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Sulfonamide-Containing Polymers: A New Class of pH-Sensitive Polymers and Gels

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SUMMARY: New pH-responsive polymers and hydrogel nanoparticles were synthesized, which bear either sulfapyridine or sulfamethoxypyridazine. The linear copolymers in water showed soluble/insoluble transition, while the hydrogel nanoparticles in aqueous solutions experienced association/dissociation transition in a narrow pH range. Their pH sensitivity was confirmed by the change in turbidity or particle size as a function of pH. The ionization of SO_2NH group in sulfonamides is responsible for aggregation of the polymers or hydrogel nanoparticles. The transition pH is determined by the amount of SO_2NH groups in the copolymers or on the hydrogel nanoparticle surface; at an optimum composition, the transitions occur near physiological pH. These systems may present a potential for various biomedical and bioengineering fields, such as pulsatile drug delivery, targeting, embolization, sensors, and bioseparation.

Introduction

Polymers that respond to a small change in pH may find a wide range of applications in pharmaceutical, biomedical, bioengineering, and other industrial areas. In particular, much attention has been paid in the last twenty years to pH-responsive polymers for drug delivery application. However, because conventional pH-sensitive functional groups provide limited pH sensitivity in polymers, their applications in biological and pharmaceutical systems, which often allow only small fluctuation in pH around 7.4, have severely been limited1).

Sulfonamide is a generic name for the derivatives of 4-aminobenzene-1-sulfonamide (sulfanilamide). This compound was discovered in 1908 and used in the dye industry. However, these compounds became more valuable as antibacterial agents twenty-five years later. At present, sulfonamides offer also other applications as electrolytes due to their electric properties2,3). Numerous substituents have been introduced into the sulfonamide group of sulfanilamide to improve their antibacterial activity, and the number of sulfanilamide derivatives reached nearly 5000 in 1948. Sulfonamides are weak acids because the amide hydrogen is readily ionized liberating a proton in solution. The oxygen atoms of sulfonyl group (SO_2), having high electronegativity, greatly attract electrons from the sulfur atom. The

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resulting electron deficiency of the sulfur atom draws the electrons of N-H bond to the nitrogen atom, resulting in ionization. The pK_a of a sulfonamide, ranging from 3 to 11, is strongly influenced by substituents at the SO_2NH group.

Our primary goal is to introduce sulfonamide group into polymers, which then respond to minute changes in pH near physiological conditions. In this study, pH-sensitive linear polymers and hydrogel nanoparticles bearing sulfonamide groups were synthesized. The linear copolymers showed pH-responsive transition in solubility and the nanoparticles exhibited a sharp association/dissociation of the particles around the physiological pH. These properties may present potential applications in various fields, including drug delivery. In particular, the physiological pH is locally changed by a disease such as in tumor tissues or by enzymatic reactions. Tumor tissues are known4) to be more acidic than normal tissues with a median pH of 7.0.

Results and discussion

Synthesis of sulfonamide monomer and copolymers

Methacryloylated sulfapyridine (SYM) was synthesized by the reaction of sulfapyridine (SY) with methacryloyl chloride. Sulfapyridine (2.5 g) was dissolved in a solution of sodium hydroxide (0.4 g) in a water/acetone mixture (1:1 v/v). Then, an excess of methacryloyl chloride (0.97 mL) was added to the solution (40 mL) under stirring at 10 °C. The reaction product precipitated during the process; it was collected by filtration and washed with water three times to remove unreacted methacryloyl chloride and methacrylic acid. The precipitate was dried in a vacuum oven for 48 h. The yield of SYM was 80 %.

The primary and secondary amino groups in SY showed three characteristic IR peaks at 3245, 3416 and 3350 cm^{-1}. On the contrary, the spectrum of SYM, as shown in Fig. 1, showed only a secondary amine peak because the N^4-amine was converted into the amide group. The chemical structure of SYM was also confirmed by ^1H NMR spectrum (ppm given): $C\underline{H}_2=C-CH_3$ 5.5, 5.8; $CH_2=C-C\underline{H}_3$ 2.0; $CON\underline{H}$ 10.1; benzene 7.7~7.8; pyridine 6.8~8.0.

