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Design and Biocatalytic Synthesis of Pluronics-based Nanomicellar Self-assembly Systems for Drug Encapsulation Applications

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Nano medicine is an emerging branch of pharmaceuticals, which is gaining considerable attention mainly due to its new and effective way of drug delivery. Various modes of drug delivery are still in their adolescent stage, polymer-based drug delivery system is one among these. In order to develop a novel and biocompatible nano-carrier for drug delivery through a "green" and, environmentally benign approach, herein, we report the design, synthesis and characterization of four novel pluronic-based amphiphilic copolymers. *Candida antartica* lipase was used to catalyze polymerization in the presence of molecular sieves under solventless conditions. The resulting copolymers were also investigated for their supramolecular organization and drug encapsulation capacity for biomedical applications.

Keywords: Pluronics, *Candida antarctica* lipase, amphiphilic polymers, curcumin, encapsulation

1 Introduction

Recent advances in nano-medicine have opened new vistas for pharmaceutical companies mainly due to drug delivery applications. This form of drug delivery not only minimizes the drug use but also enables targeted drug delivery for faster and efficient mode of action. Drug delivery systems need to be biocompatible and should also have an interaction with the drug to be delivered without affecting its therapeutic properties. Drug delivery system may alter the bio-distribution and pharmacokinetics of the associated drug. Furthermore, problems arising due to low drug solubility, degradation, fast clearance rates, non-specific toxicity, inability to cross biological barriers, can be addressed by appropriately designed drug delivery system or nano-medicine (1).

Nano medicines mainly consist of polymer micelles, polymer DNA complexes (polyplexes), nanogels and liposomes. A promising example of such polymer nano materials is represented by pluronics, a family of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers. The block copolymers with different numbers of hydrophilic ethylene oxide and hydrophobic propylene oxide units are characterized by different hydrophilic-lipophilic balance. Due to their amphiphilic character, these copolymers display surfactant properties including ability to interact with hydrophobic surfaces and biological membranes. In aqueous solutions at concentrations above critical micelle concentration (CMC), these copolymers self-assemble into micelles. The core of the micelles consists of hydrophobic PPO blocks that are separated from the aqueous exterior by a shell of hydrated hydrophilic PEO chains (2–5).

Overall, the challenge of increasing the therapeutic effect of drugs, with a concurrent minimization of side effects, can be tackled through proper design and engineering of the drug delivery system (6, 7).

To address these issues, the design and development of novel bio-catalytic approaches for the synthesis of a variety of amphiphilic copolymers has been undertaken. By varying the components, we have developed a wide variety of polymeric materials and explored their biomedical

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applications (8–11). The micelle based amphiphilic copolymers confer enhanced solubility to the encapsulated material in aqueous media, thereby increasing the bioavailability of poorly soluble or insoluble drugs for therapeutic uses.

Herein we report the synthesis of pluronic-based nanomicellar self assembly system which may find application as a novel nano carrier for drug delivery. For this purpose, a low molecular weight pluronic (BASF Pluronic® L44 NF, Mn 2200) was enzymatically copolymerized with various linkers to observe the effect of linkers on the encapsulation properties of the pluronics. Immobilized *Candida antarctica* lipase [also known as Novozyme 435 (CAL-B)] was used as biocatalyst (12, 13) in the presence of molecular sieves under solventless condition. The linkers used were 3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin or 3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4, 8-dimethylcoumarin and dimethyl 5-hydro xyisophthalate or dimethyl 5-aminoisophthalate. This methodology involves a "green" and environmentally benign approach. The synthesized pluronic based amphiphilic copolymers were evaluated for particle formation behavior, their hydrodynamic radius was measured by dynamic light scattering. Moreover these copolymers have the capacity to encapsulate various drugs and bioactive molecules. The synthesized copolymers are novel and were characterized by different spectroscopic techniques.

2 Experimental

2.1 Materials

Novozyme-435, an immobilized enzyme, was a gift from Novozymes A/S, Denmark. Dimethyl 5-hydroxyisophthalate, dimethyl 5-aminoisophthalate, molecular sieves (4Å, beads, 8–12 mesh) and solvents were purchased from Aldrich Corporation, USA. BASF Pluronic® L44 NF (Average Mw 2200, PPO 1160 and PEO 520 × 2) was purchased from BASF, Germany. All other chemicals and solvents were of analytical grade and were used as received unless otherwise noted. Dialysis membranes were purchased from Spectrum Laboratories Inc., CA.

