Key words: aliphatic polyesters; poly(ε-cprolactone); dextran; amphiphilic block copolymer; star shape polymer; micelle; unimolecular micelle; L-phenylalanine; complex; polyion; D-gluconic acid-d-lactone; polycondensation

关键词： 脂肪族聚酯；聚(ε-己内酯)；葡聚糖；两亲性嵌段聚合物；星形聚合物；胶束；单分子胶束；L-苯丙氨酸；复合物；离子聚合物；葡萄糖酸内酯；缩聚反应

Abstract

Biodegradable aliphatic polyesters have been intensively studied and widely used in biomedical applications, including sutures, implants for bone fixation, scaffolds in tissue engineering, and carriers in drug delivery, etc. [1-3] However, hydrophobicity and lack of functional groups to which bioactive molecules (drugs, recognition agents, adhesion promoters, or probes) can be covalently attached are the sources of occasional shortcomings for some applications. In contrast, naturally-occurring saccharides or polysaccharides have many active functional groups and good hydrophilicity, biocompatibility and biodegradability. In this paper, a series of amphiphilic aliphatic polyesters contained saccharide segments were synthesized and characterized.

In Chapter 1, an amphiphilic poly(ε-caprolactone)-*b*-dextran block copolymer was synthesized by Micheal addition between amino ended dextran (dextran-NH_2) and acrylate ended poly(ε-caprolactone)(PCL-$CH{=}CH_2$). The dextran-NH_2 was prepared by the reaction of ethyl diamine and dextran. The synthesis of PCL-$CH{=}CH_2$ was carried out by the reaction of the hydroxyl terminated poly(ε-caprolactone) with acryloyl chloride. The structure of the copolymers was characterized by ^{1}H-NMR. The micellization in aqueous solution was studied by transmission electron microscopy (TEM), dynamic light scattering technique (DLS) and pyrene fluorescence absorption technique. This is the first work of preparing polysaccharide block copolymer.

In Chapter 2, a novel star shape amphiphilic copolymer (sPCL-dextran) with the dipentaerythritol core, the hydrophobic poly (ε-caprolactone)(PCL) inner block arm, and the hydrophilic dextran outer block arm was synthesized by three steps. Firstly, the ring-opening polymerization of ε-caprolactone (ε-CL) initiated by the dipentaerythritol produces the star shape PCL (sPCL-OH). Secondly, the sPCL-OH reacts with acryloyl chloride to get star shaped PCL with the acrylate end group (sPCL-$CH{=}CH_2$). Finally, the amino ended dextran is attached to the sPCL-$CH{=}CH_2$ terminus by the Micheal addition reaction. The characterization with ^{1}H NMR, GPC and element analysis confirmed the structure of the sPCL-dextran. Micellization of the sPCL-dextran in aqueous solutions was investigated by

DLS. The results show that the size distribution of the micelles is related to the concentration of the sPCL-dextran in aqueous medium. The salt, pH and temperature have no obvious influences on the R_h of the micelle. This sPCL-dextran will possibly establish a useful starting point for using the polysaccharide to synthesis the star shape amphiphilic polymer.

In Chapter 3, the dextran derivatives with L-phenylalanine side groups (dex-phe) were synthesized. The structure, substituent degree (SD) and molecular weight of dex-phe were investigated by ^{1}H-NMR, element analysis and gel permeation chromatography (GPC), respectively. The polyion complex particles were obtained by dropping water to the solution of dex-phe with the carboxyl ended poly (ε-caprolactone) (PCL-COOH) in dimethyl sulfoxide (DMSO). The stability of the polyion complex particles was kept by the cooperation of hydrophobic interaction and electrostatic interaction between the L-phenylalanine side groups in dex-phe and PCL-COOH. The shape of the dry particles was showed by TEM. The average effective hydrodynamic radius (R_h) and the size distribution of the particles in aqueous solution were measured by DLS. The charge on the complex particle surface was determined by zeta-potential measurements. The particles combine several key structural features such as hydrophobic ionic core, a hydrophilic shell, nanoscale size, the safety and biocompatibility. This polyion complex particles have the potential applications in pharmaceutical fields.

In Chapter 4, a novel biodegradable polyester contained glucose units was prepared by a three-step reaction from D-gluconic acid-d-lactone (G) and maleic anhydride. Firstly, the 4,6-hydroxyl groups of D-gluconic acid-d-lactone was protected with 2,2-dimethoxy propanes (DMP) to obtain 4,6-O-isopropylidene gluconolactone (PG). Secondly, the residual two hydroxyl groups in PG reacted with maleic anhydride by the polycondensation reaction to produce the intermediate poly(4,6-O-isopropylidene gluconolactone-maleic) (PPGM). Finally, the isopropylidene group was removed from the PPGM to get the poly-(gluconolactone-maleic diester) (PGM). The products were characterized by GPC, ^{1}H NMR, FTIR and DSC. The presence of free hydroxyl and double bond groups in the PGM would provide the possibility for chemical modification of the polymer chain and coupling biologically active molecules, which expands the group of known biomedical polyesters candidates.

摘　要

生物可降解脂肪族聚酯在手术缝合线、骨钉、组织工程支架和药物载体等生物医学领域已得到广泛应用。今年来，在脂肪族聚酯中引入亲水性链段及可与药物、靶向性配体，生物探针等反应的活性位点成为生物医用高分子材料研究的重点。糖及多糖的分子结构中含有多个活性羟基并具有优良的亲水性、良好的生物相容性和生物可降解性。本文合成了一系列含糖链的两亲性脂肪族聚酯并采用多种分析方法对聚合物的结构及性能进行了表征。

第一章报道了一种新型天然多糖-脂肪族聚酯两嵌段聚合物的合成方法。即利用乙二胺与葡聚糖反应得到带有端氨基的葡聚糖(dextran-NH_2)，端羟基聚(ε-己内酯)与丙烯酰氯反应得到带有丙烯酸酯端基的聚(ε-己内酯)(PCL-CH=CH_2)。然后通过dextran-NH_2与PCL-CH=CH_2 间的Micheal加成反应合成了聚(ε-己内酯)-b-葡聚糖两嵌段聚合物。通过^{1}H-NMR表征了聚合物的结构，动态光散射和透射电镜表征了聚合物在水溶液中的胶束化行为。

第二章合成了以双季戊四醇为核，疏水性聚(ε-己内酯)为内臂，亲水性葡聚糖为外臂的星形两亲性共聚物。其反应过程分为三步：首先以双季戊四醇为引发剂引发ε-己内酯的开环聚合反应得到带有端羟基的聚(ε-己内酯)星形聚合物(sPCL-OH)。第二步通过sPCL-OH与丙烯酰氯反应得到带有丙烯酸酯端基的聚(ε-己内酯)星形聚合物(sPCL-CH=CH_2)。最后通过sPCL-CH=CH_2与端氨基葡聚糖之间的的Micheal加成反应合成了聚(ε-己内酯)-b-葡聚糖星形嵌段聚合物(sPCL-dextran)。通过核磁共振氢谱，凝胶渗透色谱、元素分析等方法表征了聚合物的结构。利用动态光散射的方法研究了该聚合物在水溶液中的胶束化行为，

结果表明：所形成胶束的粒径及粒径分布与聚合物的浓度相关；盐、pH值、温度等对胶束的有效粒径影响较小。sPCL-dextran对天然多糖为亲水性链段的星形两嵌段聚合物研究进行了有益的尝试。

在第三章中，合成了一系列带有L-苯丙氨酸侧链的葡聚糖(dex-phe)，通过核磁共振氢谱、凝胶渗透色谱、元素分析等测定了其分子量和取代度。将水滴入dex-phe和带有端羧基的聚(ε-己内酯)(PCL-COOH)的二甲基亚砜溶液时，通过dex-phe上苯丙氨酸侧基与PCL-COOH之间的疏水相互作用及电荷相互作用，得到了聚离子复合物纳米粒子。通过透射电镜测定了该复合物纳米粒子的形态。动态光散射测定了粒子的有效粒径及粒径分布。zeta-电位仪测定了粒子的表面电荷。由于该粒子具有离子化疏水性内核、亲水性的外壳以及良好的生物相容性，有望应用于药物载体。

第四章中利用葡萄糖酸内酯和顺丁烯二酸酐为主要原料通过三步反应合成了含有葡萄糖链段的脂肪族聚酯。其过程是：第一通过2,2-二甲氧基丙烷保护葡萄糖酸内酯上的4位和6位羟基得到4，6-氧-异丙叉葡萄糖酸内酯(PG)；其次利用PG上剩余的两个羟基与顺丁烯二酸酐进行缩聚，得到了4，6-氧-异丙叉葡萄糖酸内酯-顺丁烯二酸酐共聚物(PPGM)；最后PPGM脱去异丙叉保护基得到葡萄糖酸内酯-顺丁烯二酸酐共聚物(PGM)。通过磁共振氢谱、凝胶渗透色谱、红外光谱、示差扫描量热等分析方法对所得聚合物进行了表征。由于含有大量羟基及双键官能团，PGM是一种能够进行功能化修饰的新型脂肪族聚酯。

Chapter 1

Synthesis of Poly (ε-caprolactone)-*b*-Dextran Copolymers and Micellization Behavior in Aqueous Solution

Introduction

Amphiphilic block copolymers and their self-assembly in aqueous solution are currently a topic of great interest [1-9]. It is mainly motivated by their attractive applications to various research areas such as detergents, surface coating, oil recovery, drug delivery carrier technology and nanotechnology. In particular, various block copolymers consisting of poly(ethylene glycol) (PEG) and biodegradable polyesters such as poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), or their copolyesters (PLGA) have been prepared, and their self-assembled micelles have found very important uses in the biomedical materials field[10-14]. However, one drawback of these PEG-based block copolymers is the absence of reactive groups at their molecular chains, which limits further modification or ligand coupling. In contrast, naturally-occurring polysaccharides with good hydrophilicity, biocompatibility and biodegradability seem to be attractive alternatives to PEG hydrophilic segments for designing amphiphilic block copolymers. Up to now, however, only a few of studies have dealt with polysaccharide-based block copolymers. For example, Akiyoshi et al. [15] obtained the block copolymers of poly(ethylene oxide) and amylose by an enzymatic reaction; Loos [16] prepared the linear block copolymers of polystyrene and polysaccharide by a block synthesis method, and investigated their interfacial behavior; Very recently, Yang et al. [17] reported on the synthesis of diblock copolymers consisting of hyaluronan and poly(2-ethyl-2-oxazoline), a pseudopeptide block; Kamitakahara [18] reported on the preparation of diblock copolymers consisting of cellulose and a hydrophobic part, azidoalkyl carboxylic acid. To our knowledge, however, there is no information available on the amphiphilic block copolymers

consisting of a polysaccharide segment and a biodegradable polyester segment.