Copolymers of *N,N*-dimethylacrylamide (DMAAm) and SYM were synthesized by radical polymerization in DMSO. After dissolving the monomers in various ratios in 50 % aqueous DMSO and bubbling with dried nitrogen for 15 min, 2,2'-azobisisobutyronitrile (0.2 mol-% relative to the monomers) was added and the mixture was degassed for 30 min. After polymerization at 65 °C for 24 h, the polymers were precipitated with an excess (ten-fold) of ethyl acetate. The copolymers were then purified by dialysis (molecular weight cut-off 12000) against deionized water for a week. The resulting copolymers are denoted as PDSY1, PDSY2, and PDSY3, containing 10, 20, and 30 mol-% of SY in the feed, respectively. The FT-IR spectra of PDSY showed typical absorption peaks of amide, aromatics, and doublets of of sulfonyl peaks at 1680, 1600, 1340, and 1150 cm^{-1}, respectively.

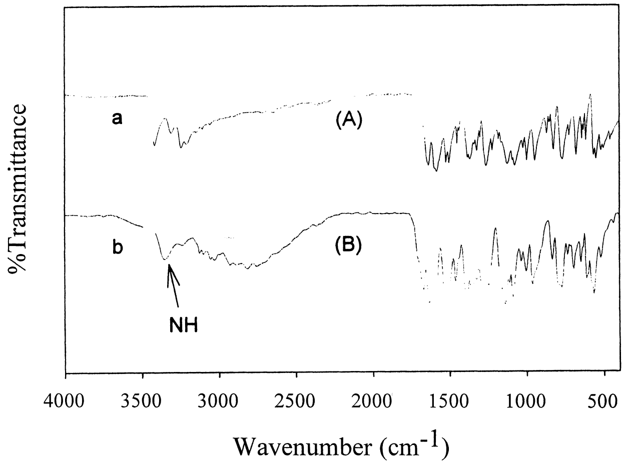

Fig. 1: FT-IR spectra of (a) sulfapyridine and (b) methacryloylated sulfapyridine.

pH-Dependent solubility

The solubilities of SY and SYM were measured at 37 °C in buffer solutions (at ionic strength 0.15) and pH varying from 5.5 to 9.0; the results are summarized in Table 1. The solubility of SY increased with increasing pH exhibiting inflection at pH around 8, which is close to the pK_a of SY[5)]. The solubility of SYM was significantly reduced when compared with SY at the same pH because the ionizable primary amino group in SY was converted to the amide group by reacting with methacryloyl chloride. However, its relative solubility was more influenced by pH than that of SY, reflecting improved pH sensitivity.

Table 1. pH-Dependent solubility (mg/100 mL) of SY and SYM at 37 °C.

pH	6.0	6.5	7.0	7.5	8.0	8.5	9.0
SY	32.0	42.0	43.0	58.5	86.0	146.0	156.0
SYM	0.14	0.18	0.23	0.35	1.59	3.15	5.22

Figure 2 illustrates the pH-dependent light (500 nm) transmittance of copolymer solutions

(0.5 wt-%) as a function of pH. As the solution pH decreased from pH 9.0, the transmittance decreased sharply at a specific pH and the solutions became opaque. The insoluble/soluble transition shifted to higher pH with higher DMAAm contents in the copolymers.

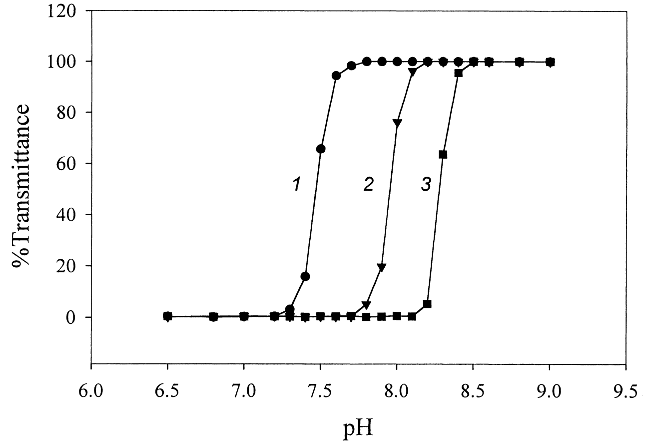

Fig. 2: Transmittance of SYM copolymer solutions in phosphate buffer (ionic strength 0.15) 37 °C as a function of pH. PDSY1 (*1*), PDSY2 (*2*) and PDSY3 (*3*).

This pH-induced transition may be caused by the aggregation of polymer molecules due to hydrophobic interaction of non-ionized sulfonamide groups. This process provides a starting point for aggregation of sulfonamides by reducing repulsive interaction among negative charges in SYM. In the aggregation, the hydrophilicity of DMAAm may require a higher degree of deionization, influencing also the pK_a of the sulfonamide group by local permittivity6). Note that PDSY1 has its transition close to physiological pH.

