1603, 1556, 1452, 1347, 1302, 1233, 1095, 1036, 948, 847, 759, 720, 509 cm⁻¹.

UV λ_{\max} (MeOH): 310 nm.

2.6 Poly[(polyoxyethylene-900)-oxy-5-(tetradecanoylamino)isophthaloyl] (**4**)

Synthesis of poly [(polyoxyethylene-900)-oxy-5-(tetradecanoylamino)isophthaloyl] (**4**) was achieved via stirring of poly[(polyoxyethylene-900)-oxy-5-aminoisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.37 g, 2.67 mmol) and tetradecanoyl chloride (0.27 g, 1.08 mmol) at room temperature.

¹H-NMR (δ_H CDCl₃, 500 MHz): 0.88–0.90 (t, 3H, *H*-14'' and 3H, *H*-14''' of end group), 1.27–1.34 [brs, 20H, (CH₂

$\times 10$), $H-4''-13''$ and $20H$ ($CH_2 \times 10$), $H-4'''-13'''$], 1.62 (m, 2H, $H-3'''$), 1.72 (m, 2H, $H-3''$), 2.32 (t, 2H, $H-2'''$), 2.42 (t, 2H, $H-2''$), 3.66–3.71 (brs, methylene protons of PEG main chain), 3.86 (t, 4H, $H-10$), 3.95 (s, 3H, $-OCH_3$ end group), 4.22 (t, 2H, $H-\alpha$), 4.50 (t, 4H, $H-9$), 8.41 (s, 1H, $H-2$), 8.49 (s, 2H, $H-4$ and $H-6$), 8.58 (brs, 1H, $-NHCO$)

$^{13}C-NMR$ ($\delta_C CDCl_3$, 125 MHz): 14.53 (CH_3), 23.09 (CH_2), 25.80 (CH_2), 29.76 (CH_2), 29.94 ($2 \times CH_2$), 30.09 ($2 \times CH_2$), 32.32 (CH_2), 32.86 (CH_2), 34.24 (CH_2), 34.57 (CH_2), 37.38 (CH_2), 64.83 (C-9, OCH_2), 69.46 (C-10, OCH_2), 70.95–71.16 (methylene carbons of PEG main chain), 125.18 ($CH \times 2$), 126.29 (CH), 131.53 ($q \times 2$), 139.58 (q), 165.90 ($-COO$), 172.44 ($-NHCO$).

IR ν_{max} : 2830, 1712, 1680, 1603, 1543, 1432, 1302, 1034, 941 cm^{-1} .

UV λ_{max} (MeOH): 310 nm.

2.7 Poly[(polyoxyethylene-900)-oxy-5-(hexadecanoyl amino)isophthaloyl] (5)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-(hexadecanoylamino)isophthaloyl] (**5**) was achieved via stirring of poly[(polyoxyethylene-900)-oxy-5-aminoisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.37 g, 2.67 mmol) and hexadecanoyl chloride (0.29 g, 1.07 mmol) at room temperature.

^1H-NMR ($\delta_H CDCl_3$, 500 MHz): 0.88–0.91 (t, 3H, $H-16''$ and 3H, $H-16'''$ of end group), 1.24–1.38 [brs, 24H, ($CH_2 \times 12$), $H-4''-15''$ and 24H ($CH_2 \times 12$), $H-4'''-15'''$], 1.64 (m, 2H, $H-3'''$), 1.71 (m, 2H, $H-3''$), 2.30 (t, 2H, $H-2'''$), 2.43 (t, 2H, $H-2''$), 3.68–3.74 (brs, methylene protons of PEG main chain), 3.85 (t, 4H, $H-10$), 3.95 (s, 3H, $-OCH_3$ end group), 4.24 (t, 2H, $H-\alpha$), 4.49 (t, 4H, $H-9$), 8.42 (s, 1H, $H-2$), 8.51 (s, 2H, $H-4$ and $H-6$), 8.68 (brs, 1H, $-NHCO$).