In chapter 1, we reported the first synthesis of a totally biodegradable amphiphilic block copolymer based on the combination of a nature polysaccharide (dextran) with a synthetic aliphatic polyester (polycaprolactone) and its micellar characteristics in aqueous solution. In order to obtain such materials, the polycaprolactone end-capped with the acryloyl group and the amino-functionalized dextran were prepared, respectively, and the subsequent coupling reaction was carried out.

Experiment

Materials

Dextran from Leuconostoc mesenteroides was obtained from Fluka, and its number average molecular weights (M_n) was determined to be 3700 g/mol by GPC. *e*-Caprolactone (CL) was purchased from Sigma (St. Louis, MO). Before the use, it was dried over CaH_2 for 24 h and distilled. Sodium cyanoborohydride was purchased from Acros. All other reagents were analytical grade and used as received.

Preparation of polycaprolactone end-capped with acryloyl group

The polycaprolactone end-capped with the acrylate ended group (Product A) was synthesized according to Scheme 1. At first, the polycaprolactone with hydoxyl end-group (PCL-OH) was obtained by the ring opening polymerization of *e*-caprolactone (*e*-CL) in the presence of *n*-butyl alcohol. For this purpose, 20 g CL, 0.5 g *n*-butyl alcohol and 0.05 g stannous octanoate were put into an ampoule with magnetic stirrer connected to the vacuum/Ar line, and the polymerizations were carried out under Ar atmosphere at 120 °C in a silicone oil bath. After 5 h, the reaction was terminated by dipping the reaction flask into ice. The resulting PCL-OH was purified by four successive precipitations using THF as the solvent and methanol as the nonsolvent, and dried at room temperature under vacuum for 24 hours. Then 1.45 g triethylamine was added into a solution of obtained PCL-OH in 150 ml

dry DMF, followed by adding slowly 1.2 g acryloyl chloride within 40 min at 0 °C. After that, the temperature was raised to 25 °C and the reaction was conducted for 2 h. Then the reaction mixture was cooled to 0 °C and poured into methanol. The product **A** was recovered by filtration, purified by three successive precipitation using THF as the solvent and methanol as the nonsolvent, and dried under vacuum. It has the average number molecular weight (M_n) of 2800 g/mol, as determined from ^{1}H-NMR analysis.

Preparation of amino-functionalized dextran

The amino-functionalized dextran (Product **B**) was prepared according to Scheme 2. The dextran (5 g, M_n = 3700 g/mol), ethylenediamine (0.2g) and aqueous sodium borate solution (100 ml, 0.1mol/l) were added into the round-bottom reaction flask equipped with stirring, and the reaction was carried out at 60 °C for 8 days in a silicone oil bath. During the reaction, sodium cyanoborohydride (20 mg) was slowly added. After the reaction completion, the reaction mixture was poured into methanol. The precipitate was recovered by filtration, and then dried in vacuum at room temperature for 48 h. The obtained product **B** was purified by three successive precipitations using water as the solvent and methanol as the nonsolvent.

Preparation of dextran-polycaprolactone diblock copolymer

For the preparation of amphiphilic block copolymer based on dextran and polycaprolactone, the Micheal reaction between Products **A** and **B** was carried out, as indicated in Scheme 3. A typical process is as follows. 1.12 g Product **A**, 2.00 g Product **B**, 50mg hydroquinone, 80 mg *p*-methyl-benzene sulfonic acid, and 20 ml freshly distilled DMSO were added into a 50 ml round-bottomed three neck flask equipped with a magnetic stirrer. The coupling reaction was carried out at 120 °C for 8 h in a silicone oil bath. After the reaction completion, DMSO was removed on a rotary evaporator under reduced pressure. The resulting product was precipitated in THF, collected by filtration, purified by three successive precipitation using water as the solvent and methanol as the nonsolvent, and dried under vacuum at ambient temperature for 24 h.

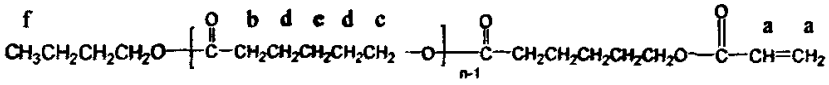
Measurements

^{1}H-NMR spectra were recorded on a Mercury-Plus 300 (Varian, USA) spectrometer at 300 MHz, using tetramethysilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. The concentration of the solution is 5 mg/ml. The morphological examination of the copolymer micelles was performed using a JEM-2010HR high-resolution transmission electron microscope. A drop of PCL-dextran block copolymer aqueous solution (2 mg/ml) containing 0.2wt % phosphotungstic acid (PTA) was deposited onto a 200 mesh copper grid coated with carbon. Excessive solution was removed with a Kimwipes delicate wipe. The shape and size of the micelles were directly determined from each transmission electron micrograph. The micellar size and size distribution were determined by dynamic light scattering (DLS) using a BI-200SM Goniometer particle size analyzer (Brookhaven, USA). Each analysis lasted for 300 s and was performed at 23 °C with angle detection of 90°. The concentration of the polymer solution was 2 mg/ml. Steady-state fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Excitation spectra were monitored at 335 nm. The slit widths for both excitation and emission sides were maintained at 0.5 nm. Sample solutions were prepared by dissolving a predetermined amount of block copolymer in an aqueous pyrene solution of known concentration, and the solutions were allowed to stand for 48 hr for equilibration.

Results and Discussion

Characterization of ^{1}H-NMR

. Figure 1 gives the ^{1}H-NMR spectrum of the PCL with acrylate ended group. As seen, three peaks appeared at around 5.80-6.38 ppm could be attributed to the proton on the C=C double bond. The signals at 0.91 ppm resulted from the proton of CH_3. All other absorption peaks were attributed to the protons of the polycaprolactone backbone.



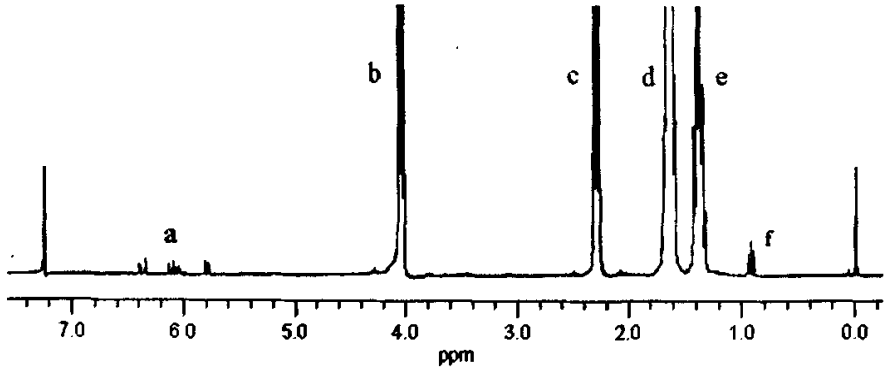
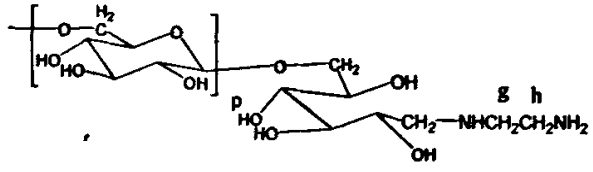


Figure 1. ^{1}H -NMR spectrum of allyl ended PCL ($CDCl_3$ as solvent)



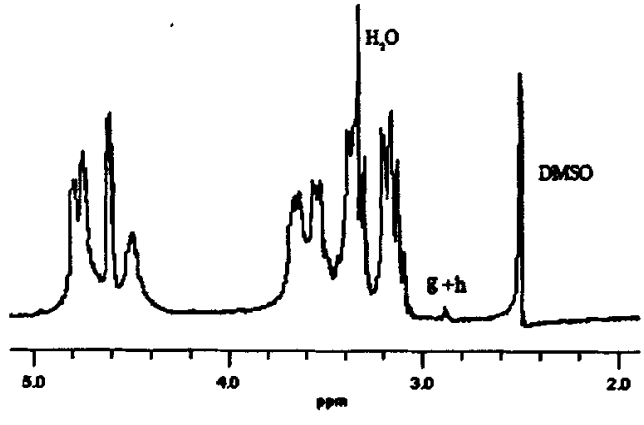


Figure 2. ^{1}H NMR spectrum of amino ended dextran (DMSO-d^6 as solvent)

Figure 2 gives the ^{1}H-NMR spectrum of the amino ended dextran. The weak signal at 2.85 ppm is for the C-g protons of ethyle diamine adjacent to the carbonxyl group, and all other

signals are for the proton of the dextran backbone.

