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# Synthesis and pH-Dependent Micellization of a Novel Block Copolymer Containing s-Triazine Linkage

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**Abstract:** Novel pH-sensitive moieties containing an s-triazine ring were synthesized with sulfonamide and secondary amino groups. The synthesized pH-sensitive moieties were used for the synthesis of a pH-sensitive amphiphilic ABA triblock copolymer. The pH-sensitive triblock copolymer was composed of diblock copolymers, methoxy poly(ethylene glycol)-poly (ε-caprolactone-*co*-D,L-lactide) (MPEG-PCLA), and pH-sensitive moiety. These copolymers could be dissolved molecularly in both acidic and basic aqueous media at room temperature due to secondary amino and sulfonamide groups. The synthesized s-triazine rings containing pH-sensitive compounds were characterized by $^{1}$H-NMR, $^{13}$C-NMR, and LC/MSD spectral data. The synthesized diblock and triblock copolymers were also characterized by $^{1}$H-NMR and GPC analyses. The critical micelle concentrations at various pH conditions were determined by fluorescence technique using pyrene as a probe. Furthermore, the micellization and demicellization study of the triblock copolymer was done with pH-sensitive groups. The sensitivity towards pH change was further established by acid-base titration.

*Keywords*: ABA triblock copolymers, s-triazine ring, pH-sensitive moiety, critical micelle concentration, micellization /demicellization.

## Introduction

Polymeric drug delivery systems can be used not only to maximize therapeutic activity while minimizing negative side effects, but also to achieve temporal and distribution controls in drug therapy.[1] Although, significant advances have been recently made in the field of intelligent polymers, the problem of optimum delivery at physiological pH remains a formidable challenge.[2,3] The small but clear difference in pH has been an interesting subject for drug delivery and various efforts has been devoted to construct pH-sensitive micelles or liposomes. However, because conventional pH-sensitive functional groups (usually carboxylic groups) provide limited pH-sensitivity in polymers, their applications in biological and pharmaceutical system have been severely restricted. It is well documented in literature that certain pH-sensitive groups like acid,[4] amino[5], and sulfonamide[6] can have pH-activity, when they are introduced in the polymeric chain.

Triazine ring have demonstrated a broad range of biological activities,[7-10] and it can be used as a linker or building block for polymer synthesis,[11-13] biological active material,[14] dyeing,[15] carbohydrate,[16] protein modifier,[17] and gene therapy.[18] The chemistry of 2,4,6-trichloro-1,3,5-triazine [s-triazine] has been extensively investigated.[19] With the advent of combinatorial chemistry, several triazine libraries have been published in the literature because of the advantage of its easy manipulation and the low price of stating material both in solid[20] and solution[21] phase. It was revealed that the selective nucleophilic displacement of chlorine atoms from s-triazine by oxygen, nitrogen or sulfur are possible under temperature control.[11,22]

In this study, we have synthesized pH-sensitive triblock copolymer composed of diblock copolymers, methoxy-poly (ethylene glycol)-poly(ε-caprolactone-*co*-D,L-lactide) (MPEG-PCLA) and s-triazine ring as building block containing sulfonamide and secondary amino groups in the main unit. We thus anticipated that, pH-dependent micellization/demicellization can be achieved by the ionization of sulfonamide and amino group at certain pH conditions. Furthermore, since the pH sensitive groups are inserted in hydrophobic segment, more sensitive response can be expected compared to that in hydrophilic segment.[23] The physiochemical properties of the micelles were investigated in terms of critical micelle concentration (CMC) and pH-sensitivity by the fluorescence of pyrene at different pH.

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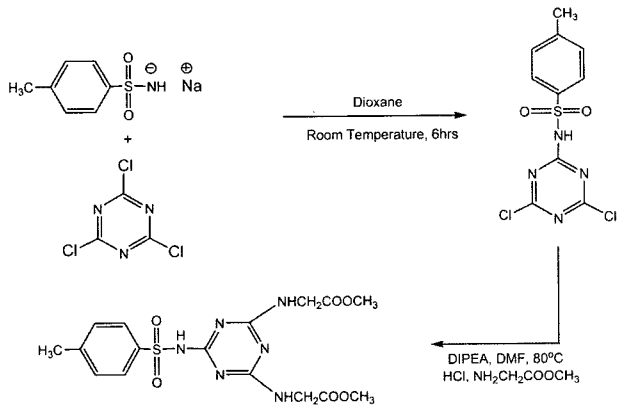
## Experimental

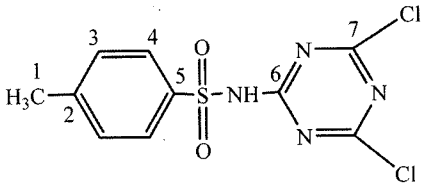
**Materials.** Mono-methoxy poly(ethylene glycol) ($M_n$= 750 and $M_n$=2,000), D,L-lactide (LA), $\varepsilon$-caprolactone (CL), sulfamethazine, anhydrous methylene chloride (MC), 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride), diisopropyl carboimide (DIPC), 4-(dimethyl amino) pyridine, *p*-toluene sulfonic acid, *p*-toluene sulfonamide, anhydrous 1,4-dioxane, diisopropyl ethyl amine (DIPEA) and *N*,*N*-dimethyl formamide (DMF) were purchased from Aldrich. The coupling catalyst (DPTS), a complex structure of 4-(dimethylamino) pyridine (DMAP) and toluenesulfonic acid (PTSA), was synthesized by reported procedure.[24] All the reagents and solvents have been used as received from Aldrich.

**Synthesis of Diacid with Sulfonamide Directly Linked to s-Triazine Ring (ST).** The diacid (ST) was synthesized by multiple step reactions of cyanuric chloride, *p*-toluene sulfonamide and glycine methyl ester. First the synthesis of *N'*-(dichloro triazinyl)-sulfonamide (1) was achieved by reaction of mono-substituted cyanuric chloride with *p*-toluene sulfonamide according to reported procedure.[25] The second step involves the nucleophilic substitution of amino group into the s-triazine ring using glycine methyl ester hydrochloride to form diester containing sulfonamide and amino groups in the main unit (Scheme I), which further undergoes hydrolysis to give desired diacid (Scheme III).

**Synthesis *N'*-(dichloro triazinyl)-sulfonamide:** The general procedure for synthesis of *N'*-(dichloro triazinyl)-sulfonamide is as follows: First, dry sodium salt of *p*-toluene sulfonamide was made by direct reaction with sodium hydroxide and *p*-toluene sulfonamide. In a 500 mL round-bottom flask charged with cyanuric chloride (28.5 g) and dioxane (220 mL). The fine powder of sodium salt of *p*-toluene sulfonamide (57.9 g) was added slowly with stirring. In the beginning, exothermic reaction was moderated by cooling. After 6 hrs shaking, the mixture was filtrated, the filter residue was washed with diethyl ether, and the sodium salt obtained was dissolved in water (800 mL). The solution was neutralized with carbon dioxide gas, filtered and the filtrate was acidified with dilute HCl to give compound (1) in 93% yields. The purification was done by crystallization from 80% ethanol. The synthesized compound was characterized by its melting temperature of 207 °C.