$^{13}C-NMR$ ($\delta_C CDCl_3$, 125 MHz): 14.20 (CH_3), 23.01 (CH_2), 25.40 (CH_2), 28.56 (CH_2), 29.12 (CH_2), 29.64 (CH_2), 29.92 ($3 \times CH_2$), 30.17 (CH_2), 31.42 ($2 \times CH_2$), 32.21 ($2 \times CH_2$), 37.68 (CH_2), 64.63 (C-9, OCH_2), 69.12 (C-10, OCH_2), 70.51–71.08 (methylene carbons of PEG main chain), 125.21 ($CH \times 2$), 126.12 (CH), 131.48 ($q \times 2$), 139.74 (q), 165.95 ($-COO$), 172.41 ($-NHCO$).

IR ν_{max} : 2862, 1725, 1691, 1607, 1556, 1448, 1347, 1095, 1036, 947, 840, 765, 720, 618 cm^{-1} .

UV λ_{max} (MeOH): 310 nm.

2.8 Poly[(polyoxyethylene-900)-oxy-5-(octadeca-9,12-dienoylamino)isophthaloyl] (6)

Synthesis of poly[(polyoxyethylene-900)-oxy-5-(octadeca-9,12-dienoylamino)isophthaloyl] (**6**) was achieved via stirring of poly[(polyoxyethylene-900)-oxy-5-aminoisophthaloyl] (**2**) (1.0 g, 0.89 mmol), anhydrous potassium carbonate (0.37 g, 2.67 mmol) and octadeca-9,12-dienoyl chloride (0.32 g, 1.07 mmol) at room temperature.

^1H-NMR ($\delta_H CDCl_3$, 500 MHz): 0.89–0.91 (t, 3H, $H-18'$), 1.28–1.34 [brs, 14H, ($CH_2 \times 7$), $H-4'-7'$ and $H-15'-17'$], 1.73 (t, 2H, $H-3'$), 2.02–2.18 (m, 4H, ($CH_2 \times 2$), $H-8'$ and $H-14'$), 2.41 (t, 2H, $H-2'$), 2.79 (t, 2H, $H-11'$), 3.65–3.72 (brs, methylene protons of PEG main chain), 3.85 (t, 4H, $H-10$), 3.95 (s, 3H, $-OCH_3$ end group), 4.50 (t, 4H, $H-9$), 5.37 (brs, 4H, $H-9'$, $10'$, $12'$ and $13'$), 8.05 (brs, 1H, $-NHCO$), 8.40 (s, 1H, $H-2$), 8.50 (s, 2H, $H-4$ and $H-6$).

$^{13}C-NMR$ ($\delta_C CDCl_3$, 125 MHz): 14.28 (CH_3), 22.94 (CH_2), 25.13 (CH_2), 25.68 (CH_2), 27.92 (CH_2), 28.28 ($2 \times CH_2$), 28.73 (CH_2), 28.94 ($2 \times CH_2$), 29.25 (CH_2), 29.40 (CH_2), 31.10 (CH_2), 64.37 (C-9, OCH_2), 69.88 (C-10, OCH_2), 70.64–71.32 (methylene carbons of PEG main chain), 125.73 ($CH \times 2$), 126.09 (CH), 128.52 (C-10' and $12'$, $2 \times CH = CH-CH_2$), 130.17 (C-9' and $13'$, $2 \times CH = CH-CH_2$), 132.94 ($q \times 2$), 138.23 (q), 165.78 ($-COO$), 173.85 ($-NHCO$).

IR ν_{max} : 2854, 1722, 1686, 1594, 1543, 1450, 1326, 1105, 1080, 1005, 928, 860, 765, 636 cm^{-1} .

UV λ_{max} (MeOH): 345 nm.