2.2 Characterization

The ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Instrument Inc. 500 MHz ARX spectrometer and 250 MHz spectrometer, respectively using TMS as an internal standard. Gradient HSQC spectrum and DEPT spectrum were recorded on Bruker 500 MHz instrument equipped with inverse probe. Static light scattering data was acquired by a laser light scattering photometer (Wyatt Technology DAWN Model F) equipped with a 632 nm He-Ne laser as the light source. Dynamic light scattering was performed on these micellar solutions using a 50mW He-Ne Laser, an avalanche photodiode detector BI-APD, a digital time correlator BI-9000 and software from Brookhaven Instruments Corporation, and dynamic light scattering software CONTIN and DOUBLE EXPONENTIAL. Infrared spectra of neat samples were recorded on a Thermo Electron Corporation Nicolet 4700 Fourier Transform Infrared (FT-IR) spectrometer. The UV spectra were recorded on a Perkin-Elmer Lambda-9-UV-Vis spectrophotometer (Norwalk, CT). The detailed synthesis and characterization of 3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin and 3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4,8-dimethylcoumarin have been reported earlier by us (8).

2.3 Synthesis of Polymers 1a-b and 2a-b

Pluronic (1 mol) and linker (1 mol) were placed in a round-bottom flask with constant stirring followed by addition of the immobilized enzyme (Novozyme 435, 10% by weight *wrt* monomers) and molecular sieves (10% by weight *wrt* monomers). The resultant reaction mixture was stirred at 90°C under vacuum (100 millitorr) for 48 h and the reaction was quenched by adding chloroform. Enzyme and molecular sieves were removed by filtration and the filtrate was concentrated to obtain the product, which was dissolved in de-ionized water and dialyzed against water using membrane (MWCO 6000-8000). The dialyzed solution was freeze dried to obtain polymers **1a–b** & **2a–b**.

2.3.1. *Polymer 1a*

Synthesis of polymer **1a** was achieved *via* reaction of dimethyl 5-hydroxyisophthalate (1.0 g, 4.7 mmol), and pluronic (9.5 g, 4.7 mmol) in the presence of molecular sieves (1.0 g) and Novozyme-435 (1.0 g).

^{1}H-NMR (δ, $CDCl_3$): 1.14 (brs, CH_3 of PPO), 3.39–3.67 (brs, OC$H_2$and OCH of PPO and OCH_2 of PEO), 3.83 (t, $COOCH_2CH_2$), 3.90 (s, end group OCH_3), 4.47 (t, $COOCH_2CH_2$), 7.72 (brs, aromatic protons), 8.19 (s, aromatic proton).

^{13}C-NMR Data (δ, $CDCl_3$): 17.81 (CH_3 of PPO), 52.61 (OCH_3 end group), 65.61, 68.89, 69.48, 70.91 – 76.57 (methylene carbons of PEO and PPO and CH carbons of PPO), 121.45, 122.48, 132.02, 157.83 (q), 166.12, 166.71.

IR ν_{max}: 3400, 2969, 2866, 1723, 1598, 1449, 1372, 1333, 1297, 1238, 1092, 932, 844, 760, 505 cm^{-1}. UV λ_{max}(MeOH): 310 nm

2.3.2. *Polymer 1b*

Synthesis of polymer **1b** was achieved *via* reaction of dimethyl 5-aminoisophthalate (1.0 g, 4.7 mmol), and pluronic (9.5 g, 4.7 mmol) in the presence of molecular sieves (1.0 g) and Novozyme-435 (1.0 g).

^{1}H-NMR (δ, $CDCl_3$): 1.15 (brs, CH_3 of PPO), 2.20 (brs, D_2O exchangable NH_2),

3.40-3.69 (brs, OC$H_2$and OCH of PPO and OCH_2 of PEO), 3.83 (t, $COOCH_2CH_2$), 3.9 (s, end group OCH_3),

4.48 (t, COOCH_2CH$_2$), 7.53 (brs, aromatic protons), 8.04 (s, aromatic proton).

^{13}C-NMR Data (δ, $CDCl_3$): 19.09 (CH_3 of PPO), 53.77 (OCH_3 end group), 65.84, 68.89, 69.53 (CH_2), 70.95 -76.57 (methylene carbons of PEO and PPO and CH carbons of PPO), 121.27, 121.35, 131.80, 148.51, 166.37, 166.92.