**Scheme I**



(1)

FTIR (KBr, $cm^{-1}$): 3350-3250 (-NH-); 1570-1550 (-C=N-, -Ar-NH-); 1330, 1140 (-S=O), Figure 1(A)

$^1$H-NMR (500 MHz, DMSO-$d_6$): 7.75 (2H,d,$H_4$); 7.30 (2H,d,$H_4$); 4.2 (1H,s,-NH-); 2.40 (3H,s,$H_1$), Figure 2(A)

$^{13}$C-NMR (500 MHz, DMSO-$d_6$): 150.0 ($C_6$); 148.50 ($C_7$); 142.40 ($C_2$); 138.05 ($C_5$); 130.00 ($C_3$); 127.50 ($C_4$); 20.14 ($C_1$), Figure 3(A)

LC/MSD [negative; m/e (relative intensity)]: $M^+$ 317.97, Figure 4

**Synthesis of Diester with Sulfonamide Directly Linked to s-Triazine Ring ($ST_{diester}$):** The general procedure for synthesis of $ST_{diester}$ is as follows: The 100 mL two neck round-

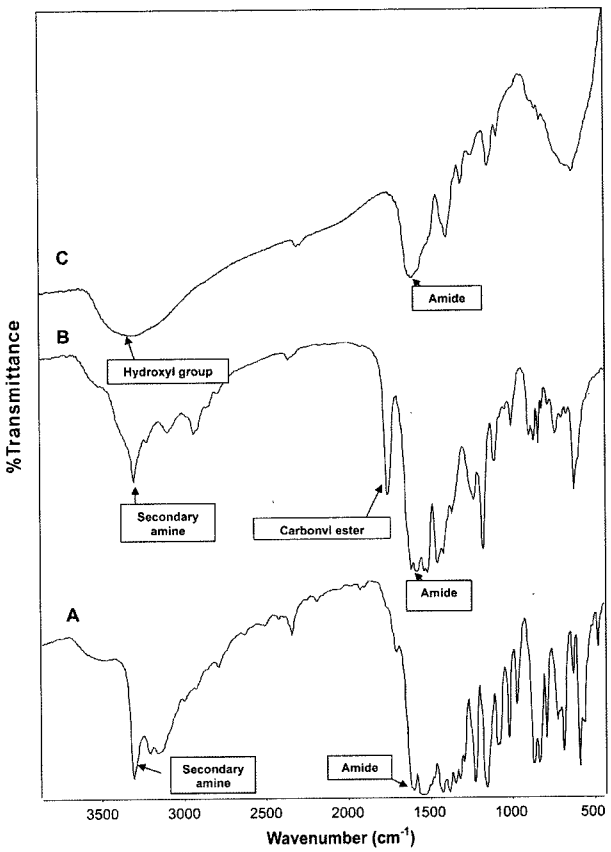



**Figure 1.** FTIR spectra of (A) *N'*-(dichloro triazinyl)-sulfonamide, (B) $ST_{diester}$, and (C) ST using KBr pellet technique.

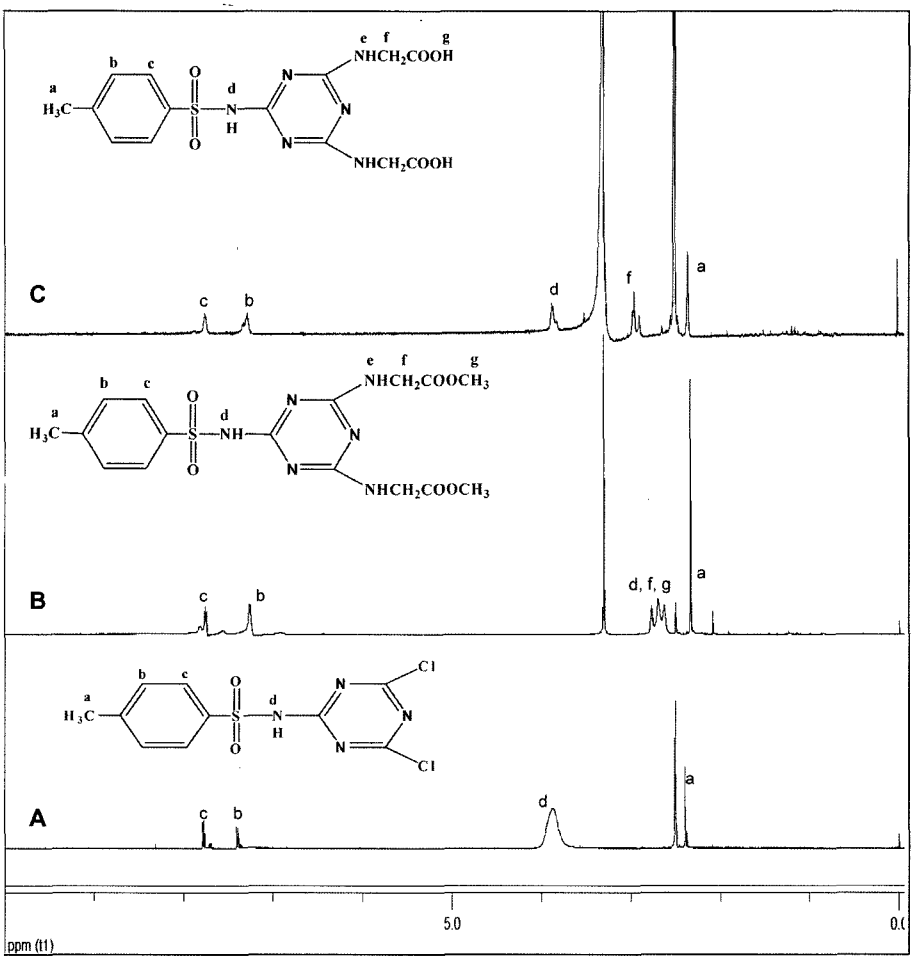



**Figure 2.** $^{1}$H-NMR spectra of (A) *N'*-(dichloro triazinyl)-sulfonamide, (B) $ST_{diester}$, and (C) ST in DMSO-$d_6$.