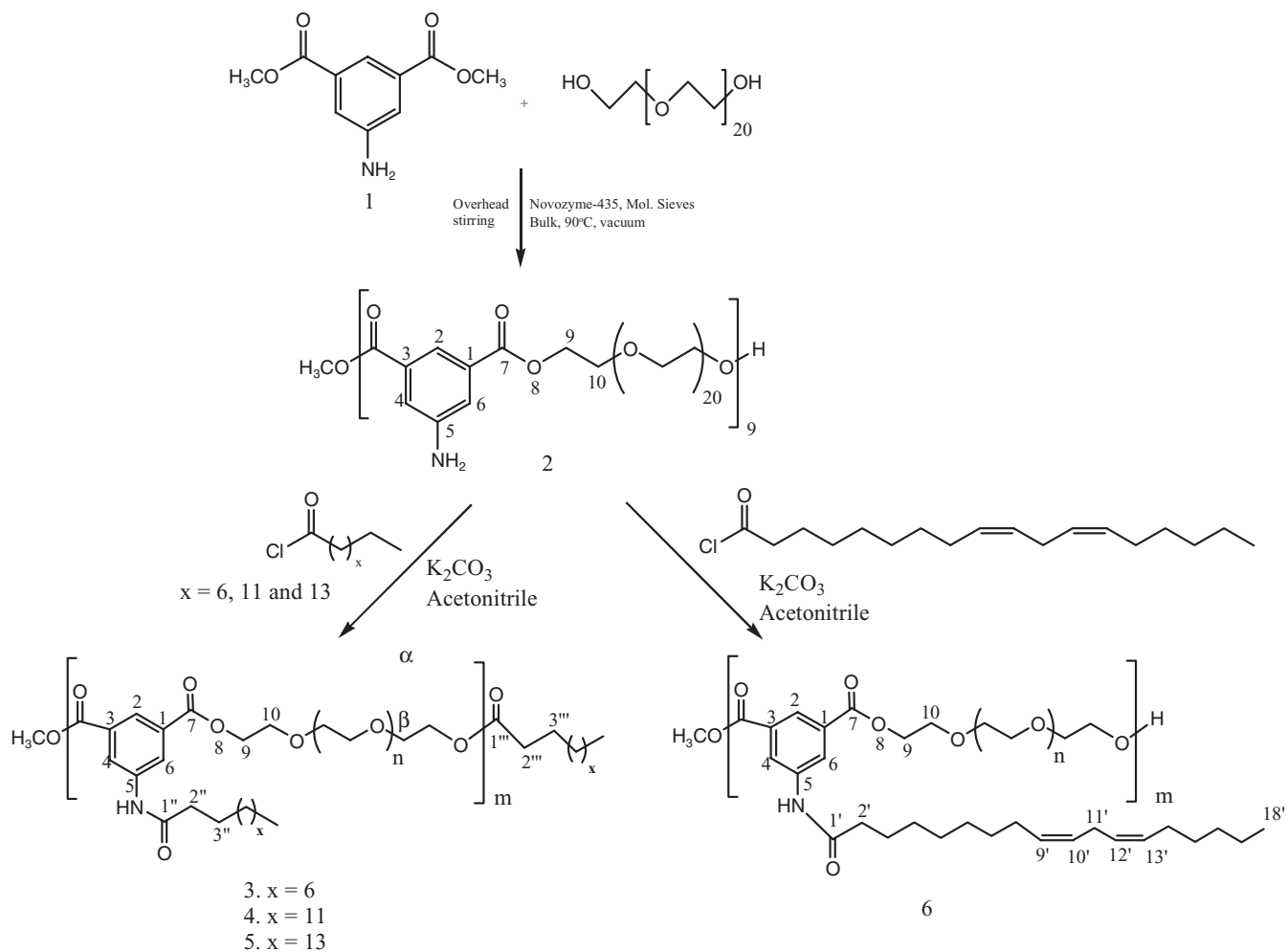
2.9 Method for Encapsulation of Curcumin and β -carotene