IR ν_{max}: 3357, 2969, 2866, 1720, 1603, 1452, 1372, 1346, 1235, 1091, 1012, 933, 863, 759, 722 cm^{-1}. UV λ_{max}(MeOH): 343 nm.

2.3.3. *Characterization of Polymer 2a*

Synthesis of polymer **2a** was achieved *via* reaction of 3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin (1.00 g, 2.7 mmol), and pluronic (5.5 g, 2.7 mmol) in the presence of molecular sieves (0.65 g) and Novozyme-435 (0.65 g).

^{1}H-NMR (δ, $CDCl_3$): 1.15 (brs, CH_3 of PPO), 1.26 (t, OCH$_2$$CH_3$ end group), 2.43 (s, 3H, -CH_3), 2.63 (t, 2H, CH$_2$), 2.94 (t, CH$_2$), 3.39–3.65 (brs, OCH_2 and OCH of PPO and OCH_2 of PEO), 3.78 (t, COOCH$_2$CH_2), 4.16 (q, OCH_2CH$_3$ end group), 4.20 (t, COOCH_2CH$_2$), 4.57 (s, OCH$_2$CO), 6.76 (brs, CH), 6.91 (brs, CH), 7.55 (brs, CH).

^{13}C NMR Data (δ, $CDCl_3$): 14.38 (CH_3 end group), 15.29 (CH$_2$), 17.69 (CH_3), 23.49 (CH$_2$), 32.90 (CH$_2$), 57.70 (OCH$_2$ end group), 61.73 (CH$_2$), 64.03 (CH$_2$), 68.86 (CH$_2$x 2), 69.40 (CH$_2$), 70.64–75.95 (methylene carbons of PEO and PPO and CH carbons of PPO), 103.40 (CH), 113.75 (CH), 121.45, 125.93 (CH) 148.20, 153.96, 162.02, 162.32 (q), 173.13, 175.50.

IR ν_{max}: 2969, 2866, 1710, 1609, 1452, 1372, 1347, 1295, 1246, 1089, 933, 846 cm^{-1}.

UV λ_{max}(MeOH): 323, 278 nm.

2.3.4. *Characterization of Polymer 2b*

Synthesis of polymer **2b** was achieved via reaction of 3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4, 8-dimethylcoumarin (1.00 g, 2.6 mmol), and pluronic (5.3 g, 2.6 mmol) in the presence of molecular sieves (0.63 g) and Novozyme-435 (0.63 g).

^{1}H-NMR (δ, $CDCl_3$): 1.15 (brs, CH_3 of PPO), 1.26 (t, OCH$_2$$CH_3$ end group), 2.31 (s, 3H, $-CH_3$), 2.41 (CH_3), 2.65 (t, CH$_2$), 3.01 (t, CH$_2$), 3.41–3.67 (brs, OCH_2 and OCH of PPO and OCH_2 of PEO), 3.77 (t, COOCH$_2$CH_2), 4.14 (q, OCH_2CH$_3$ end group), 4.20 (t, COOCH_2CH$_2$), 4.61 (s, OCH$_2$CO), 6.77 (brs, CH), 7.33 (brs, CH).

^{13}C-NMR Data (δ, $CDCl_3$): 14.38 (CH_3 end group), 14.58 (CH_3), 15.29 (CH$_2$), 17.69 (CH_3), 23.58 (CH$_2$), 33.09 (CH$_2$), 58.78 (OCH$_2$ end group), 61.89 (CH$_2$), 64.03 (CH$_2$), 68.86 (CH$_2$), 69.40 (CH$_2$), 70.64–75.95 (methylene carbons of PEO and PPO and CH carbons of PPO), 100.01 (CH), 115.50 (q), 122.32 (q), 122.87 (CH), 147.64 (q), 158.17(q), 162.08 (q), 168.87 (q), 173.29, 175.50.

IR ν_{max}: 2969, 2866, 1706, 1668, 1601, 1451, 1373, 1347, 1280, 1248, 1093, 935, 844, 779, 529 cm^{-1}. UV λ_{max}(MeOH): 323, 278 nm.