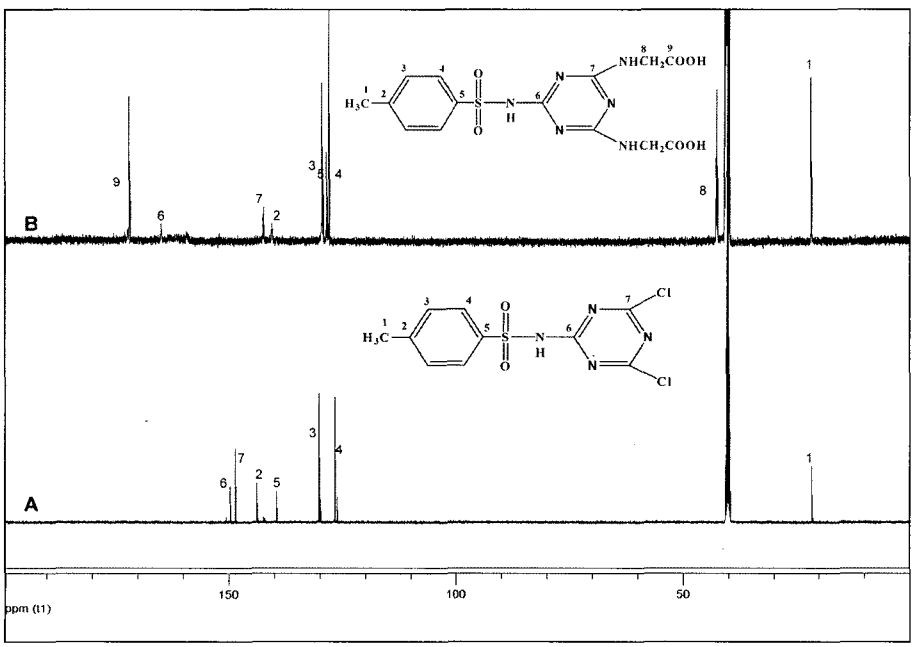



**Figure 3.** $^{13}$C-NMR spectra of (A) *N'*-(dichloro triazinyl)-sulfonamide and (B) ST in DMSO-$d_6$.

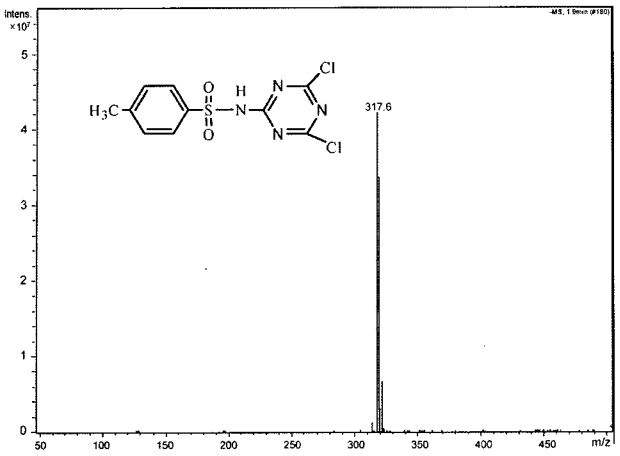



**Figure 4.** LC/MSD spectra of *N'*-(dichloro triazinyl)-sulfonamide using DMF as solvent.

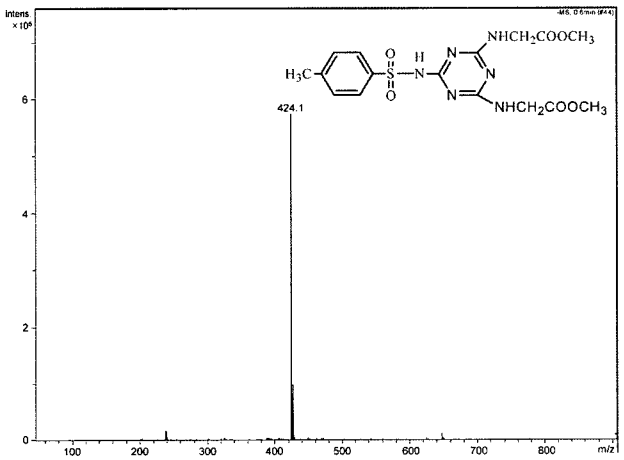



**Figure 5.** LC/MSD spectra of $ST_{diester}$ using DMF as solvent.

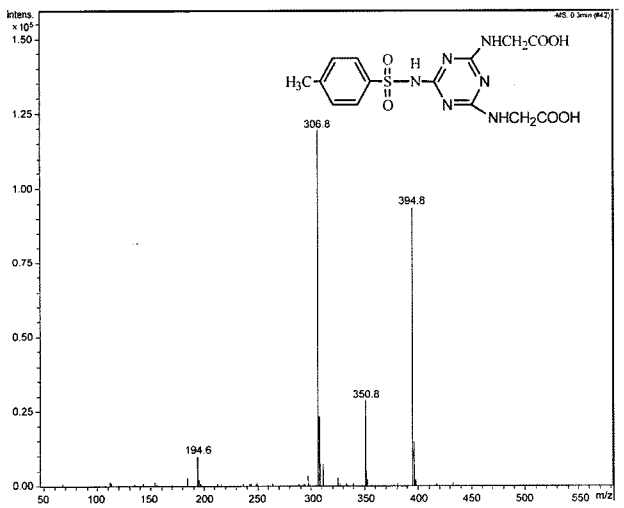
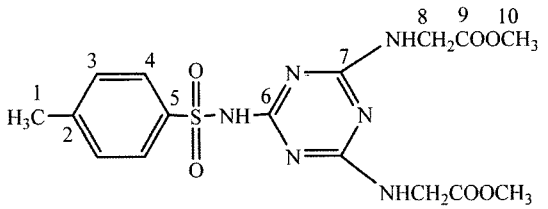



**Figure 6.** LC/MSD spectra of ST using DMF as solvent.

bottom flask containing glycine methyl ester hydrochloride (3.53 g) was charged with nitrogen. Anhydrous DMF (50 mL) and DIPEA (10.2 mL) were added and stirred at room temperature for 2 hrs. The solution of compound (1) in DMF (20 mL) was added to flask, and stirred at 45 °C for 5 hrs and then the temperature was increased to 80 °C for 45 hrs. The reaction content was poured into water and filtered. The crude diester was obtained in 85% yield. The purification of diester was achieved by silica column using ethyl acetate as an eluent.



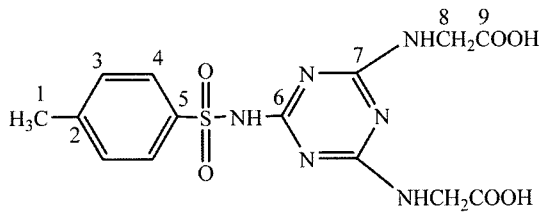
(2)

FTIR (KBr, $cm^{-1}$): 3350-3250 (-NH-); 1745 (-C=O); 1570-1550 (-C=N-,-Ar-NH-); 1330,-1140 (-S=O); 1190-1095 (-C-O-), Figure 1(B)

$^1$H-NMR (500 MHz, DMSO-$d_6$): 7.75 (2H,d,$H_4$); 7.30 (2H,d,$H_3$); 3.80 (3H,s,-NH-); 3.75(4H,d,H8); 3.70 (6H,s,$H_{10}$); 2.40 (3H,s,$H_1$), Figure 2(B)

LC/MSD [negative; m/e (relative intensity)]: $M^+$ 424.1, Figure 5

**Synthesis of Diacid with Sulfonamide Directly Linked to s-Triazine Ring (ST):** The synthesized $ST_{diester}$ was further applied for hydrolysis of compound (2) using LiOH and MeOH at reflux temperature for 5 hrs. The methanol was evaporated under vacuum and then aqueous layer was neutralized using dilute HCl at pH 7.0. The white solid compound, ST, was obtained in 50% yield.



(3)

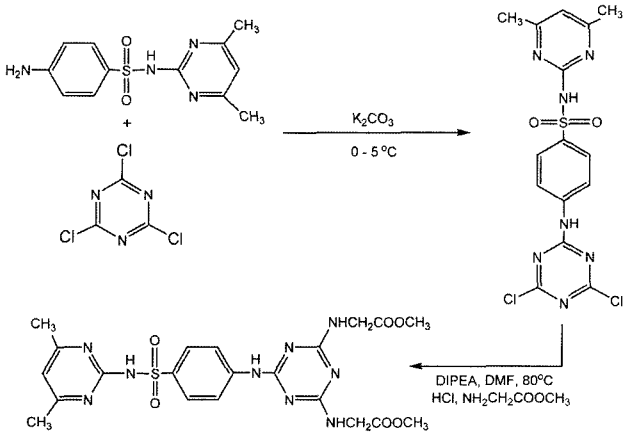
FTIR (KBr, $cm^{-1}$): 3450 (-OH); 3350-3250 (-NH-); 1710 (-C=O); 1570-1550 (-C=N-,-Ar-NH-); 1330-1140 (-S=O); 1240 (-C-O-), Figure 1(C)

$^1$H-NMR (500 MHz, DMSO-$d_6$): 7.75 (2H,d,$H_4$); 7.30 (2H,d,$H_3$); 3.85 (3H,s,-NH-); 3.05 (4H,d,H8); 2.40 (3H,s,$H_1$), Figure 2(C)

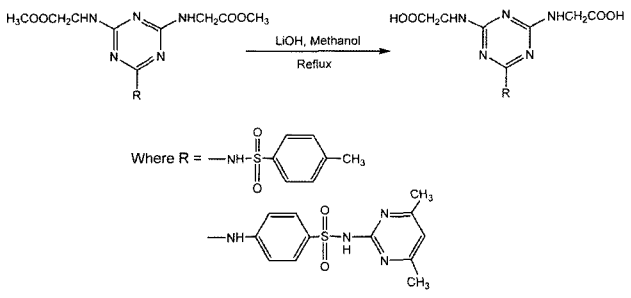
$^{13}$C-NMR (500 MHz, DMSO-$d_6$): 178.45 ($C_6$); 176.50 ($C_7$); 173.50 ($C_9$); 142.25 ($C_2$); 135.05 ($C_5$); 130.00 ($C_3$); 127.50 ($C_4$), 43.57 (C8); 20.50 ($C_1$), Figure 3(B)

LC/MSD [negative; m/e (relative intensity)]: $M^+$ 394.8, Figure 6

**Scheme II**

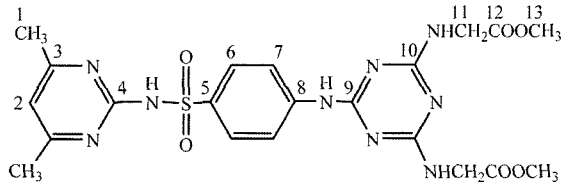



**Scheme III**




**Synthesis of Diacid with Sulfamethazine Directly Linked to s-Triazine Ring (SMT).** The diacid SMT was synthesized by similar procedure as described for synthesis of diacid ST except the first step. First, the synthesis of *N'*-(dichloro triazinyl)-sulfamethazine (4) was achieved by reaction of mono substituted cyanuric chloride with sulfamethazine using $K_2CO_3$. The second step involves the nucleophilic substitution of amino group into s-triazine ring using glycine methyl ester hydrochloride to form diester containing sulfonamide and amino groups in the main unit (Scheme II), which was further undergoes hydrolysis to give desired diacid (SMT) (Scheme III) were done by same procedure as described above.