The copolymers **3–6** and curcumin/ β -carotene were dissolved in methanol to obtain 1:2 drug/polymer (w/w ratio) and the contents were stirred for 1 h. Organic solvent was removed at room temperature under vacuum. The resulting viscous mixture of curcumin/ β -carotene and polymer was dissolved in water with vigorous stirring for 2 h to form nanoparticles. Non-incorporated curcumin/ β -carotene were separated by filtration of the nanoparticle suspension through a 0.2 μm filter (curcumin/ β -carotene crystals cannot pass through the filter unless the curcumin/ β -carotene is solubilized by nanoparticles). The curcumin/ β -carotene concentration in the filtrate was estimated by UV spectroscopy using a calibration curve for curcumin/ β -carotene in methanol.

3 Results and Discussion

PEG-isophthalate copolymer was synthesized by enzyme catalyzed condensation polymerization of dimethyl-5-aminoisophthalate and PEG 900 under bulk conditions using molecular sieves and Novozyme-435 as shown in Scheme 1. The detailed synthesis and characterization of polymer **2** has been published by us earlier (6). 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, we have utilized the free functional amino group of the polymer to make it amphiphilic by reacting the polymer with saturated long chain fatty acid chlorides (C-9, C-14, and C-16) and unsaturated C-18 acid chloride having two double bonds using anhydrous potassium carbonate as a base and acetonitrile as a solvent (Sch. 1). These



Sch. 1. Synthesis of amphiphilic pegylated copolymers 3–6.

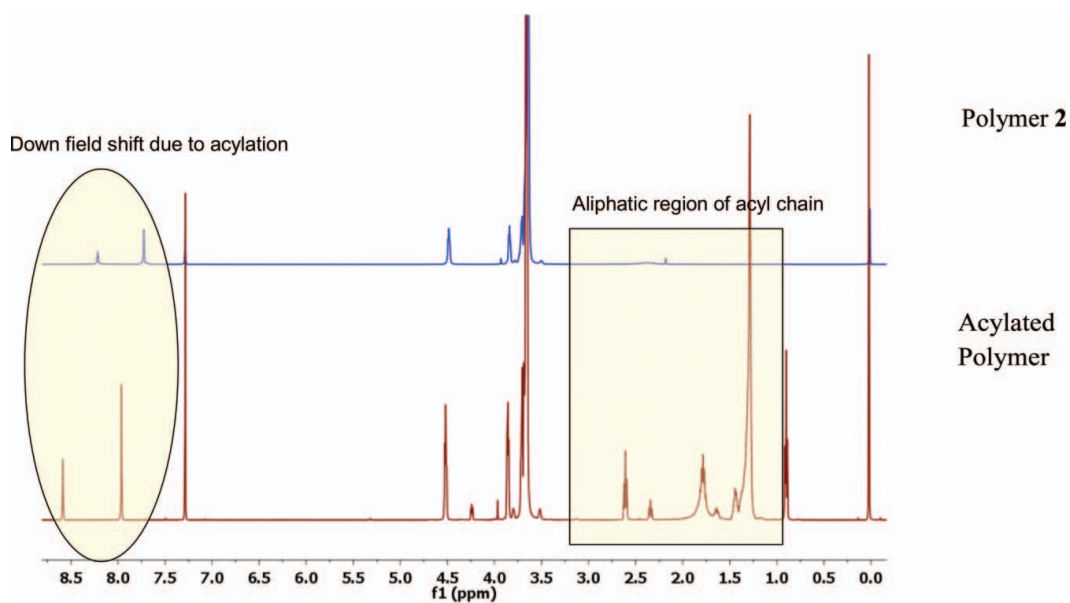


Fig. 1. The ¹H-NMR spectra of polymer **2** before and after acylation. (Color figure available online.)