3 Results and Discussion

3.1 Synthesis and Characterization

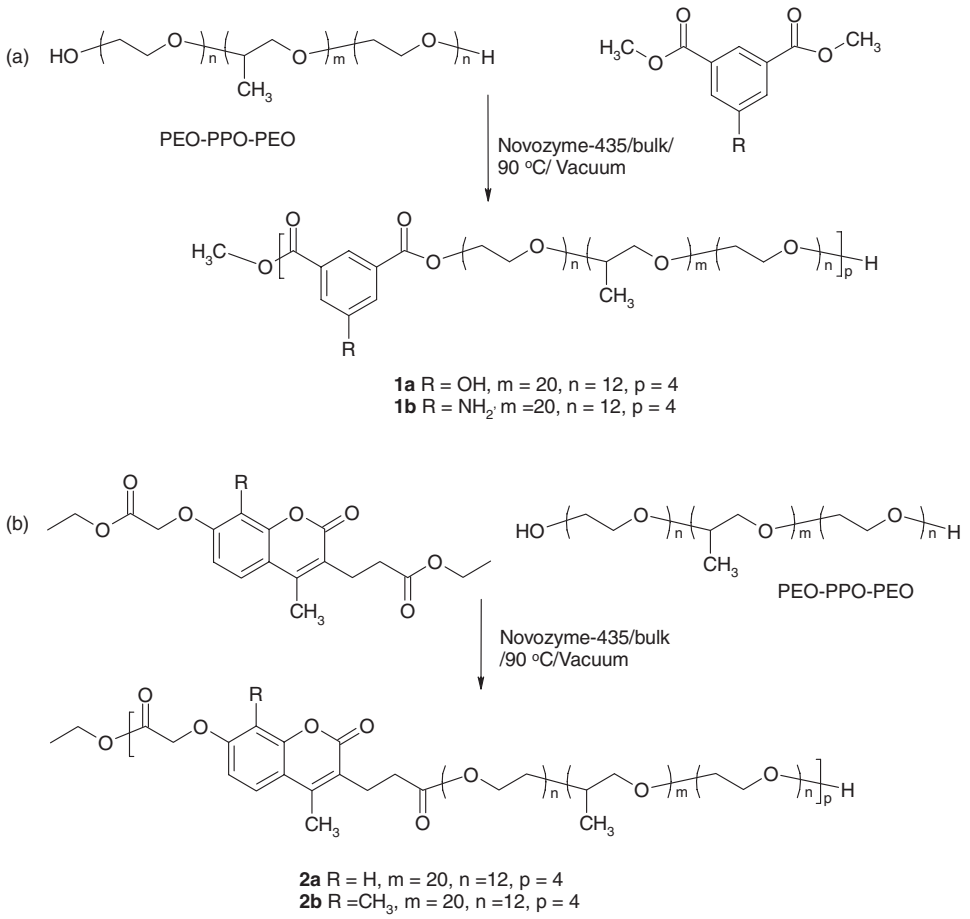
The Novozyme - 435 (immobilized *Candida antarctica* lipase B) catalyzed condensation of dimethyl ester of 5-amino/5-hydroxy isophthalate or 3-(2-ethoxycarbonyl) ethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin/3-(2-ethoxycarbonyl)ethyl-7-ethoxycarbonylmethoxy-4,8-dimethylcoumarin and pluronic (M_n2200) under solventless conditions gave the polymers **1a-b** and **2a-b** in 80% isolated yields (Sch.1(a, b)). The

4-methylcoumarin monomers were synthesized by the condensation of resorcinol/2-methylresorcinol with diethyl 2-acetylglutarate using polyphosphoric acid as the condensing reagent (8). The polymers **1a** and **1b** were characterized by the presence of a peak at δ 4.47 and 4.48 ppm (Fig. 1), respectively due to the *trans* esterification between the hydroxyl group of the pluronic and isophthalate. Formation of the ester bond was supported by ^{13}C-resonances at δ 65.61 and 65.84 ppm, respectively for polymer **1a** and **1b**. This observation was further supported by correlation of the ^{1}H-resonance at δ 4.47 ppm with the ^{13}C-resonance at δ 65.84 ppm in the gradient HSQC NMR of polymer **1b**. The UV, IR, ^{1}H - ^{1}H COSY and DEPT-135 carbon NMR also supported the above characterization.

The structures of the polymers **2a-b** were also similarly established. The proton NMR spectrum of polymer **2a** displayed a resonance at δ 4.20 ppm due to the formation of new ester bond (Fig. 2). This was supported by the carbon NMR spectrum which showed a resonance at 64.03. Both these resonances correlated with each other in the gradient HSQC NMR of polymer **2a**. The DEPT-135 carbon NMR of polymer **2a** showed three aromatic CH carbons at δ 103.40, 113.75 and 125.93 ppm along with other CH, CH$_2$ and CH_3 carbons. Molecular weights of these polymers were determined by GPC and also by end group analysis and came out in the range of 10 KD.