**Synthesis of *N'*-(dichloro triazinyl)-sulfamethazine:** The general procedure for synthesis of compound (4) is as follows: A 500 mL round-bottom flask containing 50 mL of distilled water was cooled to 0 °C. Then cyanuric chloride (22.13 g) dissolved in acetone 200 mL was added dropwise and the temperature was maintained at 0 °C. After 30 min stirring, the solution of sulfamethazine (27.83 g) and $K_2CO_3$ (6.91 g) dissolved in water (300 mL) was added dropwise at the same temperature. The reaction mixture was stirred for 6 hrs and acidified with dilute HCl. Further the solid compound was filtered and dried. The dried compound was washed with dichloromethane (500 mL) to remove unreacted cyanuric chloride and vacuum dried to give the compound (4) in 95% yields.



(4)

FTIR (KBr, $cm^{-1}$): 3350-3250 (-NH-); 1570-1550 (-C=N-, -Ar-NH-); 1330-1140 (-S=O), Figure 7(A)

$^1$H-NMR (500 MHz, DMSO-$d_6$): 10.5 (1H, s, $-SO_2NH-$); 7.95 (2H,d,$H_6$); 7.75 (2H,d,$H_7$); 6.70 (1H,s,$H_2$); 2.25 (6H,s,$H_1$), Figure 8(A)

$^{13}$C-NMR (500 MHz, DMSO-$d_6$): 169.85 ($C_9$); 166.50 ($C_{10}$); 158.05 ($C_4$); 155.04 ($C_3$); 142.10 (C8); 130.02 ($C_6$); 122.85 ($C_5$); 120.50 ($C_2$); 112.68 ($C_7$); 21.50 ($C_1$), Figure 9(A)

LC/MSD [negative; m/e (relative intensity)]: $M^+$ 425, Figure 10

**Synthesis of Diester with Sulfamethazine Directly Linked to s-Triazine Ring ($SMT_{diester}$):** This compound $SMT_{diester}$ was synthesized according to the procedure used

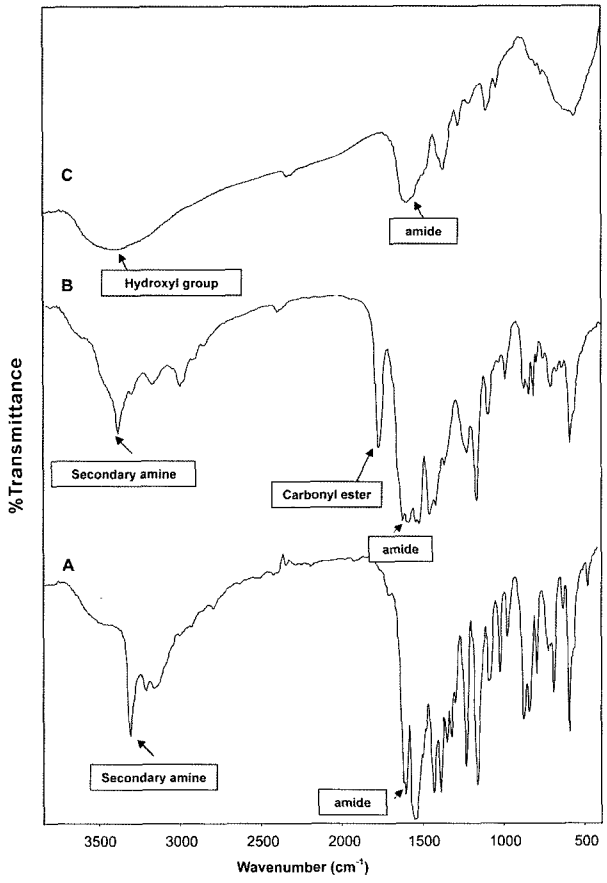



**Figure 7.** FTIR spectra of (A) *N'*-(dichloro triazinyl)-sulfamethazine, (B) $SMT_{diester}$, and (C) SMT using KBr pellet technique.

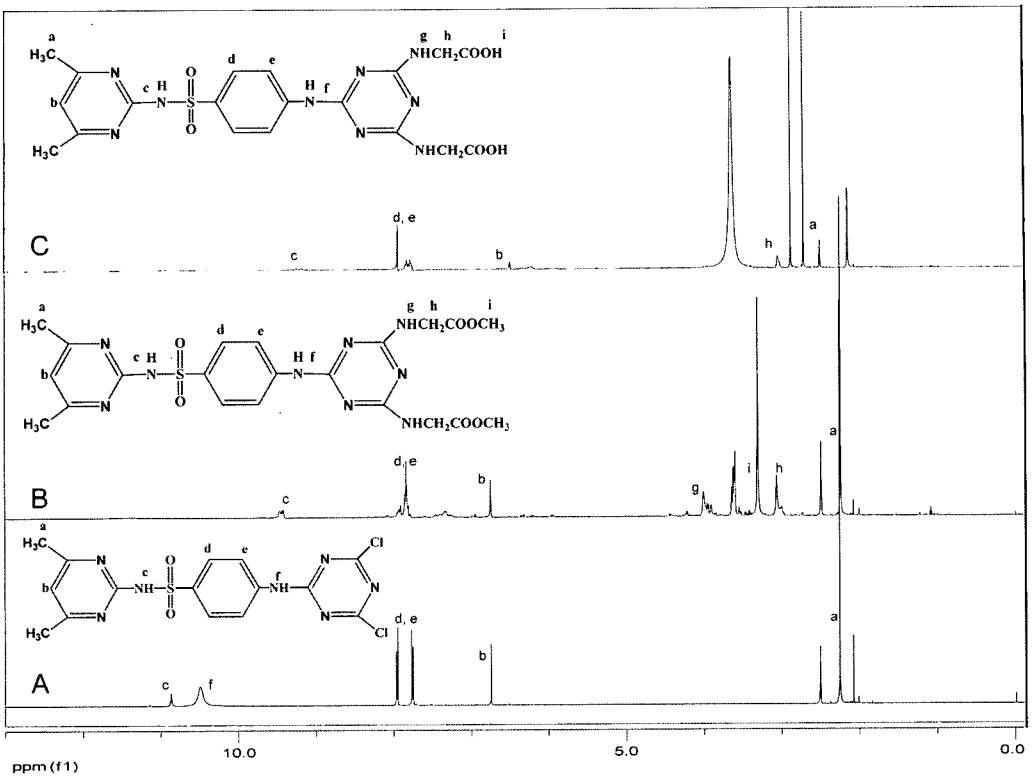



**Figure 8.** $^{1}$H-NMR spectra of (A) *N'*-(dichloro triazinyl)-sulfamethazine, (B) $SMT_{diester}$, and (C) SMT in DMSO-$d_6$.