Synthesis of pullulan acetate (PA) containing sulfamethoxypyridazine (SMP)

Pullulan is a linear polysaccharide composed of maltotriose units connected by (1→6)-α-linkages7). Because pullulan is water-soluble, it is necessary to hydrophobize it for the preparation of self-assembled hydrogel nanoparticles. PA/SMP was synthesized in two steps by acetylation of pullulan and subsequent substitution with SMP.

We prepared hydrophobized pullulan by substituting hydroxy groups of glucose units with acetyl groups (pullulan acetate, PA). Pullulan (2 g) was suspended in 20 mL of formamide and dissolved by vigorous stirring at 50 °C. Pyridine (60 mL) and acetic anhydride (150 mL) were added and the mixture was stirred at 54 °C for 48 h. The product was obtained by precipitation in 200 mL of water[8)]. The synthesized PA was identified using FT-IR and NMR. The spectra showed the presence of acetate groups, indicated by C=O (1752 cm^{-1}) and CH_3 (1375 cm^{-1}) absorption. NMR study showed the degree of acetylation 1.27 acetyl groups per glucose unit of pullulan.

For the conjugation with SMP, PA was carboxylated with succinic anhydride. PA (5.0 g) was dissolved in 200 mL of dry 1,4-dioxane and succinic anhydride (2.2 g) was added. 4-(dimethylamino)pyridine (2.0 g) and the same amount of triethylamine were used as catalysts. The reaction mixture was stirred for 24 h at room temperature under nitrogen atmosphere. After the reaction, the product was concentrated using a rotary evaporator and the concentrate was dissolved in 300 mL of tetrachloromethane to remove the unreacted anhydride. The solution was filtered, the filtrate was concentrated to 100-150 mL using a rotary evaporator and the product was precipitated with cold diethyl ether. The degree of carboxylation was 0.54 per glucose unit of PA. Finally, SMP was coupled to carboxylated PA using dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSU) forming amide linkages. Different amounts of SMP (400-900 mg), DCC (350-800 mg) and HOSU (250-500 mg) were added to 100 mL of dry DMSO containing 1 g of carboxylated PA and reacted for 24 h at room temperature. The precipitated dicyclohexylurea was removed by filtration. After 24 h, the reaction mixture was filtered and then dialyzed using a dialysis tube (molecular weight cut-off 12 000) against distilled water for three days. The products were lyophilized using a freeze-drier. To remove unreacted compounds, the lyophilized samples were dissolved in DMSO and dialyzed against distilled water. This process was repeated three times. The degrees of substitution (DS), defined as the number of SA groups per 100 glucose units and determined by ^{1}H NMR and ^{13}C NMR were 6.9, 12.5 and 28.7, respectively.

Preparation of PA/SMP self-assembled nanoparticles and their pH sensitivity

Nanoparticles were prepared by the following process. Each PA/SMP derivative (50 mg) was dissolved in 25 mL of DMSO. The solution was stirred at room temperature and dialyzed against borax buffer (ionic strength 0.15) for three days using a dialysis tubing (molecular weight cut-off 2000). The solution was filtered through a 0.45 μm filter to remove precipitated material. Particle stability or aggregation in solutions was monitored by measuring transmittance changes as a function of pH. The results are shown in Fig. 3, where the transmittance is related to the refractive index and particle size of the nanoparticles. When pH of the solution containing nanoparticles (1 mg/mL) decreased from 7.5 to 6.0, the magnitude and sharpness of the changes in transmittance increased with increasing SMP content. The transmittance of DS 28.7 decreased from 100 at pH 7.4 to 35 at pH 6.4. The change in transmittance with increasing SMP content is assumed to be due to an increase in particle aggregation as a consequence of increased hydrophobicity of hydrogel nanoparticles.