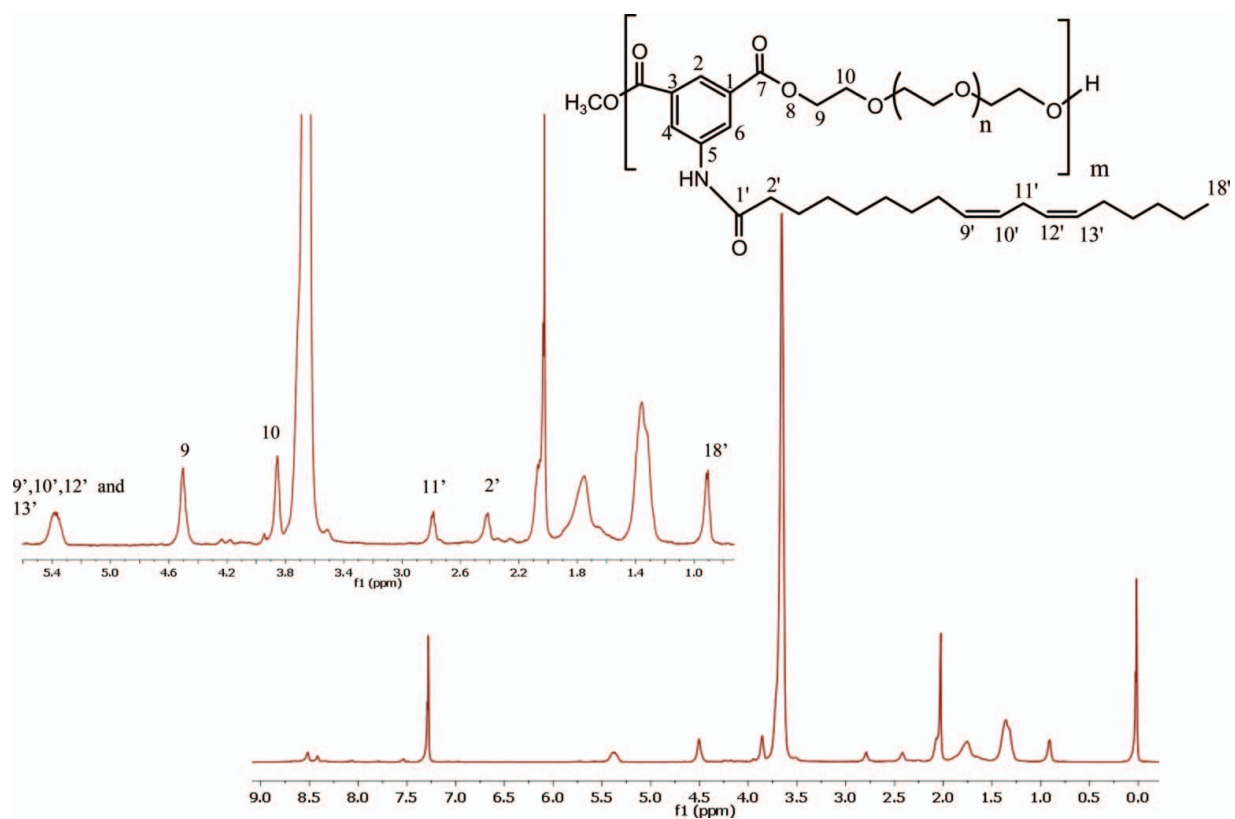


Fig. 2. The ^1H -NMR Spectra of polymer 6. (Color figure available online.)

reactions gave the acylated copolymers **3–6** in 85–90% isolated yields. The structures of these polymers were established on the basis of their ^1H -NMR and ^{13}C -NMR spectra.