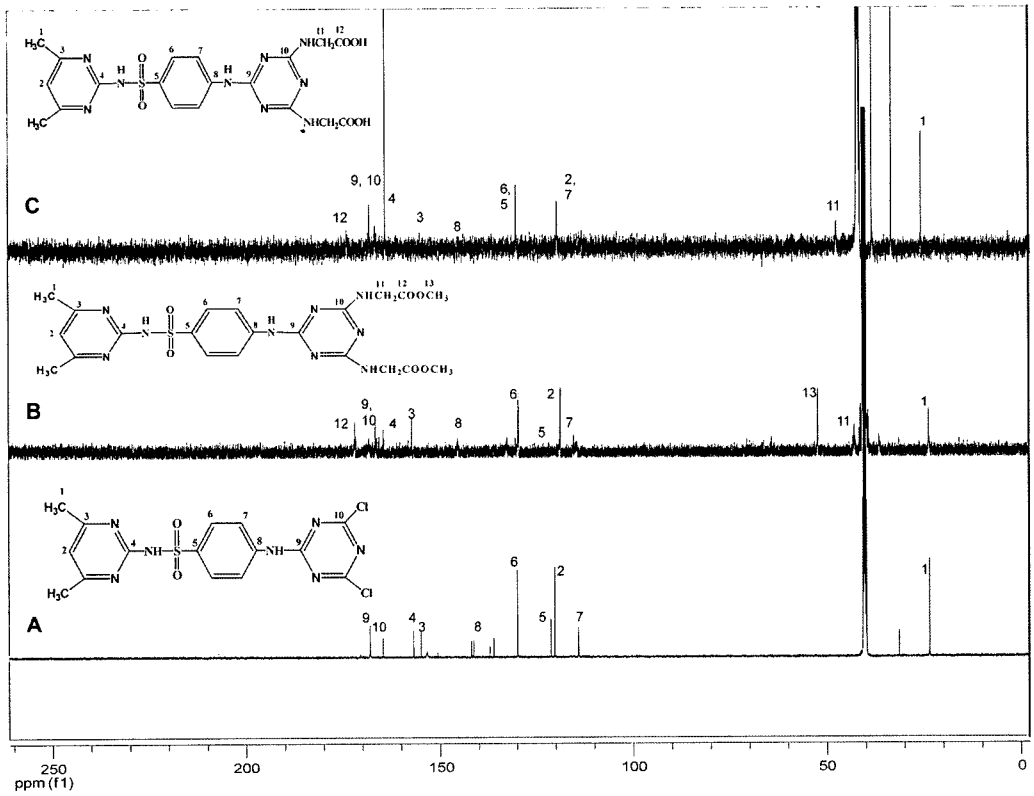



**Figure 9.** $^{13}$C-NMR spectra of (A) *N'*-(dichloro triazinyl)-sulfamethazine, (B) $SMT_{diester}$, and (C) SMT in DMSO-$d_6$.

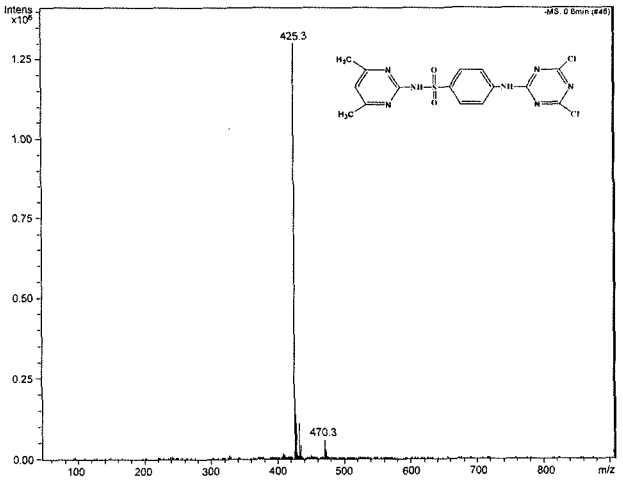



**Figure 10.** LC/MSD spectra of *N'*-(dichloro triazinyl)-sulfamethazine using DMF as solvent.

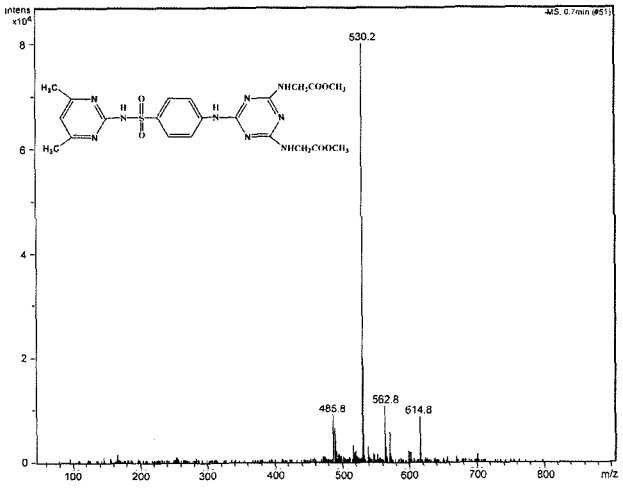



**Figure 11.** LC/MSD spectra of $SMT_{diester}$ using DMF as solvent.

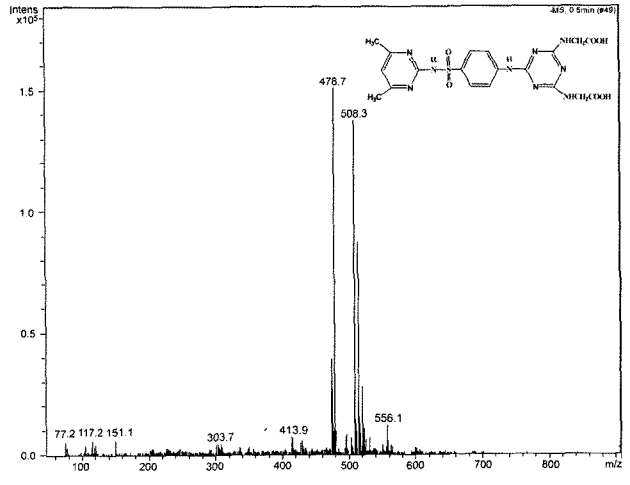
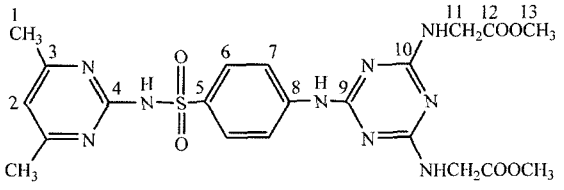



**Figure 12.** LC/MSD spectra of SMT using DMF as solvent.

for synthesis of $ST_{diester}$.



(5)

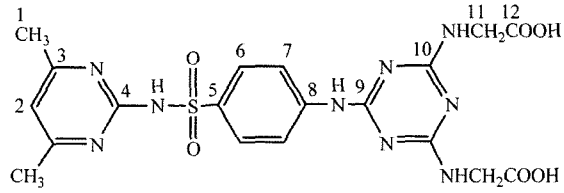
FTIR (KBr, $cm^{-1}$): 3350-3250 (-NH-); 1745 (-C=O); 1570-1550 (-C=N-,-Ar-NH-); 1330-1140 (-S=O); 1190-1095 (-C-O-), Figure 7(B)

$^1$H-NMR (500 MHz, DMSO-$d_6$): 9.5 (1H,s, $-SO_2NH-$); 7.85 (2H,d,$H_6$); 7.80 (2H,d,$H_7$); 6.70 (1H,s,$H_2$); 4.5 (3H,s,-NH-); 3.35 (6H,s,$H_{13}$); 3.05 (4H,d,$H_{11}$); 2.25 (6H,s,$H_1$), Figure 8(B)

$^{13}$C-NMR (500 MHz, DMSO-$d_6$): 172.085 ($C_9$); 168.50 ($C_{12}$); 167.50 ($C_{10}$); 162.20 ($C_4$); 157.64 ($C_3$); 142.05 (C8); 131.02 ($C_6$); 130.05 ($C_5$); 120.50 ($C_2$); 112.68 ($C_7$); 54.57 ($C_{11}$); 45.50 ($C_{13}$); 21.50 ($C_1$), Figure 9(B)

LC/MSD [negative; m/e (relative intensity)]: $M^+$ 531, Figure 11

**Synthesis of Diacid with Sulfamethazine Directly Linked to s-Triazine Ring (SMT):** This compound SMT was synthesized according to the procedure used for synthesis of diacid (ST).