3.2 Supramolecular Organization

The supramolecular organization of polymers **1a-b** and**2a-b** in aqueous and organic media was studied by static light scattering (SLS) and dynamic light scattering (DLS). SLS showed radius of gyration (R_g) in the range of 55.5 to 57.1 nm in the case of isophthalate based polymers **1a–b** and 49.0 to 50.8 nm in the case of coumarin polymers **2a-b**. The hydrodynamic radius obtained from DLS measurements showed a smaller size, in the range of 23.2 to 25.9 nm, for all four polymers. This difference can be attributed to the higher influence of unmicellized polymers in SLS than DLS measurements (14). Typically for a solid colloidal particle the radius of gyration is smaller than the hydrodynamic radius. However, if the micelle formed has a smaller condensed core behaving as a solid particle and



Sch. 1. Synthesis of pluronics-based amphiphilic copolymers.

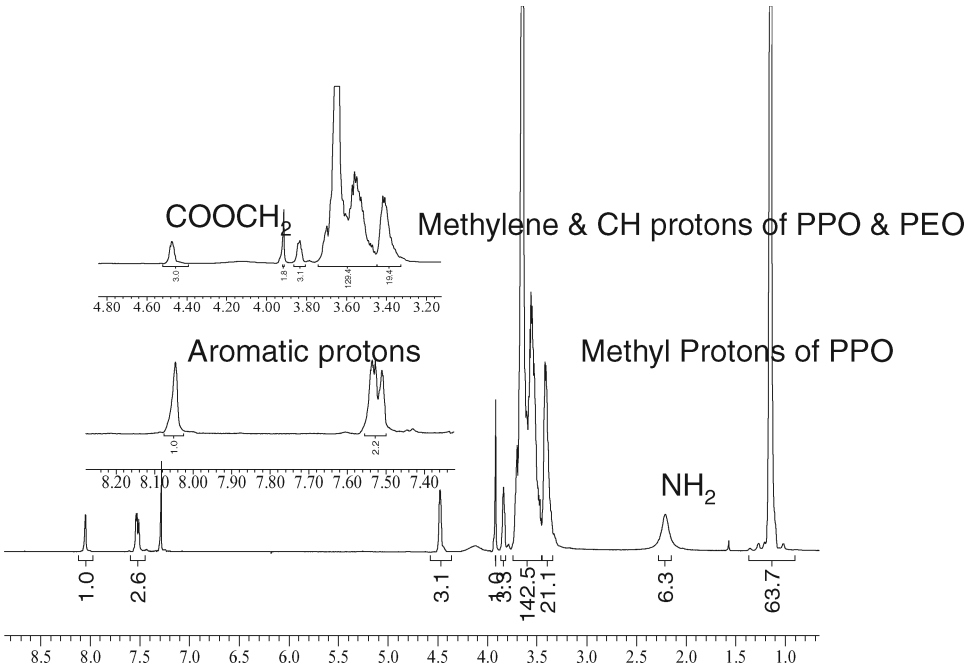


Fig. 1. ^{1}H-NMR spectrum of copolymer **lb**.