Acylation of amino polymers were confirmed by the down field shifts of the aromatic protons at δ 7.46–7.51 (s, 2H, *H*-4 and *H*-6) and δ 7.96–8.03 (s, 1H, *H*-2) before acylation to δ 8.37–8.39 (s, 1H, *H*-2) and δ 8.50–8.51 (s, 2H,

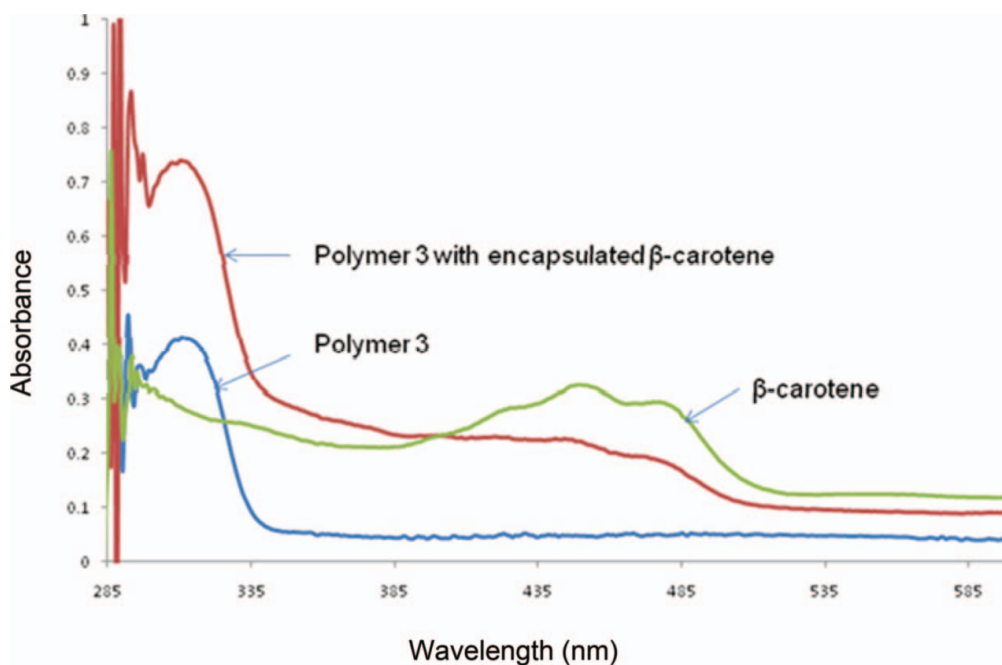


Fig. 3. UV spectra of β -carotene, polymer 3 and encapsulated β -carotene in polymer 3. (Color figure available online.)