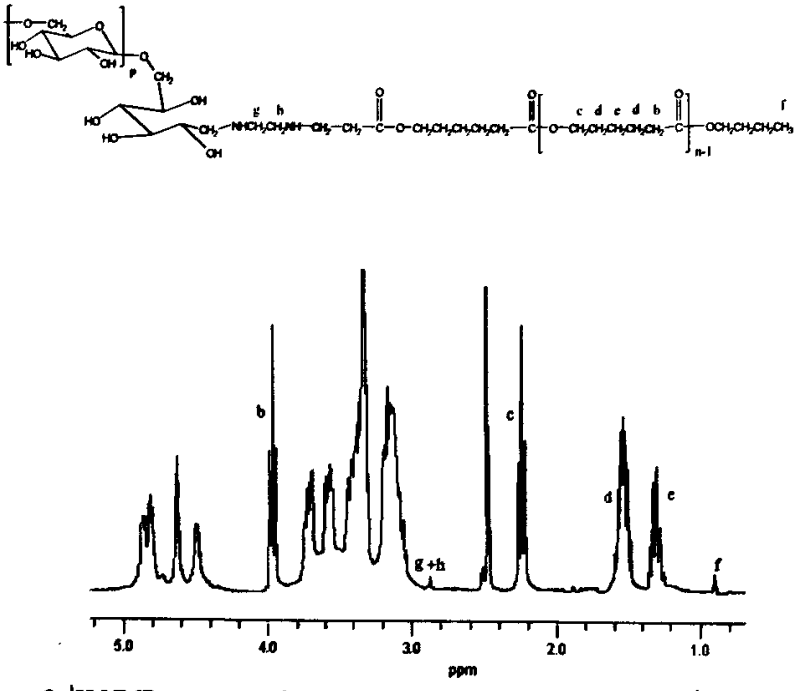


Figure 3. ^{1}H-NMR spectrum of PCL-b-dextran copolymers (DMSO-d^6 as solvent)

Figure 3 gives the ^{1}H-NMR spectrum of the dextran-polycaprolactone block copolymer. The signals at 3.10 and 4.86ppm are for the dextran, and the signals at 1.28, 1.51, 2.22, 3.96 ppm are for the polycaprolactone. This indicates that the Micheal rection between the acryloyl group of Product A and the amino end group of Product B has occurred.

Determination of critical micelle concentration

The microscopic characteristics of resultant amphiphilic block copolymer in aqueous medium were investigated using a fluorometer in the presence of pyrene as a fluorescent probe. It is known that the variation in the ratio (I_1/I_3) of intensity of first (372 nm) to the third (383 nm) vibronic peaks, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where the pyrene is located.[21-23] **Figure 4** gives the excitation spectra of pyrene in its aqueous solutions with various concentrations and the change of I_1/I_3 with the concentration. At lower concentrations, the I_1/I_3 values remain nearly unchanged.

Further increasing the concentration, the intensity ratio starts to decrease, implying the micelle formation. The critical micelle concentration (*cmc*) was determined to be 0.06 mg/ml by the interception of two straight lines. Compared with low molecular weight surfactants, the resultant amphiphilic block copolymer has a lower *cmc* value, indicating the stability of the micelles from this polysaccharide block copolymer at aqueous solution.

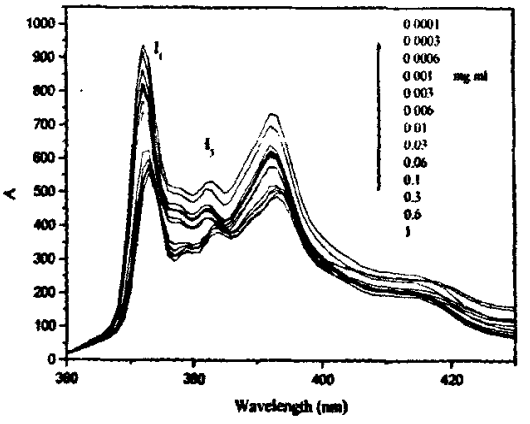


Figure 4. Emission spectra of pyrene as function in an aqueous solution of PCL-b-dextran at various concentrations

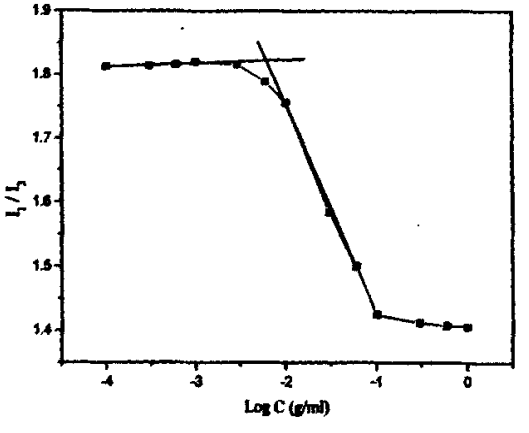


Figure 5. Plot of the I_1/I_3 ratio of pyrene excitation spectra in water as a function of PCL-b-dextran copolymer concentration

Characterization of TEM and DLS

Further work was carried out on the morphology of the formed micelles by the transmission electron microscopy (TEM) technique. From Figure 6, it can be confirmed that the resulting polymeric micelles in water are spherical in shape, with the diameters ranged from 20 to 50 nm. The size distribution of the micelles was also investigated by the dynamic light scattering (DLS) technique. As shown in Figure 7, a relative narrow unimodal distribution was obtained.

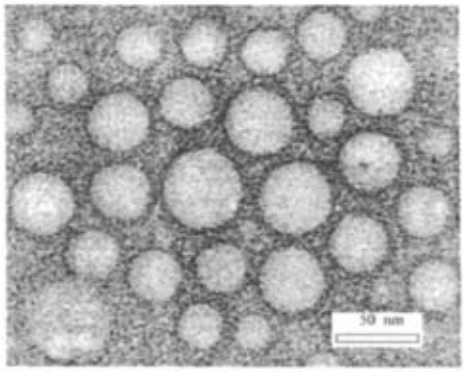


Figure 6. Micelles observed by TEM for PCL-b-dextran Copolymers

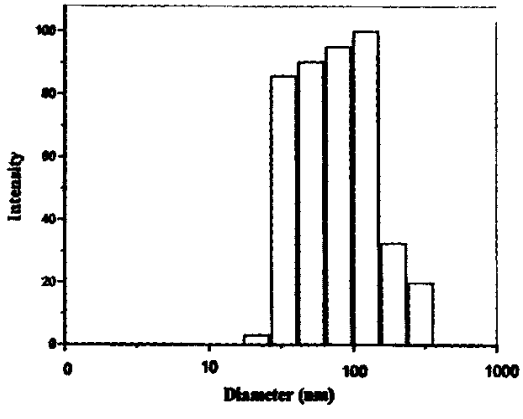


Figure 7. Particle size distribution of micelles formed by PCL-b-dextran Copolymers

Conclusions

In conclusion, a totally biodegradable amphiphilic block copolymer was synthesized for the first time by the coupling reaction between the amino-functionalized dextran and the polycaprolactone end-capped with the acryloyl group. It could self-assembly in water to form the bioresorbable polymeric micelles without any organic solvent or surfactant. Due to good hydrophilicity, biocompatibility, biodegradability and multifunctional conjugation capability of the used dextran, such polysaccharide derivative will hold greater advantages as the nanoscale container for hydrophobic drugs and genes when compared with widely used amphiphilic block copolymer of PCL and polyethylene oxide or polyethylene glycol.

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Chaper 2

Synthesis of Star-Shape Amphiphilic Block Copolymer and Its Micellar Properties in Aqueous Solution

Introduction

Polymeric nano-micelles have been extensively studied and applied in biomedical fields in recent years. The major factors that influence the performance of polymeric micelles for drug delivery are loading capacity, release kinetics, circulation time, biodistribution, size, and stability [1]. Recent studies have shown that the anti-tumor activity of a drug incorporated into the polymer micelles is positively correlated with the stability of micelles in vitro [2]. The formation of classical micelles is thermodynamically favorable only above a specific concentration of the amphiphilic molecules (critical micelle concentration, *cmc*). Above the *cmc*, the micelles are in dynamic equilibrium with the free copolymer molecules in solution. When the concentration of the copolymer is below the *cmc*, micelles tend to disassemble. Such thermodynamic instability of micelles below the *cmc* is one of the concerns for their application in vivo. A delivery system is subject to a severe dilution upon intravenous injection into an animal or human subject. In the bloodstream, under dilution, micelles begin to disassemble, causing changes in micelle structure and size. Sudden dissociation of micelles may cause serious toxicity problems due to potentially large fluctuations in drug concentrations. Cross linking of the core is an effective method to prevent dissociation of the block copolymer micelle. Kataoka's group has successfully employed this idea [3]. In their study, the micelles were prepared from an amphiphilic block copolymer in which the hydrophobic block contains a polymerizable end group. After the micellization, the end groups on the hydrophobic block were polymerized to form a stable core for the star-shaped polymer structure. The resulting micelles showed fairly high stability and maintained small size.

Another approach was developed by Uhrich et al. [4] In this case, both star-shaped and

branched amphiphilic polymers are expected to generate unimolecular micelles under certain conditions, which have better thermodynamic stability in aqueous solutions.[5] In recent years, a series of star amphiphilic block copolymers with the number of arms ranging from three to eight have been synthesized. The arms are composed of block copolymer with PEG as the hydrophilic block and biodegradable polyester as the hydrophobic block. For example, Cai, et.al [6] synthesized a series of well-defined poly(L-lactide)(PLLA) and poly(ethylene glycol) (PEG) block copolymers with 1-,2-,4-,and 6-branch arms; , Kim and coworkers [7-9] studied the micellization and gelation of star-shaped PLLA-b-PEG block copolymers in aqueous medium. Choi[10] investigated the application of this type of copolymer as an injectable drug delivery system. Wang [11] synthesized a star shape amphiphilic block copolymer from poly(ε-caprolactone) (PCL) and PEG. A cytotoxicity assay demonstrates that the star-PCL-PEG copolymer is nontoxic in cell culture and can be used as a drug delivery carrier.

Dextran and its derivatives have good biocompatibility and biodegradability, and have been used as a plasma expander, gene transfer vector and drug carrier[12-15] in biomedical fields. Combining dextran with hydrophobic segments can offer amphiphilic polymers, which may self-assemble into micelles which is considered as the potential drug carrier. [16-19]. In this work, a star shape polymer with amphiphilic block arms (sPCL-dextran) has been synthesized. The core of this star polymer is dipentaerythritol, and the inner block in the arm is hydrophobic PCL, as well as the outer block in the arm is hydrophilic dextran. The micellization of the sPCL-dextran in aqueous solutions were investigated by dynamic light scattering.