(6)

FTIR (KBr, $cm^{-1}$): 3450 (-OH); 3350-3250 (-NH-); 1710 (-C=O); 1570-1550 (-C=N-,-Ar-NH-); 1330-1140 (-S=O); 1240 (-C-O-), Figure 7(C)

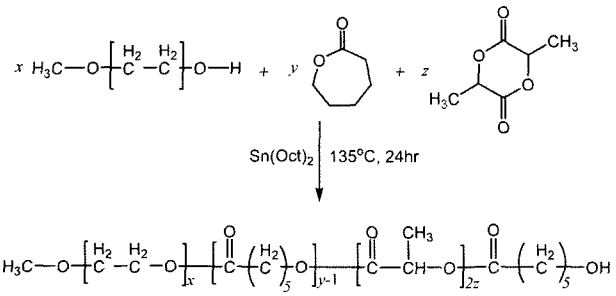
$^1$H-NMR (500 MHz, DMSO-$d_6$): 7.95 (2H,d,$H_6$); 7.75 (2H,d,$H_7$); 6.60 (1H,s,$H_2$); 3.05 (4H,d,H11); 2.15 (6H,s,$H_1$), Figure 8(C)

$^{13}$C-NMR (500 MHz, DMSO-$d_6$): 178.85 ($C_9$); 172.55 (C12); 168.50 (C10); 162.50 ($C_4$); 160.24 ($C_3$); 150.05 (C8); 131.02 ($C_{6,5}$); 120.50 ($C_{2,7}$); 40.20 (C11); 21.68 ($C_1$), Figure 9(C)

LC/MSD [negative; m/e (relative intensity)]: $M^+$ 504, Figure 12

**Synthesis of Biodegradable Diblock Copolymer.** Synthesis of MPEG-PCLA diblcok copolymer was carried out through a one step ring opening polymerization, using initiator via the synthetic pathway shown in Scheme IV. A typical example for ring opening polymerization for MPEG-PCLA ($M_n$ of MPEG: 750, MPEG/PCLA=1) is performed as fol-

**Scheme IV**




lows. A 100 mL round bottom flask was charged with MPEG (10 g) and $Sn(Oct)_2$ (0.05% w/w) and then vacuum-dried at 80 °C for 2 hrs. The solution was charged with nitrogen gas and cooled. The CL (4.557 mL) and LA (3.87 g) were added and the reaction mixture was stirred for 30 min at 80 °C. After that the temperature was gradually increased to 135 °C and kept for 16 hrs. The additional CL (1.139 mL) was added and the reaction mixture was stirred for another 6 hrs at 135 °C. Finally the contents was cooled to room temperature and diluted with methylene chloride. The reaction content was poured into hexane (500 mL) to precipitate the diblock copolymer. The hexane was filtrated and the polymer was vacuum dried for 12 hrs.

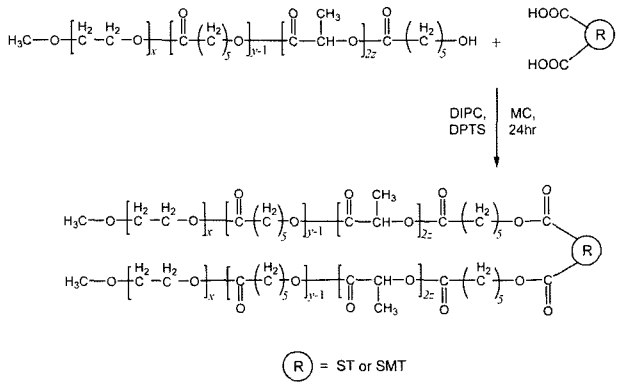
To determine the composition of the diblock copolymer, 500 MHz $^1$H-NMR measurements were carried out in $CDCl_3$. The molecular weight of the PCLA segment in the block copolymer was estimated from a $^1$H-NMR spectrum by examining the peak intensity ratio of the PLA (-$COCH(CH_3)O$; $d$=5.2 ppm), PCL (-$COCH_2(CH_2)_4O$: $d$=2.2 ppm) and the methylene protons of the PEG segments ($OCH_2CH_2$: $d$= 3.6 ppm), which was based on the known number averaged molecular weight ($M_n$) of PEG determined from GPC measurements. A series of MPEG-PCLA diblock copolymers with different molecular weight and MPEG/PCL ratio were synthesized as shown in Table I.

**Synthesis of pH-Sensitive Triblock Copolymer.** The

**Table I. MPEG-PCLA Diblock Copolymer with Different Molecular Weight Used in This Study**

| Molecular Weight of MPEG | MPEG-PCLA Weight Ratio | Molecular Weight | |
|---|---|---|---|
| | | $^1$HNMR | GPC |
| 750 | 1/0.5 | 1,105 | 1,094 |
| 750 | 1/1 | 1,394 | 1,312 |
| 2,000 | 1/0.5 | 2,867 | 2,945 |
| 2,000 | 1/1 | 3,958 | 3,935 |

**Scheme V**




syntheses of different pH-sensitive triblock copolymer were achieved by coupling reaction of different molecular weight diblock copolymer and pH-sensitive moieties such as; (ST) and (SMT) (Scheme V). The general outline for the synthesized is given as follows: MPEG-PCLA ($M_n$ of MPEG: 2,000, MPEG/PCLA=2) diblock copolymer (5 g), DPTS (0.85 g, 40% w/w of DIPC) and SMT (0.42 g) were put in two-neck flask and then dried under vacuum at 80 °C for 30 min. Then mixture was charged with nitrogen and cooled to room temperature, thereafter methylene chloride (60 mL) was added and stirred. The DIPC (0.52 mL) was added to reaction mixture and stirred at room temperature for another

**Table II. Critical Micelle Concentration (CMC) of pH-Sensitive Triblock Copolymer in Various pH Conditions**

| Diblock Used for Synthesis of Triblock Copolymer | Molecular Weight[a] | pH-Sensitive Group | CMC (mg/mL)[b] | | |
|---|---|---|---|---|---|
| | | | pH 6.5 | pH 7.4 | pH 8.5 |
| MPEG(750)-PCLA(1/0.5) | 2,582 | ST | 1.0 | 1.15 | 0.75 |
| MPEG(750)-PCLA(1/1) | 3,082 | ST | 0.5 | 0.75 | 0.25 |
| MPEG(2k)-PCLA(1/0.5) | 6,210 | ST | 0.55 | 0.45 | 0.38 |
| MPEG(2k)-PCLA(1/1) | 7,955 | ST | 0.15 | 0.20 | 0.10 |
| MPEG(750)-PCLA(1/0.5) | 2,645 | SMT | 1.05 | 1.25 | 0.90 |
| MPEG(750)-PCLA(1/1) | 3,168 | SMT | 0.3 | 0.5 | 0.28 |
| MPEG(2k)-PCLA(1/0.5) | 6,296 | SMT | 0.25 | 0.3 | 0.20 |
| MPEG(2k)-PCLA(1/1) | 7,845 | SMT | 0.10 | 0.18 | 0.13 |

[a]Determined by GPC analysis. [b]Determined by fluorescence spectroscopy.

24 hrs. The resulting product was filtered and evaporated under vacuum. The product was dissolved in tetrahydrofuran, then filtered again to eliminate urea and then precipitated in hexane (500 mL). Precipitated product was dried under vacuum at 40 °C for 12 hrs. All the other pH-sensitive triblock copolymers were synthesized by similar procedure. The molecular weight of triblock copolymer was determined from GPC as shown in Table II.

**Characterization.** $^1$H-NMR spectra were recorded on a Varian-Unity Inova 500NB operated at 500 MHz and DMSO-$d_6$ and $CDCl_3$ were used as a solvent. FTIR spectra were recorded on Unicam 5000 spectrometer using KBr pellet technique. To measure molecular weight, LC/MSD trap and GPC were used. LC/MSD trap (Agilent-1100) was used to measure low molecular weight of product (below 500). Molecular weights of diblock and synthesized pH-sensitive polymers were measured by GPC with two styragel columns (Shodex-KF802.5, KF-803L).

**Fluorescence Measurements.** First a buffer solution of 0.1 N Borax and potassium phosphate was made. A stock solution of pyrene in tetrahydrofuran is poured in a buffer solution and heated to 40 °C for 2 hrs to eliminate tetrahydrofuran. The final concentration of pyrene was $1.0 \times 10^{-6}$ M. Emission spectra of pyrene were recorded at 350 to 440 nm using fluorescence spectrometer (A MINCO.BOWMAN® Series2).

**Acid-Base Titration.** The polymers (1 mg/mL) were dissolved in 50 mL deionized water and the solution was adjusted to pH 12.0 with 1 M NaOH. The diluted solution was titrated by stepwise addition of 1 M HCl solution to obtain the titration profile. The average value of triplicate titrations was plotted.

## Results and Discussion

**Synthesis and Characterization of pH-Sensitive Moiety.** 2,4,6-Trichloro-1,3,5-triazine is a cheap and commercially available molecule for many application. The selective nucelophilic substitution in the s-triazine ring can be achieved at selective temperature.[19] The diacids containing s-triazine ring with sulfonamide and amino group were synthesized from several steps as shown in Scheme I, II, and III. The mono-substitution into the s-triazine ring was achieved by reaction with *p*-toluene sulfonamide or sulfamethazine at selective reaction temperature. Further, these mono-substituted s-triazines were used for synthesis of diester with sulfonamide and amino in the main chain using glycine methyl ester hydrochloride by nucleophilic displacement of two chlorine atoms. The pH-sensitive diacid containing s-triazine ring with sulfonamide and amino groups were obtained after hydrolysis of diester compounds.