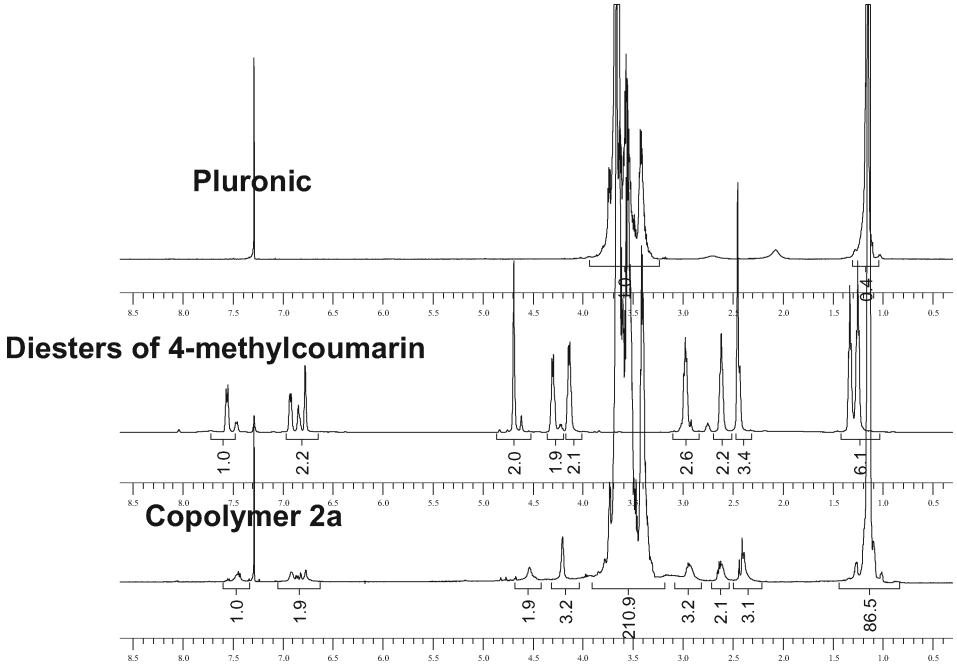


Fig. 2. [1]H-NMR spectrum of copolymer **2a**.

a long coronal region is mobile in the solvent, R_H can be smaller than R_g.

Also, we studied the drug encapsulation potential of our novel polymeric systems by attempting the encapsulation of antitumor hydrophobic drug "curcumin" (Fig. 3). The copolymers **1a–b/2a–b** and the hydrophobic drug curcumin were dissolved in acetonitrile, in a 1:2 drug/polymer w/w ratio, and mixed for 15 min. The organic solvent was then evaporated under vacuum. The viscous mixture of drug and polymer was then dissolved in water with extensive vortexing. Non-incorporated curcumin was filtered off the nanoparticle suspension through a 0.2 μm filter (curcumin cannot pass through the filter unless the drug is solubilized in nanoparticles). As seen by UV-spectroscopy, the polymers have no absorption above 400 nm while curcumin has an absorbance max at 425 nm. The concentration of curcumin in the filtrate could then be estimated by measuring absorbance at 425 nm and using a calibration curve for curcumin in methanol. The percentage solubilization/encapsulation of curcumin was found to be in the range of 2.7–5.7% *wrt* the weight of the different polymers (Table 1). Thus, it can be concluded that changing the linker on pluronics based polymer changes the percentage of encapsulation of curcumin.

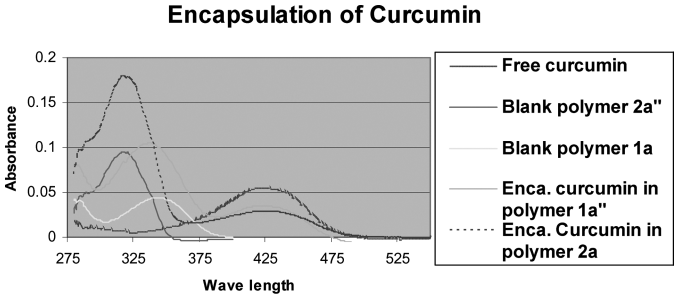


Fig. 3. UV-spectra of polmers **1a** and **2a** before and after encapsulation of the hydrophobic anti-tumor drug curcumin, using methanol as a solvent.

Table 1. Radius of gyration, hydrodynamic radius and molecular wt. of copolymers **1a-b** and **2a-b**

Polymer	*Radius of gyration (R_g)*	*Hydrodynamic radius (R_H)*	*Percentage of encapsulation of curcumin (by wt%)*	*Average molecular wt. (by NMR)*	*Concentration used for measurements of Rg and R_H*
1a	57.1 $\pm$ 8.7 nm	24.6 $\pm$ 0.03 nm	5.7%	10 KD	2.9 mg/mL
1b	55.5 $\pm$ 9.7 nm	24.6 $\pm$ 0.03 nm	4.2%	10 KD	2.9 mg/mL
2a	50.8 $\pm$6.3 nm	25.9 $\pm$ 0.17 nm	3.3%	10 KD	2.9 mg/mL
2b	49.0 $\pm$ 2.7 nm	23.2 $\pm$ 0.50 nm	2.7%	10 KD	3.4 mg/mL

4 Conclusions

An environmentally benign approach has been designed and developed to make novel materials by using a low molecular wt. pluronic and different aromatic linkers. Our methodology follows greener approach and utilizes the properties of both pluronic and aromatic linkers for drug encapsulation. The potential applications of the synthesized polymeric materials were touched upon and it was demonstrated that these new copolymers may potentially be useful in biomedical applications. The developed polymeric materials are capable of solubilizing/encapsulating the hydrophobic drug curcumin.

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