Experiment

Materials

Dextran was obtained from Fluka, and its number average molecular weights (M_n) was determined by GPC. ε-Caprolactone (ε-CL) was purchased from Sigma (St. Louis, MO). Before the use, it was dried over CaH_2 for 24 h and distilled. Sodium cyanoborohydride was purchased from Acros. Dipentaerythritol was purchased from Alfa Aesar. All other reagents

were analytical grade and used as received.

Measurements

Element analysis

EA3100 (Analytik Jena AG, German) was used for the **element analysis.**

^{1}H NMR Measurement

^{1}H NMR spectra were recorded on Mercury-Plus 300 (Varian, USA) spectrometer at 300 MHz, using tetramethysilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. The concentration of the solution is 5mg/ml.

Transmission Electron Microscopy (TEM)

The morphological examination of the copolymer micelles was performed using a JEM-2010HR high-resolution transmission electron microscope. A drop of 1mg/ml sPCL-dextran aqueous solution containing 0.2 wt % phosphotungstic acid (PTA) was deposited onto a 200 mesh copper grid coated with carbon. Excessive solution was removed with a Kimwipes delicate wipe. The shape and size of the micelles were directly determined from each transmission electron micrograph.

Dynamic Light Scattering Measurement (DLS)

The micelle size and size distribution were determined by dynamic light scattering (DLS) using a BI-200SM Goniometer particle size analyzer (Brookhaven, USA). Each analysis lasted for 300 s and was performed at 25 °C with angle detection of 90°. The concentration of the polymer solution was 2 mg/ml.

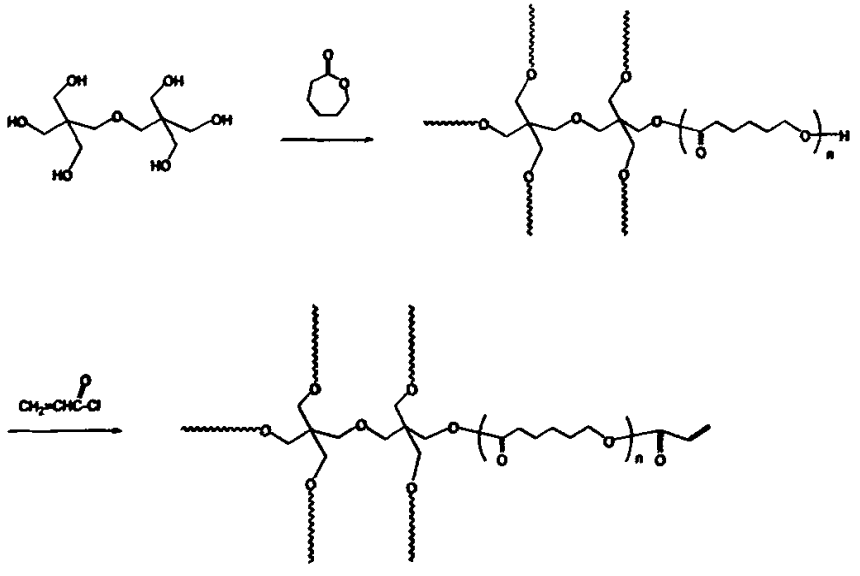
Synthesis of the star shape (6 arms) PCL with hydoxyl ended groups (sPCL-OH) [20]

sPCL-OH was obtained by the ring opening polymerization of ε-CL using the dipentaerythritol as the initiator and stannous octanoate ($SnOct_2$) as the catalyst. A typical example as follows: 160 mg dipentaerythritol initiator was added to the 5 g ε-CL monomer. The tube was then connected to an argon gas line, where an exhausting–refilling process was repeated three times. A certain amount of $SnOct_2$ ([ε-CL]/[$SnOct_2$] = 1000/1, mol/mol) in dry

toluene was added to the mixture and the exhausting–refilling process was carried out again for the removal of the toluene. The polymerization was carried out in bulk at 120 °C for 24 h. Then, the resulting product was dissolved in 20 mL of THF and added dropwise into 200 mL of cold methanol under vigorous stirring at room temperature. The precipitate was filtered and dried in vacuum at room temperature for 24 hours.

Synthesis of star shape PCL with acrylate ended group (sPCL-CH=CH_2)

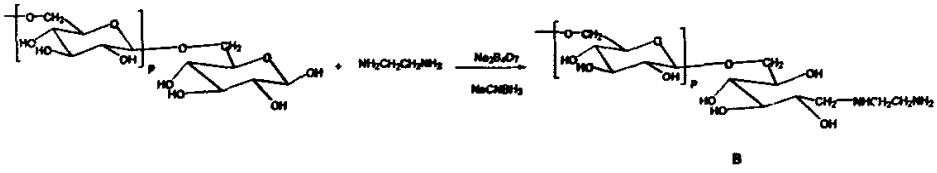
0.6 g triethylamine was added to a solution of the 4.0 g sPCL-OH in 60ml N,N-dimetylformamide, and then 0.5 g acryloyl chloride was added drop by drop into the flask within 40 min at 0 °C under stirring . The temperature was raised to 25°C and kept for 2 h. After cooling to 0°C, the mixture was poured into methanol to obtain the precipitate of sPCL-CH=CH_2, then the precipitate was purified by three successive precipitations using THF as the solvent and methanol as the nonsolvent. After dried in vacuo at room temperature for 24 hours, 3.683 g (wt %, 92% yield) of white solid product was obtained. The synthetic route was shown in **Scheme 1.**



Scheme 1. Synthesis of sPCL-CH=CH_2

Synthesis of the dextran with amino end-group (dextran-NH_2)

5g Dextran and 0.6g ethylene diamine and 100 ml of 0.1M sodium tetraborate in aqueous solution was added into a round-bottomed reaction flask equipped with stirring, and the reactions were conducted at 60 °C for 8 days in a silicone oil bath. During the reaction, 20 mg sodium cyanoborohydride was added one times a day. After the completion of the reaction, most of water and unreacted ethylene diamine were removed by evaporation. The product was poured into methanol and the precipitate was recovered by filtration and then dried in vacuum at room temperature for 48 h. The obtained dextran-NH_2 was purified by three successive precipitations using water as the solvent and methanol as the nonsolvent. The precipitate was filtered and dried in vacuo at room temperature for 48 hours. The synthetic route was shown in Scheme2.



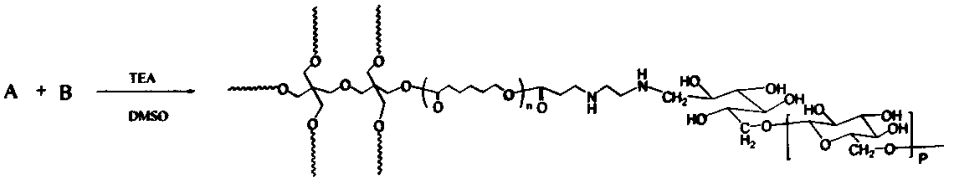
Scheme 2. Synthesis of dextran-NH_2

Synthesis of the sPCL-dextran(Scheme 3)

0.5g sPCL-CH=CH_2 and the calculated dextran-NH_2 ([sPCL-CH=CH_2]/[dextran-NH_2]=1/10, mol/mol) were dissolved in 20 ml DMSO and transferred to a three-necked flask with electromagnetic stirrer. To this solution, 50 mg hydroquinone (inhibitor) and 120 mg acetic acid (catalyst) were added. The reaction was carried out at 130 °C for 24 hours in an Ar atmosphere. After the flask cooled to room temperature, the reaction mixture was poured into a beaker and added 200 ml methanol, the obtained precipitate was filtrated and washed three times with acetone and dried in vacuum for 24 hour under room temperature.

The fractionation was used to purify the product. Briefly, the product was dissolved in 50 mL of water followed by the gradual addition of methanol until the mixture became cloudy. The resulting mixture was then heated in a 45 °C bath followed by the addition of methanol until a stable cloudy point was reached and transferred into a separation funnel. The lower layer was diluted with distilled water and precipitated into methanol. After drying, 0.67 g of

white solid product was obtained.



Scheme 3. Synthesis of sPCL-CH=CH_2

Preparation of Micelles from sPCL-dextran

The sPCL-dextrantran polymer was dissolved in DMSO of 10 mg/mL and stirred at room temperature. Water was added dropwise to the solution until the desired water content was achieved (water/DMSO=5/1, v/v). The obtained solution was stirred overnight and then dialyzed against distilled water. The dialysate water was exchanged every hour for the first 4 h and then every 6 h for the next 12 h. The product was stored at 4℃ for characterization of the micelle.

Results and Discussion

Characterization of dextran-NH_2

The product of dextran-NH_2 was characterized by GPC, ^{1}H NMR and element analysis. GPC results show that the M_n, M_w and poly dispersion index (M_w/M_n) are 1340g/mol, 1860g/mol and 1.394, respectively. The nitrogen percentage ($N\%_{exp}$) of the dextran-NH_2 is 1.987% by element analysis. The theoretic nitrogen percentage of dextran-NH_2 ($N\%_{cal}$) is calculated by the following equation:

$N\%_{cal} = 2\times14/M_n$ of dextran-NH_2 $\times100\%$=2.089%

The nitrogen percentage from element analysis is lower than that of theoretic content. This is perhaps due to the dextran-NH_2 containing some unreactive dextran. The dextran-NH_2 content was estimate by the following equation:

$$\text{dextran-NH}_2\,\% = (N\%_{,\,exp} / N\%_{,\,cal}) \times 100\%$$

From the equation, the dextran-NH_2 content of 95.12% was obtained. This result indicated that the reaction of ethyl diamine and dextran processed effectively.