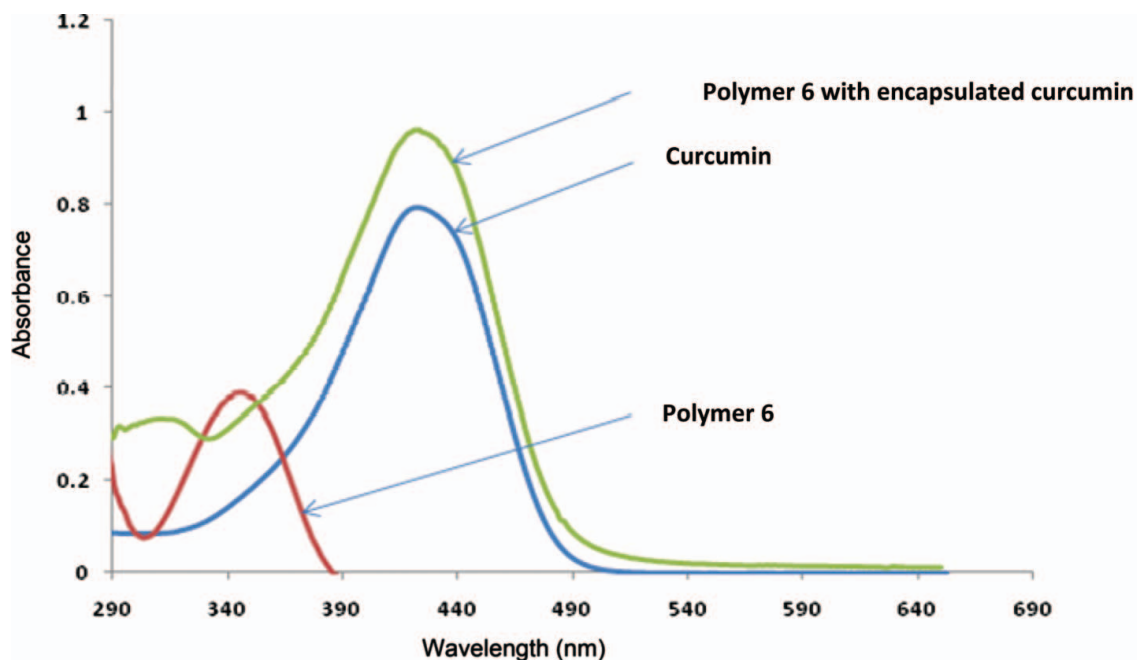


Fig. 4. UV spectra of curcumin, polymer 6 and the encapsulated curcumin in polymer 6. (Color figure available online.)

H-4 and H-6) after acylation. The methylene protons (H-9) of the amide bond shifted from δ 4.40–4.42 to δ 4.48–4.49. Furthermore, the ^{13}C -NMR spectrum displayed signals at δ 172 for C-1" indicating the attachment of acyl side chain through amide linkage. In the course of acylation reactions, the presence of a peak at δ 4.23 (H- α) indicated the end group acylation. The ^1H NMR spectrum (Fig. 1) shows the expected changes in the aliphatic region upon acylation. On the attachment of octadeca-9,12-dienoyl chloride to polymer **2**, apart from the peaks that were observed in the case of attachment of saturated long chain acid chlorides, we observed a peak at δ 5.37 corresponding to four protons attached to unsaturated carbon atoms. The methylene protons (H-11') flanked by two double bonds were observed downfield at δ 2.79. Protons H-8' and H-14' which were present next to double bonded carbons also appeared separately from rest of the aliphatic chain at δ 2.02. The NMR spectrum shown in Figure 2 displays all these peaks observed upon acylation

Table 1. Percentage of encapsulation of curcumin and β -carotene in amphiphilic copolymers

Sidechain in the amphiphilic copolymer	Encapsulation of curcumin (Wt%)	Encapsulation of β -carotene (Wt%)
Nonanoyl	4.86%	1.81%
Tetradecanoyl	5.61%	4.04%
Hexadecanoyl	6.27%	4.29%
Octadeca-9,12-dienoyl	8.24%	5.51%

These amphiphilic polymers self assemble in their aqueous solution to form nano-particles. The bioactive compound curcumin which has considerable aromatic unsaturation and β -carotene which has considerable double bond unsaturation were encapsulated in these amphiphilic copolymers to measure encapsulation capacity of these nano-particles. We have studied further the effects of the introduction of double bond in the side chain on the percentage of encapsulation. The percentage of encapsulation was estimated by UV spectroscopy using a calibration curve for curcumin and β -carotene in methanol. The UV spectra (Figs. 3 and 4) clearly show the encapsulation of active moieties in the nano-particles. It was found that the nature of the side chain influenced the amount of encapsulation. As compared to β -carotene, curcumin was encapsulated in higher percentage as shown in Table 1.

4 Conclusions

In summary, novel PEG amino based amphiphilic copolymers were synthesized by a green chemistry approach using *Candida antarctica* lipase (Novozyme 435) catalysis and were 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. Bioactive hydrophobic compounds: β -carotene and curcumin were encapsulated in these micelles. In amphiphilic copolymers having saturated aliphatic side chain, percentage of encapsulation of curcumin varied from 4.8 to 6.2 and encapsulation of β -carotene from 1.8 to 4.2 percent as side chain length was

increased from C-9 to C-16 carbon atoms. In amphiphilic copolymer having unsaturated aliphatic side chain, percentage of encapsulation of curcumin was found to be 8.2 and encapsulation of β -carotene was found to be 5.5. As we moved from saturated C-9 side chain to unsaturated C-18 side chain having two double bonds, there was considerable enhancement in the percentage of encapsulation. Thus, it was found that the nature of the side chain influences the amount of encapsulation in these polymers.

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