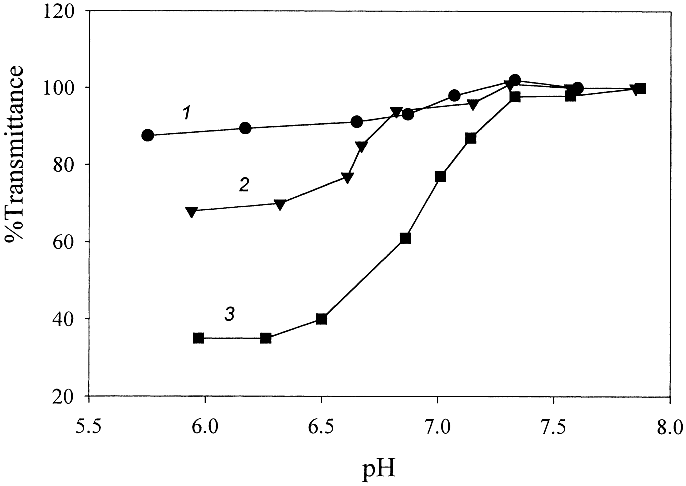

Fig. 3: Changes in relative transmittances of PA/SMP hydrogel nanoparticles as a function of pH. (Degree of substitution: 6.9 (*1*), 12.5 (*2*) and 28.7 (*3*)).

The outer shell of hydrogel nanoparticle is composed of hydroxy groups, carboxyl groups and SMP. Once the pH of solution containing SMP decreased below the pK_a of SMP, the hydrophilic/hydrophobic balance of the particle was altered, resulting in decreased stability of hydrogel nanoparticles. The formation of hydrophobic groups on the outer shell surface induced hydrophobic interactions of hydrogel nanoparticles$^{9)}$. This caused particle aggregation, the degree of aggregation increasing with increasing SMP content and decreasing pH. This was supported by the result of dynamic light scattering (DLS) study. DLS experiments were performed with an argon ion laser beam (DLS, Malvern Instruments, Series 4700) tuned at a wavelength of 488 nm. Each sample was filtered through an 0.45 μm filter directly into a cleaned cylindrical cell 10 mm in diameter. The intensity of autocorrelation was measured at a scattering angle of 90° at 25 °C. The sample concentration was kept at 1 mg/mL. The mean diameters of particles of DS 6.9, 12.5, and 28.7 in borax buffer (pH 8.0, ionic strength 0.15) were 146 ± 129, 263 ± 254 and 275 ± 268 nm, respectively. Figure 4 shows the effect of pH on the size of particles. The particle size significantly changed with increasing SMP content and solution pH decreasing from 7.4 to 6.0. At pH 6.0, the sizes of particles of DS 6.9, 12.7, and 28.7 were 356, 976 and 2213 nm, respectively.

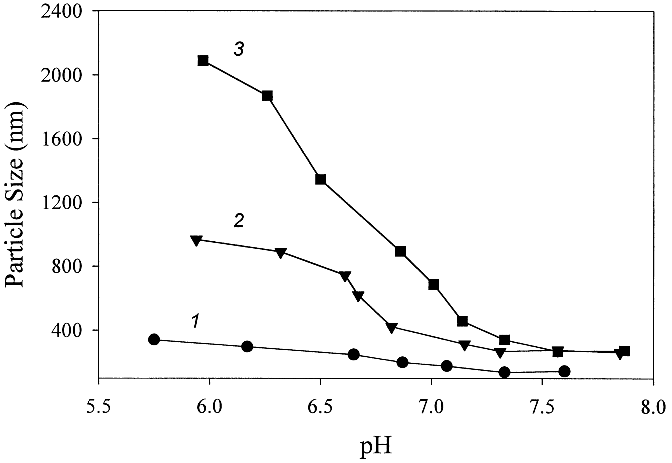

Fig. 4: Changes in particle sizes of PA/SMP hydrogel nanoparticles as a function of pH. DS: 6.9 (*1*), 12.5 (*2*) and 28.7 (*3*).

Conclusions

New pH-sensitive polymers and hydrogel nanoparticles based on sulfapyridine or sulfamethoxypyridazine were synthesized. All their copolymers with a hydrophilic comonomer DMAAm showed a sharp, pH-induced soluble/insoluble transition in a narrow pH range. The copolymer composition determined the transition pH, which was shifted to higher pH with increasing contents of DMAAm in the copolymers. This allowed to design a pH-sensitive polymer with a specific transition pH, such as physiological pH. Hydrogel nanoparticles were prepared from pullulan acetate substituted with SMP. The particle size and turbidity in buffer solution were strongly influenced by pH ranging from 6.0 to 7.4. Hydrophobic interaction by de-ionization of SMP groups on the particle surface resulted in the aggregation of the nanoparticles. The aggregation was controlled by the degree of substitution with SMP. The responsive properties of the polymers around physiological pH could be utilized in various biomedical and bioengineering fields, such as pulsatile drug delivery, targeting, embolization, sensors, and bioseparation.

Acknowledgment

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