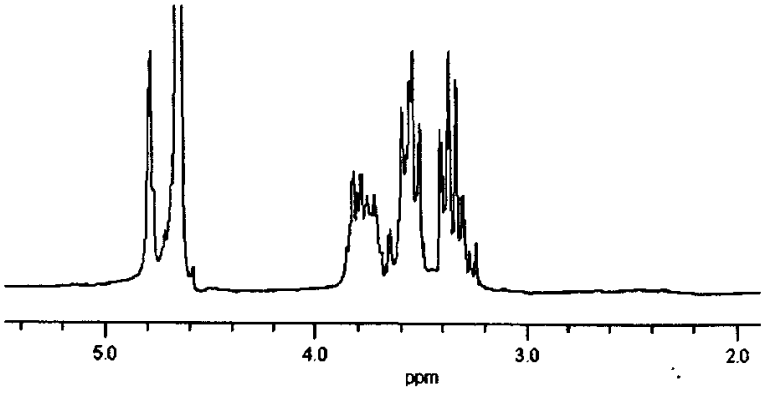
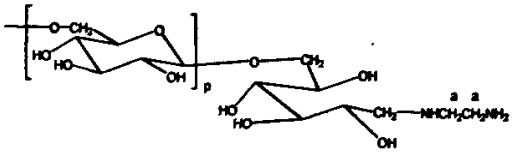


Figure 1. ^{1}H NMR spectrum of dextran (D_2O as solvent)



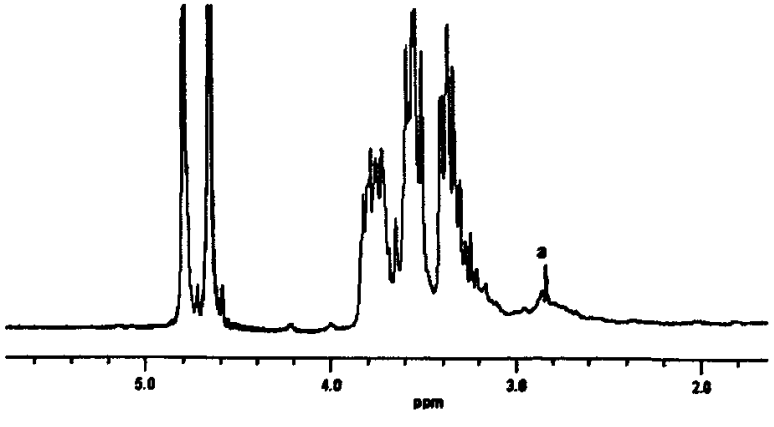


Figure 2. ^{1}H NMR spectrum of dextran-NH_2 (D_2O as solvent)

Figures 1 and 2 are the H^1 NMR spectra of dextran and dextran-NH_2, respectively. In **Fgure 1**, the signals at 3-5ppm are for the protons of the dextran backbone. In **Figure 2**, the appearance of weak signals at 2.84 ppm is due to the protons of methylene in the ethyle diamine end group. All other signals are for the protons of the dextran backbone.

Characterization of sPCL-OH

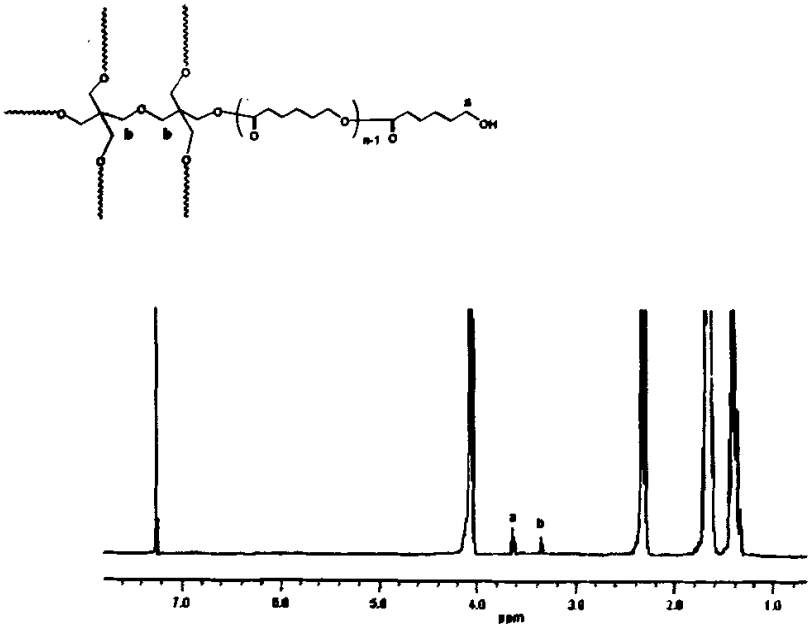


Figure 3. ^{1}H NMR spectrum of sPCL-OH(CD_3 as solvent)

For the synthesis of sPCL-OH, a commercial dipentaerythritol compound was chosen as a multifunctional initiator and $SnOct_2$ as a catalyst. , M_n of the sPCL-OH was controlled by the feed ratio of ε-CL to dipentaerythritol. **Figure 3** is the ^{1}H NMR spectrum of the sPCL-OH. The peak at 3.62 ppm is owed to the proton on the end hydroxyl methylene group (CH_2OH). The small signal at 3.34 ppm is due to the protons on the methyleneoxy groups (CH_2OCH_2) assigned to the dipentaerythritol initiator residue. All others absorption peaks are attributed to the protons of the sPCL-OH backbone. Moreover, the integral ratio of the proton signal on the methylene adjacent end hydroxy group to the methyleneoxy group is 3.1, which indicates the star shape PCL with six arms is obtained[20].

From the integration ratio of proton signal on the methylene end group (CH_2OH, 3.62 ppm)

to the methylene proton signal of the main chain, the average arm length (L_{arm}) in sPCL-OH is obtained and showed in **Table 1**. In addition, the theoretic average arm length is obtained by the following equation and also showed in **Table 1**.

L_{arm} = [ε-CL]/[dipentaerythritol](mol/mol)×114.14/ 6

where 114.14 is the molecular weight of ε-CL, 6 is the number of the arms in sPCL-OH. From the **Table 1**, we can find that the L_{arm} of ^{1}H NMR is closed to the theoretic L_{arm}. This further proves the sPCL-OH having six arms.

Table 1. Average arm length of sPCL-OH from ^{1}H NMR and theoretic calculating

sample	[ε-CL]/ [dipentaerythritol] (mol/mol)	L_{arm} (^{1}H NMR)	L_{arm} (theory)
$sPCL_{60}$-OH	60	1380	1270
$sPCL_{120}$-OH	120	2710	2540

Characterization of sPCL-CH=CH$_2$

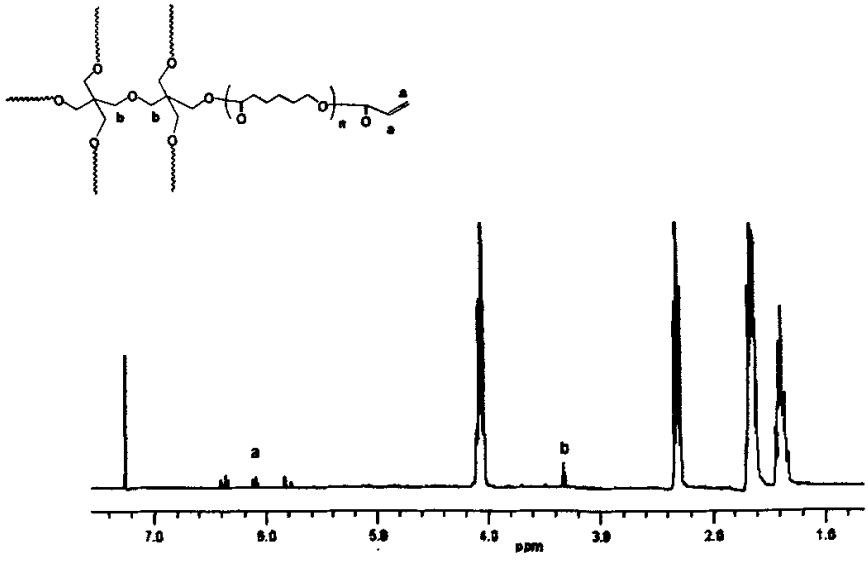


Figure 4. ^{1}H NMR spectrum of sPCL-CH=CH$_2$ (CD_3Cl as solvent)

Figure 4 is the ^{1}H NMR spectrum of sPCL-CH=CH$_2$. The signals at 1.39, 1.65, 2.35 and

4.10 ppm are due to the protons of the main chain. The signals at 5.80-6.38 ppm are assigned to the protons of the acrylate end group ($CH=CH_2$, 5.80-6.38 ppm), and the signals at 3.31 ppm is due to the protons on the methyleneoxy groups (CH_2OCH_2) from the dipentaerythritol initiator residue.

Table 2 gives the GPC results of the sPCL-$CH=CH_2$. For the reactants of $sPCL_{60}$-OH and $sPCL_{120}$-OH, the M_n of the corresponding $sPCL_{60}$-$CH=CH_2$ and $sPCL_{120}$-$CH=CH_2$ are 7800 and 14600 g/mol with the M_w/M_n of 1.28 and 1.33, respectively.