The structure of mono-substituted s-triazine, diester, and diacids with sulfonamide groups were ascertained by FTIR, $^1$H-NMR, and $^{13}$C-NMR spectroscopy. All the spectral data are given in experimental section above.

**Synthesis of Block Copolymer.**

**Synthesis of Biodegradable Diblock Copolymer:** Ring opening polymerization of D,L-lactide and $\varepsilon$-caprolactone using MPEG in the presence of $Sn(Oct)_2$ have been reported in a number of literatures. Ring opening polymerization is primarily initiated by coordinate ionic initiator. The mechanism of this reaction varies according to monomer and initiator. In this study, MPEG-PCLA diblock copolymer with various molecular weight were synthesized by control of the feed ratio of MPEG, D,L-lactide, and $\varepsilon$-caprolactone as shown in Table I. While synthesis of diblock copolymer, CL (20 wt%) was added afterward to achieve the primary hydroxyl end group. The synthesized diblock copolymers were characterized by $^1$H-NMR and GPC analysis. The characteristic peak of methyl protons ($-OCH_3$) of MPEG in $^1$H-NMR of MPEG-PCLA is appeared at 3.5 ppm, whereas the peaks at 5.1 and 2.2 are assigned for (-CH) of LA and ($-CH_2$) of CL, respectively.

**Synthesis of pH-Sensitive Triblock Copolymer:** The diblock copolymer can be degraded upon moisture and heat due to the presence of ester linkage, thus the coupling reaction should be done at room temperature. To overcome this problem we have selected DIPC and DPTS were used as a coupling reagent and a catalyst respectively. After the coupling reaction, $^1$H-NMR of the synthesized block copolymer shows the peaks in aromatic region, which indicates the formation of pH-sensitive block copolymer, whereas in case of diblock copolymer had no the peaks in this region (Figure 13). The further characterization of pH-sensitive triblock copolymer was achieved by GPC analysis (Table II).

**Critical Micelle Concentration.** The effect of pH on the CMC of triblock copolymer with different pH-sensitive groups was carried out by fluorescence spectroscopy. The emission spectra of pyrene in the presence of the triblock copolymer is shown in Figure 14.

The effect of the triblock copolymer concentration on $I_3/I_1$ in the emission spectrum is shown in Figures 15, 16, and 17. At hydrophobic environment, peak intensity at 384 nm ($I_3$) becomes higher compared to that at 374 nm ($I_1$). When the micelles are formed, the condition of the pyrene was changed to hydrophobic environment, thus, the $I_3/I_1$ band ratio increased. A CMC value was taken from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentrations. The CMCs of pH-sensitive triblock copolymers in pH 6.5, 7.4, and 8.5 are listed in Table II. The CMC of MPEG(2k)-PCLA(1/0.5)-ST-PCLA-MPEG at 6.5 was 0.55 mg/mL while that of MPEG(2k)-PCLA(1/0.5)-SMT-PCLA-MPEG was 0.25 mg/mL.

The synthesized pH-sensitive triblock copolymers respond readily to the basic and acidic pH change owing to sulfonamide and amino group. The effect of pH on the CMC was examined and the results are presented in Figure 18. At pH

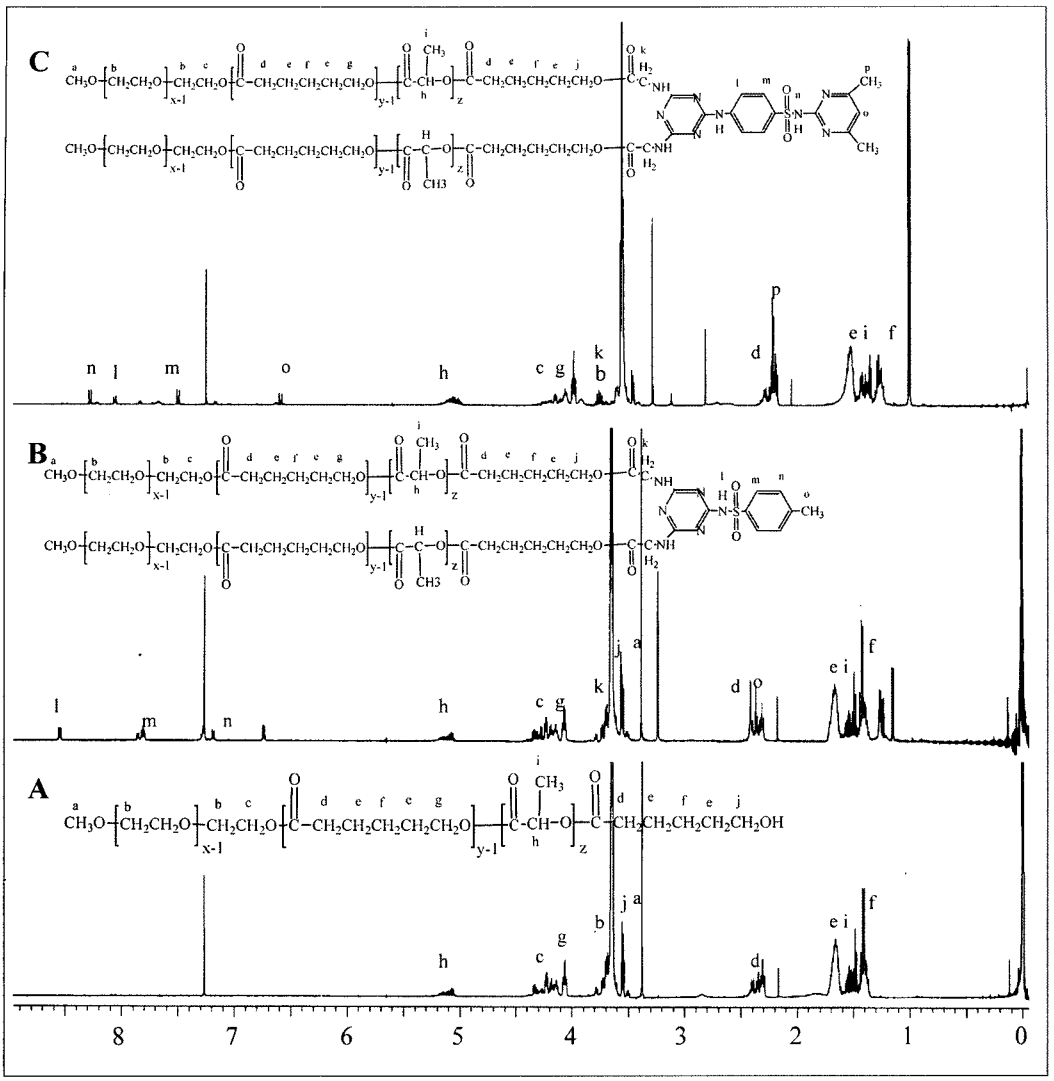



**Figure 13.** $^1$H-NMR of (A) MPEG-PCLA, (B) MPEG-PCLA-ST-PCLA-MPEG, and (C) MPEG-PCLA-SMT-PCLA-MPEG in $CDCl_3$.

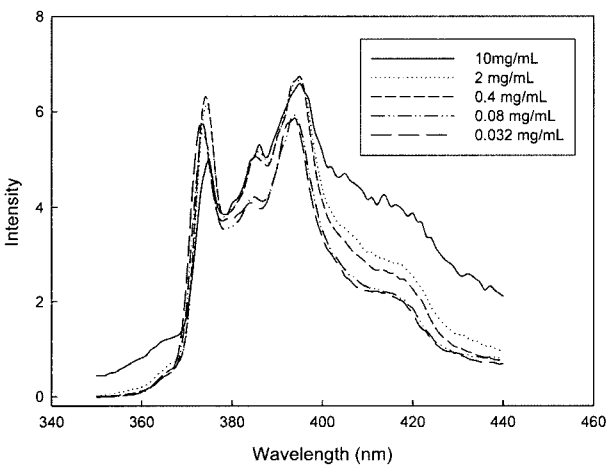



**Figure 14.** Emission spectra of pyrene ($1.0 \times 10^{-6}$ M) at pH 6.5 in the presence of MPEG (750)-PCLA[1/0.5]-ST-PCLA-MPEG as a function of polymer concentration.

