Synthesis and pH-Dependent Micellization of Sulfonamide-Modified Diblock Copolymer

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Abstract: The main objective of this study was to develop and characterize pH-sensitive biodegradable polymeric materials. For pH-sensitivity, we employed three kinds of moieties: 2-amino-3-(1H-imidazol-4-yl)-propionic acid (H), N-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]succinamic acid (SM), and 2-{3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]carbamoyl}-propionylamino)-3-(3H-imidazol-4-yl)-propionic acid (SH). The pH-sensitive diblock copolymers were synthesized by ring opening polymerization and coupling reaction from poly(ethylene glycol) (MPEG), ε-caprolactone (CL), D,L-lactide (LA) and pH-sensitive moieties. The pH-sensitive SH molecule was synthesized in a two-step reaction. The first step involved the synthesis of SHM, a methyl ester derivative of SH, by coupling reaction of SM and L-histidine methyl ester dihydrochloride, whereas the second step involved the hydrolysis of the same. The synthesized SM, SHM and SH molecules were characterized by FTIR, 1H-NMR and 13C-NMR spectroscopy, whereas diblock copolymers and pH-sensitive diblock copolymer were characterized by 1H-NMR and GPC analysis. The critical micelle concentrations were determined at various pH conditions by fluorescence technique using pyrene as a probe. The micellization and demicellization studies of pH-sensitive diblock copolymers were also done at different pH conditions. The pH-sensitivity was further established by acid-based titration and DLS analysis.

Keywords: diblock copolymer, pH-sensitive moieties, critical micelle concentration, micellization /demicellization.

Introduction

Although some significant advances have been recently made in the field of intelligent polymers, the problem of optimum delivery at physiological pH remains a formidable challenge. The polymers that respond to a small change in pH may find a wide range of applications in pharmaceutical, biomedical, bioengineering, and other industrial areas. The micelles have been attractive as a carrier for poorly water-soluble drugs and due to small size (< 100 nm) and the evading capability from scavenges by mononuclear phagocyte system in the body. For these such micelles have been employed as an anticancer drug carrier, combining with tumor targeting capability by enhanced permeability and retention (EPR) effect. The pH-sensitivity is one of the most interesting properties of polymers used for drug delivery systems and have been extensively investigated.

It becomes generally known that the extracellular pH of tumors is lower than that of normal tissues; pH value of about 7.0 in tumors and 7.4 in normal tissues. The small but clear difference in pH has been an interesting subject for tumor targeting and various efforts has been devoted to construct pH-sensitive micelles or liposomes. However, because conventional pH-sensitive functional groups (carboxylic groups) provide limited pH-sensitivity in polymers, their applications in biological and pharmaceutical systems, which often only small fluctuation in pH around 7.4, have severely been limited.

It is well documented in literature that certain pH-sensitive groups like sulfonamide and imidazole ring have pH-activity, even when they are located in the polymeric chain. Sulfonamide, a generic name for the derivatives of para-amino benzene sulfonamide, shows weak acidic nature, whereas the imidazole ring has an electron pair on the unsaturated nitrogen that endows histidine with amphiteric nature by protonation-deprotonation, which leads to pKb value and pH-solubility properties.

In this study, we have synthesized pH-sensitive diblock copolymers, which is composed of methoxy poly(ethylene glycol)-poly(ε-caprolactone-co-D,L-lactide) (MPEG-PCLA) and pH-sensitive moieties such as 2-amino-3-(1H-imidazol-4-yl)-propionic acid (H), N-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]succinamic acid (SM), and 2-{3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]carbamoyl}-propionylamino)-3-(3H-imidazol-4-yl)-propionic acid (SH). The physiochemical properties of the micelles made from...
these copolymers were investigated in terms of size, critical micelle concentration (CMC) and pH-sensitivity.

**Experimental**

**Materials and Methods.** All the reagents and solvents have been used as received from Aldrich. Methoxy poly (ethylene glycol) (MPEG), $M_n$=750, 2,000), D,L-lactide (LA), ε-caprolactone (CL), sulfamethazine ((4-amino-N-4,6-dimethyl-pyrimidin-2yl)-benzenesulfonamide), anhydrous methane chloride, dicyclohexyl carboimide (DCC), disopropyl carboimide (DIPC), succinic anhydride, 2,4-(dimethyl amino) pyridine, toluene-sulfonic acid, 1,4-dioxane (anhydrous), N,N-dimethyl formamide (DMF) and L-histidine (LH) were used as received from Aldrich. Whereas, N-acetyl-histidine [2-amino-3-(1H-imidazol-4yl)-propionic acid] (H) was acquired from TCI (Tokyo Kasai Kogyo Co. Ltd., Japan). The coupling catalyst (DPTS) was a complex structure of 4-(dimethylamino) pyridine (DMAP) and p-toluene sulfonic acid (PTSA). DPTS was synthesized as the reported procedure.\(^2\)\(^3\) L-Histidine methyl ester hydrochloride was synthesized from L-histidine according to the reported procedure.\(^2\)\(^4\)

$^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian-Unity Inova 500NB operated at 500 MHz. DMSO and CDCl\(_3\) were used as solvent. The FTIR spectra were recorded on Unicam-5000 spectrometer using KBr pellet technique. Molecular weights of diblock and synthesized pH-sensitive polymers were measured by GPC with two styragel columns (Shodex-KF802.5, KF-803L). CMC (critical micelle concentration) and CMP (critical micelle pH) of diblock copolymer were measured by fluorescence spectrometer (AMINCO·BOWMAN® Series2). And micelle sizes were determined by DLS (dynamic light scattering).

**Synthesis of N-[4-(4,6-dimethyl-pyrimidin-2-sulfamoyl)phenyl] succinic acid (SM):** SM was synthesized by carboxylation reaction of amino group of sulfamethazine. The detailed synthetic route was outlined in the Scheme I.

The 250 mL round-bottom flask containing sulfamethazine (5 g) and succinic anhydride (2.69 g), equipped with reflux condenser and septum with nitrogen atmosphere was charged with 100 mL of 1,4-dioxane and 2-dimethylamino pyridine (10% w/w). The mixture was stirred with heating at reflux temperature for 10 hrs and then the reaction content was dried. After that the solid compound was well washed with water to remove unreacted succinic anhydride and 2-dimethyl amino pyridine and dried in vacuum. The synthesized compound was obtained in 95% yield.

![Scheme I](image)

**Synthesis of 2-[3-[4-(4,6-dimethyl-pyrimidin-2-ylsul-}

![Figure 1](image)