Table 2. GPC results of the sPCL-$CH=CH_2$

sample	M_n	M_w	M_w/M_n
$sPCL_{60}$-$CH=CH_2$	7800	10900	1.28
$sPCL_{120}$-$CH=CH_2$	14300	21000	1.33

Characterization of sPCL-dextran

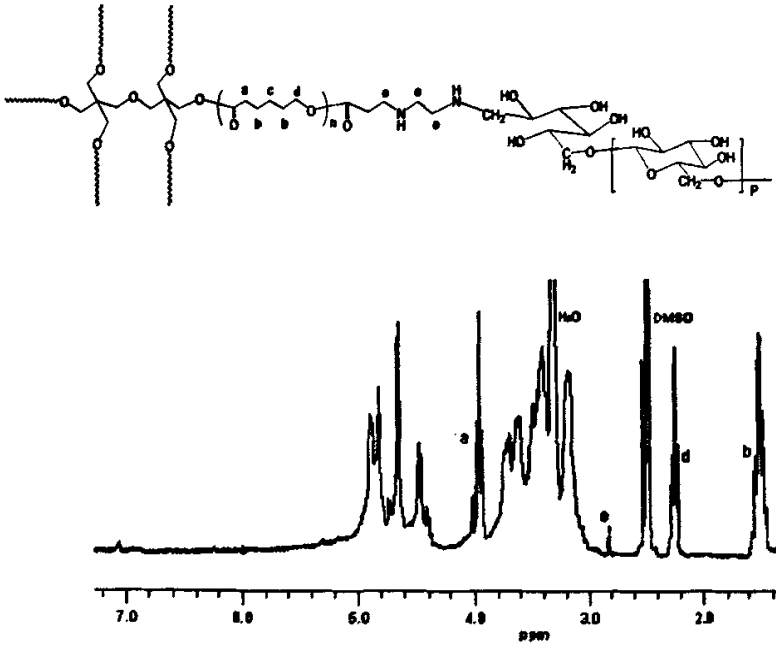


Figure 5. H^1 NMR spectrum of sPCL-dextran(DMSO-d_6 as solvent)

The sPCL-dextran was synthesized by the Micheal addition reaction of acrylate group in the sPCL-$CH=CH_2$ coupled with amino group in dextran-NH_2. **Figure 5** is the H^1 NMR

spectrum of sPCL-dextran. In **Figure 5**, the peaks at 4.0, 2.22, 1.5 and 1.22 ppm are attributed to the protons of the PCL segment in sPCL-dextran. The small signal at 2.81ppm is due to the protons of methylene in the ethyle diamine segment, and the peaks at 3-5ppm are attributed to the protons of the dextran segment. These results indicate that the dextean-NH_2 is successfully coupled with sPCL-CH=CH_2.

From the nitrogen percentage by element analysis and M_n of sPCL-CH=CH_2 by GPC, the average number arms of the dextran in sPCL-dextran is obtained from the following equation:

$$N\% = No_{,arm} \times 2 \times 14 / (M_{n,\ sPCL\text{-}CH=CH_2} + No_{,arm} \times M_{n,\ dextran\text{-}NH2} + 60)$$

where, $N\%$ is the weight percentage of the nitrogen in sPCL-dextran. $No_{,arm}$ is the number of the dextran arms in sPCL-dextran. $M_{n,\ sPCL\text{-}CH=CH2}$ is the number average molecular weight of sPCL-CH=CH_2 and $M_{n,\ dextran\text{-}NH2}$ is the number average molecular weight of the dextran-NH_2. 14 is the atomic weight of the nitrogen and 60 is the molecular weight of ethyl diamine.

Table 3. Number of dextran arms in sPCL-dextran determined by element analysis

Samiple	Nitrogen content (w%)	number of dextran arm
$sPCL_{60}$-dextran	0.98	5.5
$sPCL_{120}$-dextran	0.66	5.1

In **Table 3**, for the [ε-CL]/[dipentaerythritol] of 60 and 120, the nitrogen contents are 0.98% and 0.66, the number of dextran arms are 5.2 and 5.0, correspondingly. That gives a quantitatively evidence which the double bond in PCL-CH=CH_2 has coupled with amino group in dextran-NH_2 and the outer block in the arm of the obtained sPCL-dextran is hydrophilic dextran.

Micellar Properties of sPCL-dextran

A dialysis method was employed to prepare polymeric micelles. The polymer was first dissolved in DMSO, which is a good solvent for both PCL and dextran segments, and micellization was induced by the dropwise addition of water and followed by dialysis. The

micelle effective hydrodynamic diameter (R_h) with different concentrations is determined by DLS and shown in Figure 6. For the $sPCL_{120}$-dextran, the R_h of the micelle is about 30nm at the concentration less than 1mg/mL, and the R_h of the micelle increas from 30 to about 180 nm with the concentration from 1mg/mL to 22mg/mL. This is perhaps due to the aggregating of the micelles at the high concentration. The R_h of the micelle formed by $sPCL_{60}$-dextran is lightly lower than that formed by $sPCL_{120}$-dextran.

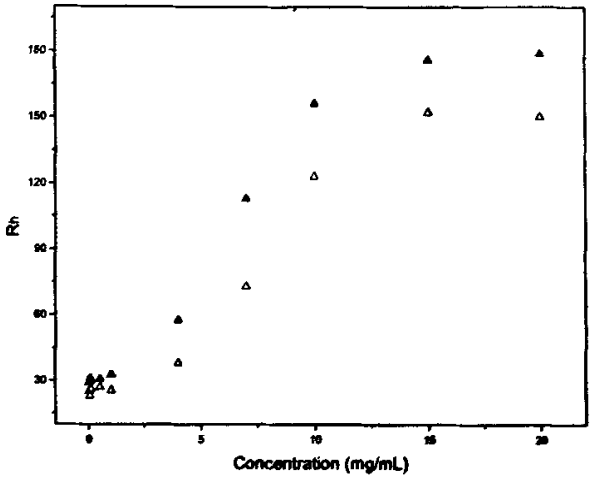
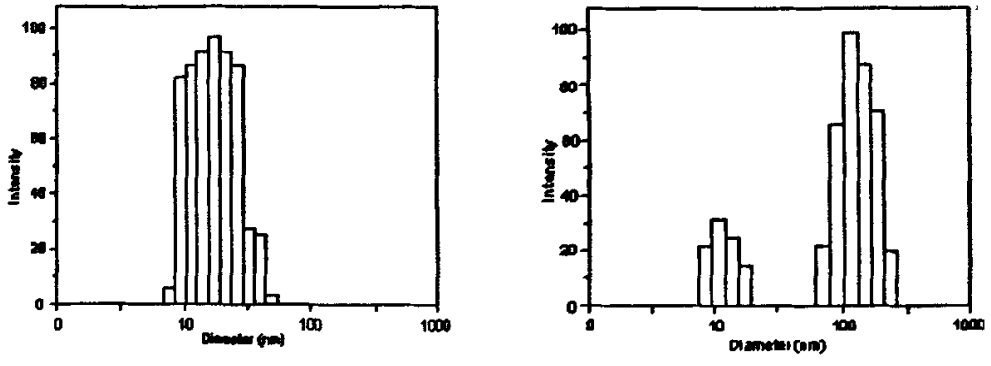


Figure 6. Relationship between R_h and concentration of sPCL-dextran in water
? $sPCL_{120}$-dextran ? $sPCL_{60}$-dextran



A B

Figure 7. Histograms showing the size distribution of (A) 1 mg/ml (B) 10mg/ml

Figure 7A and **Figure 7B** give the size distribution of the micelles formed by

$sPCL_{120}$-dextran. At the concentration of 1mg/mL (**Figure 7A**), a unimodal distribution appears with an overall R_h of 32.3nm and polydispersity index of 0.137. When the concentration increased to 10 mg/ml (**Figure 7 B**), a bimodal distribution appears with a smaller size component of 30.5 nm (20%) and a large size component of 156 nm (80%). The small species in DLS with the diameter of 30.5 nm is identified as unimolecular micelle, and the large species with 156 nm diameter is assigned to aggregated micelles.

A schematic picture of a possible aggregated micelle structure is shown in **Figure 8**. In the case of a star block copolymer with a low arm density (<16 arms), the hydrophilic outer dextran shell is too loose to outweigh the intermolecular association of the hydrophobic PCL blocks and cause intermolecular micelle formation.

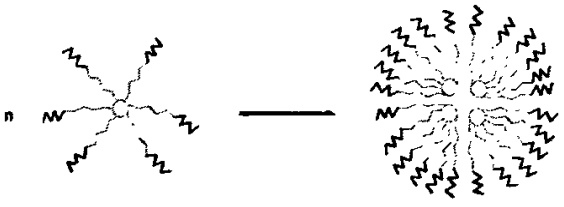


Figure 8. Formation of the aggregated micelle

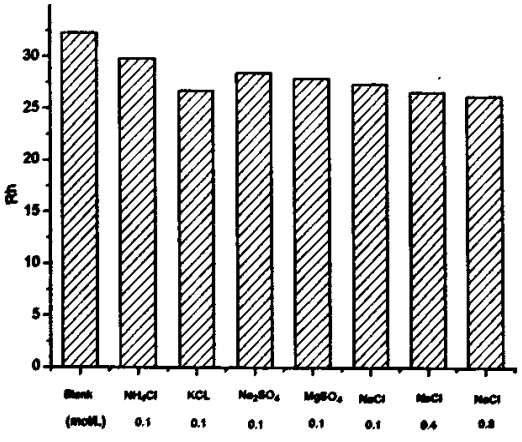


Figure 9. R_h of the micelle in differential salt solutions

The stability of the micelle formed by $sPCL_{120}$-dextran in salt solutions was investigated by DLS (**Figure 9**). The micelle in differential salt solutions (0.1mol/L) has the similar R_h of

about 30nm. In the NaCl aqueous solutions, R_h of the micelle hardly changes when the concentration increased from 0.1 to 0.8 mol/L. Dextran generally shows extended conformation in different salt solutions, so the shell of the micelles formed by dextran hydrophilic segments can extend in the salt solution and guarantee the stability of the micelle.

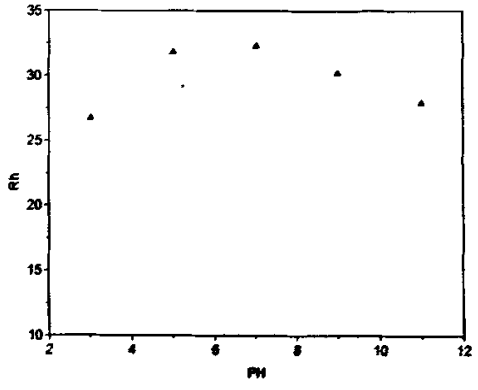


Figure 10. R_h of the micelle in different pH solution

Figure 10 gives the R_h of the micelle formed by $sPCL_{120}$-dextran in differential pH solutions, the R_h of the micelle increase from 26.7 to 32.2nm when the pH value changed from 3 to 11, indicating the micelle is stable in different pH solutions.