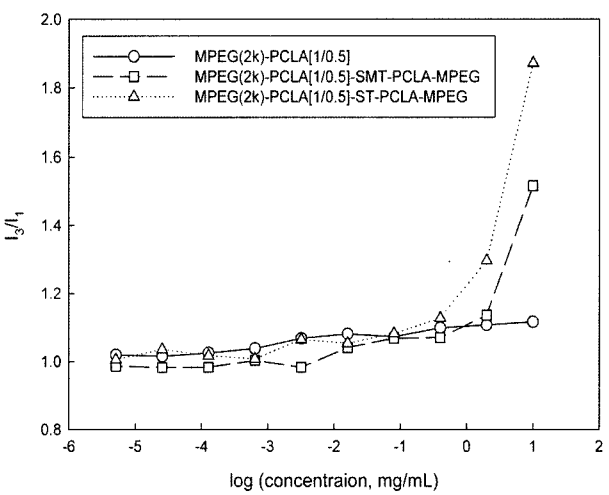



**Figure 15.** The intensity ratio of $I_3/I_1$ in the emission spectra as a function of the block copolymer concentration at pH 6.50.

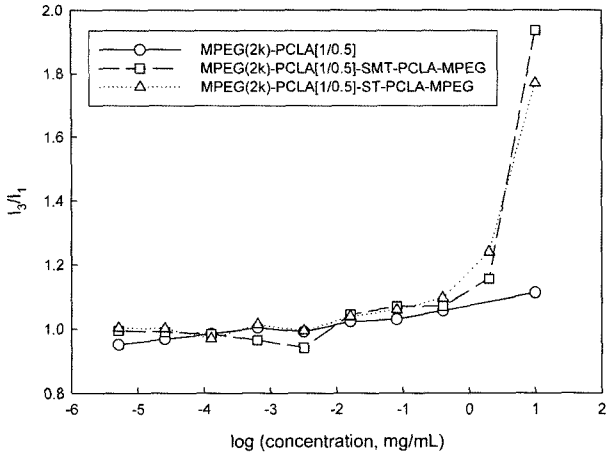



**Figure 16.** The intensity ratio of $I_3/I_1$ in the emission spectra as a function of the block copolymer concentration at pH 7.40.

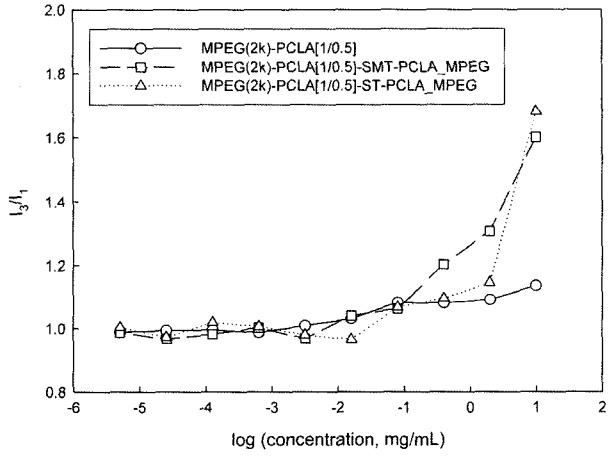



**Figure 17.** The intensity ratio of $I_3/I_1$ in the emission spectra as a function of the block copolymer concentration at pH 8.50.

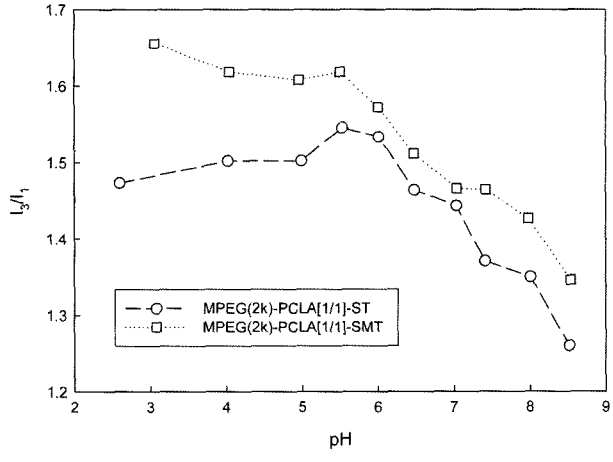



**Figure 18.** Comparison of pH-sensitivity of triblock copolymer with ST and SMT as pH-sensitive moieties.

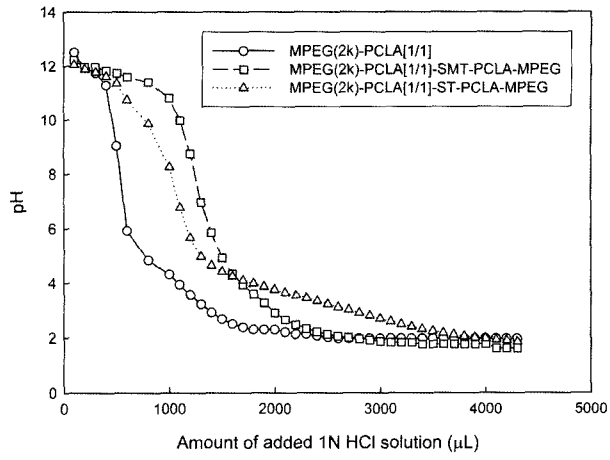



**Figure 19.** Titration curves of diblock copolymer and pH-sensitive triblock copolymer at 25 °C.

condition between 5.8~8.5, the CMC of MPEG(2k)-PCLA(1/0.5)-ST-PCLA(1/0.5)-MPEG(2k) and MPEG(2k)-PCLA(1/0.5)-SMT-PCLA -MPEG micelle increased. However below pH 5.8 or higher to 8.5, demicellization starts to begin. The fact can be evidence due to the presence of both sulfonamide and amino group together in the main chain. The evident result says that, these pH sensitive groups showing their resultant value and responsible for collapse of micelle below pH 5.8 or higher to 8.5.

**Titration of pH-Sensitive Triblock Copolymer.** The acid-base titration profile of diblock copolymer and pH-sensitive triblock copolymer (MPEG-PCLA-ST-PCLA-MPEG and MPEG-PCLA-SMT-PCLA-MPEG) with s-triazine ring with pH-sensitive groups are presented in Figure 19. All the pH-sensitive triblock copolymer solution exhibited a buffering effect between pH 4 and 9. The titration curve confirmed that the polymer with SMT as a pH-sensitive moiety had a little higher buffer capacity towards the physiological pH range of pH 6.0-8.0 compared to the others. This fact can be explained by the essential difference in electronic configuration between s-triazine ring and benzene as a consequence of greater electronegativity of the nitrogen atoms as compared to that of carbon atoms. s-Triazine ring (41.2 Kcal/mol) has got more aromatic character than normal benzene ring (36 Kcal/mol). Thus, the sulfonamide group directly attached to s-triazine ring as in case of ST shows less pH change as compared to SMT, because in that case the electron of sulfonamide groups was delocalized into the s-triazine ring due to resonance effect, while in case of SMT they were far away from it.

## Conclusions

In this paper, we suggested a novel pH-sensitive triblock copolymer, synthesized from diblock copolymer and s-triazine ring having sulfonamide and amino groups. From the

micellization of the synthesized block copolymer, it is confirmed that the stability of micelle depends on the resultant hydrophobicity at specified pH. Among the synthesized pH-sensitive triblock copolymer with s-triazine ring containing sulfonamide and amino group, MPEG-PCLA-SMT-PCLA-MPEG shows relatively sharp pH change towards physiological pH, when compared to MPEG-PCLA-ST-PCLA-MPEG. This effect can be evident because the essential difference exists in electronic configuration between s-triazine ring and benzene as a consequence of greater electronegativity of the nitrogen atoms as compared to that of carbon atoms. Thus, due to resonance effect the sulfonamide group of ST shows less pH change as compared to SMT.

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