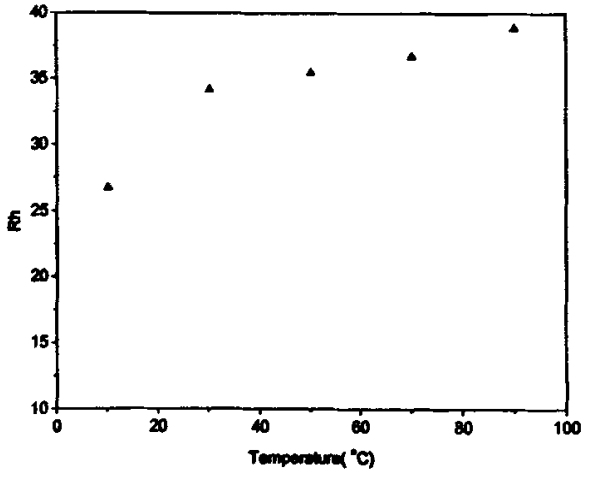


Figure 11. R_h of the micelle in different temperature

The micelle is also stable in different temperatures. In **Figure 11**, the R_h increases from

26.7 to 38.9 nm when the solution temperature changed from 10 to 90 °C.

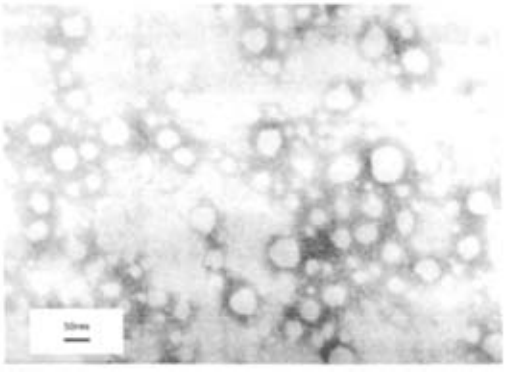


Figure 12. TEM image of micelles formed by $sPCL_{120}$-dextran

Figure 12 shows the TEM image of micelles formed by $sPCL_{120}$-dextran at 1mg/mL. It can be seen that the micelles take a spherical shape and a broad size distribution. The diameter about 50 nm of some micelle is perhaps due to the aggregation of the micelles during the drying.

Conclusions

A star polymer with six amphiphilic block copolymer branch arms has been synthesized and characterized. Characterization with SEC, ^1H NMR, FTIR, TGA, and DSC confirmed that the core of the star polymer is dipentaerythritol, the inner block in the arm is hydrophobic PCL, and the outer block in the arm is hydrophilic dextran. At the concentration of the polymer solution below 1 mg/ml, the R_h about 30nm and a unimodal distribution was observed. When the concentration of polymer solution increased from 1 to 20mg/ml, a bimodal distribution appears, and the R_h of the micelle increased from 30 to about 180 nm. The micelle is stable in different salt solutions. The pH and temperature have no apparent influence on the R_h. This sPCL-dextran will possibly establish a useful starting point for using the polysaccharide to synthesis the star shape amphiphilic polymer.

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Chapter 3

Polyion Complex Particles from the Dextran Derivative with L-phenylalanine Side Groups and Carboxylic Acid Ended Poly (ε-caprolactone) in Aqueous Medium

Introduction

The nanoparticle with shell-core structure can be formed from block or graft copolymers by self-assembling in selective solvents. The driving forces include the repulsive interactions between one of the blocks (or grafts) and the solvent, [1-3] the electrostatic interaction between a pair of oppositely charged copolymers, [4-6] and complexation of the metal with polymer [7, 8] or hydrogen bondings [9, 10] of the groups on the polymer.

Polyion complex particles with core shell structure in aqueous solution are unique for biomedical applications. [4-6, 8] For example, various charged substances such as ionic drugs, proteins, and nucleic acids can be selectively concentrated in ionic inner-core through electrostatic interaction. The hydrophilic outer-shell of the particle can protect drug-incorporating inner-core from the protein adsorption or attack of reticuloendothelial systems (RES) and increase the blood circulation time of the particles.

However, the polyion complex particles are usually prepared in non polarity organic solvent. [11-14] In recent years, several groups reported the formation of complex particles in aqueous solution. For example, Kataoka [4-6] prepared stable polyion complex particles in an aqueous solution through electrostatic interaction between a pair of oppositely charged block copolymers of poly(ethylene glycol)-b-poly(L-lysine) and poly(ethylene glycol)-b-poly(aspartic acid). Bronich reported [8] the block ionomer complexes formed between poly(ethylene oxide)-*b*-polymethacrylate anions and divalent metal cations can be utilized as templates for the synthesis of the cross-linked micelles.

Dextran is a biocompatible and water soluble polysaccharide, and has been used widely in biomedical field [15, 16]. Recently, some literatures reported the nanoparticles formed by amphiphilic dexrtran derivatives in aqueous solution [17, 18]. In chapter 3, a series of dex-phe

were synthesized by the grafting L-phenylalanine onto the dextran. When water was drop to the solution of dextran derivatives with L-phenylalanine side group (dex-phe) and carboxyl ended poly(ε-caprolactone) (PCL-COOH) in DMSO, the complex nanoparticles were formed by the cooperation of hydrophobic interaction and electrostatic interaction between the dex-phe and PCL-COOH segments. The image of transmission electron micrograph (TEM) shows that the complex particles take an approximately spherical shape with the diameter of 60-120 nm and a core–shell structure.

Experiment

Materials

ε-caprolactone (ε-CL)(SIGMA) was dried over CaH_2 for 24 hours and distilled under reduced pressure prior to use. Dextran (Fluka), L-leucine (Shang hai Bio-reagent Limited Co. China), Boc-L-phenylalanine (Boc-phe)(Analytical grade, Alfa Aesar), and all other reagents were of analytical grade and used as received.

Measurements

^{1}H NMR

^{1}H NMR spectra were recorded on Mercury-Plus 300 (Varian, USA) spectrometer at 300 MHz, using tetramethysilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. The concentration of the solution was 5mg/ml.

Gel permeation chromatography (GPC)

Number average molecular weight (M_n) and polydispersity index (M_w/M_n) of PCL-COOH were measured with a Waters high-performance liquid chromatography system, a model 2410 refractive-index detector, and Shodex K802.5 with shodex K-G Guard column. The measurements were performed in chloroform at 25°C and calibrated with polystyrene standards, and the value of M_n was determined by the universal calibration method using a viscosity detector. M_n and M_w/M_n of the dextran and Dex-Phe were measured at 40°C by using a Waters 515-410 gel permeation chromatograph equipped with Waters 410 detector

and Ultrhydrogel column. Water was used as the eluent at a flow rate of 0.6 ml/min.

Titration of carboxyl end group in PCL-COOH

Purified PCL-COOH was dissolved in a mixture solvent of isopropyl alcohol and 1,4-dioxane(V/V= 1/4) and titrated with 0.012 mol/L potassium hydroxide in the mixture solvent of isopropyl alcohol and 1,4-dioxane(V/V =1/4) with 1% phenolphthalein/pyridine as the indicator, and the number of the carboxyl groups in the PCL was calculated according to the following equation:

$$No. = (V_{KOH} \times 10^{3} \times 0.012) / (Mass\ of\ PCL / M_n)$$

Where N_O. is the number of carboxyl group, V_{KOH} is the volume of KOH (ml) consumed in the titration, 0.012 is the molar concentration of KOH (mol/L), and M_n is the number-average molecular weight of the PCL-COOH by GPC.

Determination of the substitution degree of L-phenylalanine in the dex-phe

The substitution degree (SD) of L-phenylalanine in dex-phe can be determined from the following equation.

$$A\% = \frac{SD \cdot W_N}{W_{C_6H_{10}O_5} + SD \cdot W_{(C_9H_{11}NO_2)} - SD \cdot W_{H_2O}}$$

where $A\%$ is the weight percent of the nitrogen of dex-phe, $W_{C_6H_{10}O_5}$ and $W_{C_9H_{11}NO_2}$ are the molecular weight of dextran and L-phenylalanine, respectively, and W_{H_2O} is the molecular weight of water which lost in the reaction.

Solubility test

1 g dex-phe was placed in the flask and a total of 10 ml of solvent was added in three portions. Between each portion, the sample was stirred for 35-40 minutes at 25 °C. During this time, the status of the solution was observed.

Zeta-Potential measurements

Zeta-potentials of the polyion complexes particles were determined by a Zeta Potential

Analyzer instrument (JS94H Shanghai zhongchen digital technic apparatus co. China).

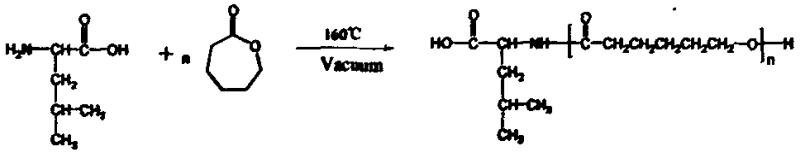
Dynamic light scattering measurements

The complex particle size and size distribution were determined by dynamic light scattering (DLS) using a BI-200SM Goniometer particle size analyzer (Brookhaven, USA). Each analysis lasted for 300 s and was performed at 25 °C with angle detection of 90°. The concentration of the polymer solution was 2 mg/ml.

Transmission electron microscopy (TEM) measurements

The morphological examination of the complex particles was performed using a JEM-2010HR high-resolution transmission electron microscope. A drop of dex-phe and PCL-COOH (weight ration of PCL-COOH to dex-phe of 0.5) in aqueous solution (1.2 mg/ml, based on dex-phe) containing 0.2wt % phosphotungstic acid (PTA) was deposited onto a 200 mesh copper grid coated with carbon. Excessive solution was removed with a Kimwipes delicate wipe. The shape and size of the particles were directly determined from transmission electron micrograph.

Synthesis of PCL-COOH (Scheme 1)

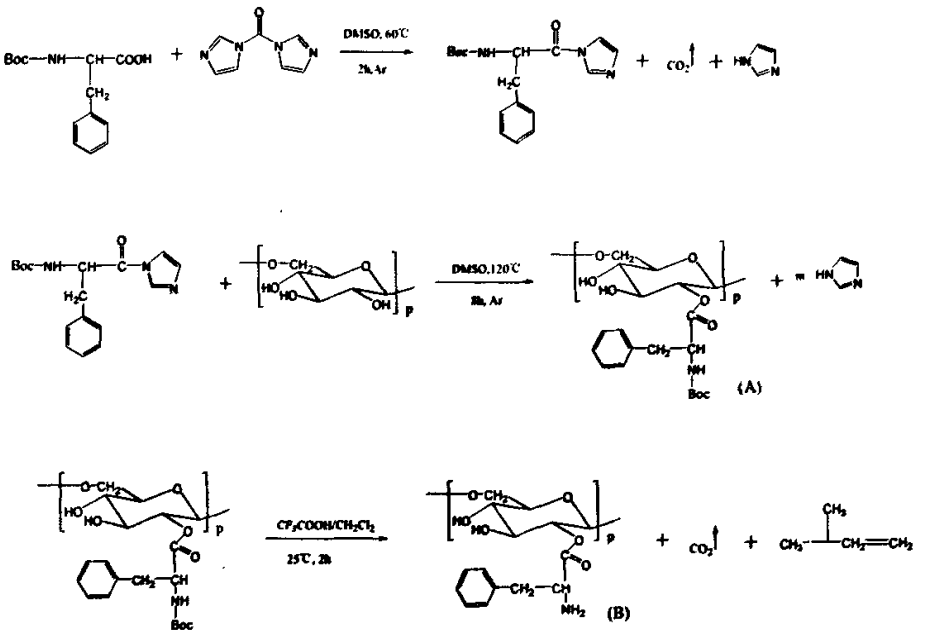


Scheme 1. Synthesis of PCL-COOH

PCL-COOH was synthesized by ring opening polymerization of ε-CL initiated by L-leucine[19]. The typical procedure is described as following: 1.5g ε-CL with 0.1g of amino acid and a mini electromagnetic stirrer in vacuum-sealed ampoule (40 Pa) was polymerized in an oil bath at 160 °C. The ampoules were removed at given time and cooled in ice water to stop the polymerization. After cooled down, the obtained product was dissolved in tetrahydrofuran (THF) and precipitated by a mixture of methanol and distilled water (v/v=4/1)

at room temperature. The precipitate was filtrated and dried in vacuum at 30°C for 24 h.

Synthesis of dex-phe (Scheme 2)



Scheme 2. Synthesis of dextran with L-phenylalanine side group

2 g N-(tert-butoxycarbonyl)-L-phenylalanine (Boc-phe) was dissolved in 20 ml DMSO and transferred to a three-necked flask with mini electromagnetic stirrer. To this solution, 1.35g *N, N*-carbonyldiimidazole (CDI) ([CDI]/[Boc-phe]=1.1/1, *mol/mol*) was added. The reaction was conducted at 60 °C for 2 hours in an Ar atmosphere. Then the calculated amount of dextran ([dextran]/[Boc-phe] =0.5-1.6, mol/mol) was added, and the contents were heated to 120 °C in an Ar atmosphere for 8 hours. Then the flask was cooled to room temperature, a mixture of methanol and distilled water (v/v=2/1) was added. The resulting precipitate was filtrated, washed three times with distilled water, and dried in vacuum for 24 hours to obtain A.

The product A was dissolved in the mixture of trifluoroacetic acid and dichloromethane (*v/v*=1/1) to take off the tert-butoxycarbonyl protective group. After 2 hours at 25 °C, the trifluoroacetic acid and dichloro methane were removed using a rotary evaporator. The

obtained solid B was dissolved in 4% (*w%*) aqueous acetic acid solution and precipitated in a mixture solvent of methanol/triethylamine (*v/v*=4/1), then washed with methanol three times and dried in vacuum at 30°C for 24 hours. The reaction process is shown in Scheme 2.

Preparation of complexes particles

10 mg PCL-COOH and the calculated amount of the dex-phe ([PCL-COOH]/[dex-phe]=0.1-3, *w/w*)were dissolved in 1ml DMSO, then adding water drop by drop to the solution with a mild stir. In the initial 2ml, the interval time is 10 s per drop. After 2 ml, the interval time decrease to 5 s per drop. In the final solution, the volume ratio of water to DMSO was 10. The solution was transferred into dialyser that does not pass the molecules which molecular weight above 500 and dialyzed against distilled water of 2L for 24 hours to obtain the purified particles. Distilled water was refreshed at 2, 5, 8, and 24 hours respectively. The obtained product was stored at 4℃ for TEM and DLS tests.

Results and Discussion

Preparation and characterization of PCL-COOH

Considering the good biocompatibility of the natural amino acid, the L-leucine was selected to initiate the ring opening polymerization of the ε-caprolactone (ε-CL) to obtain the PCL-COOH[19]. The results in **Table 1** show that the PCL-COOH with M_n of 2300, 3100 and 5700 g/mol have been obtained by adjusting the feed ratio of ε-CL to L-leucine, and all the PCL-COOHs have approximate molecular weight distributions about 1.65.

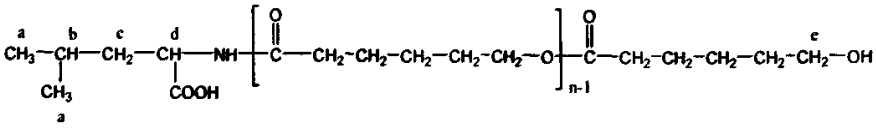
Table 1. Preparation of PCL-COOH initiated by L-leucine

PCL-COOH	[ε-CL]/[L-leucine]	Yield [a] (%)	M_n	M_w/M_n
1	20	91.4	2300	1.66
2	30	94.2	3100	1.63[a] 1.80[b]
3	50	95.1	5700	1.65

[a] after purification

[b] without purification

Structure of obtained PCL-COOH was characterized by ^{1}H-NMR spectroscopy. The result is shown in **Figure 1**.



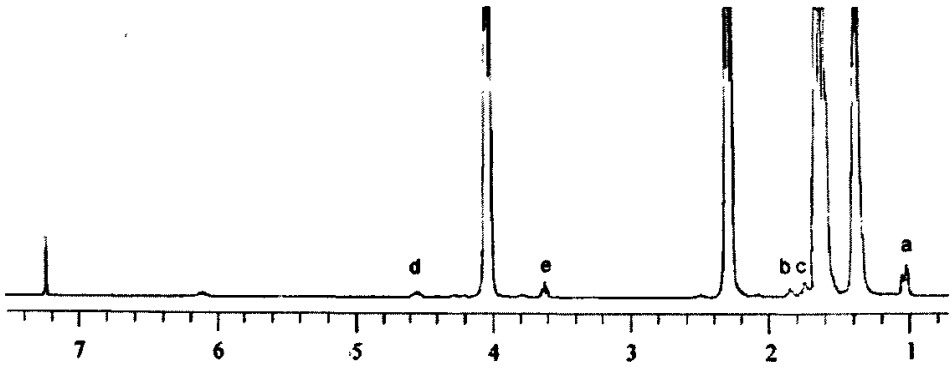


Figure 1. ^{1}H-NMR spectrum of PCL-COOH obtained by using L-leucine as the initiator (molar ratio of [ε-CL]/[L-leucine]=30)

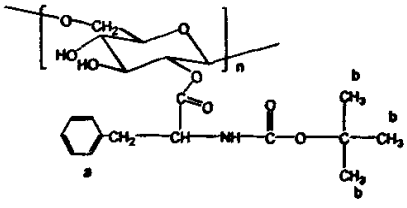
In **Figure 1**, the peaks at 1.0, 1.8, 1.7 and 4.5 ppm are attributed to the proton of CH_3–(a), –CH–(b), –CH_2–(c) and –CH–(d) of the L-leucine end group. The triplet at 3.6 ppm arose from the protons in the –CH_2OH end group. All other peaks are due to the backbone of PCL-COOH. The results of ^{1}H NMR indicate that the L-leucine has incorporated into the polymer chain.

Table 2. Content of carboxyl group in PCL-COOH (analyzed by titration)

PCL-COOH	M_n of PCL (g/mol)	KOH (ml)	Mass of PCL (g)	No. of carboxyl group
1	2300	16.7	0.505	0.91
2	3100	12.8	0.510	0.93
3	5700	6.4	0.502	0.87

The content of the carboxyl end group in the PCL-COOH was analyzed by titration. In Table 2, the number of the carboxyl group of the PCL-COOH with the number average molecular weight of 2300, 3100 and 5700 g/mol were 0.91, 0.93 and 0.87, respectively. It is demonstrated that the carboxyl group exists in the polymer chain, consisting with the ^1H NMR result.

Characterization of dex-phe



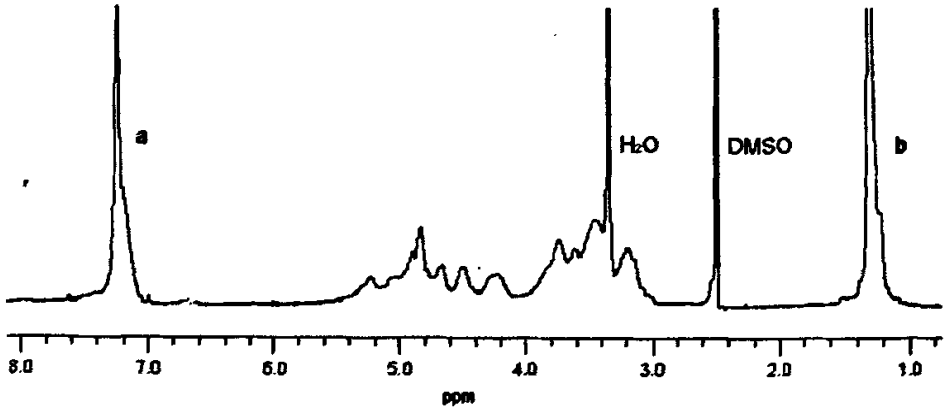
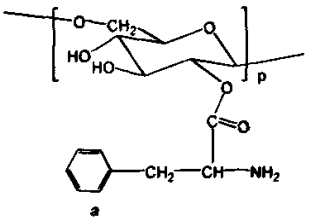


Figure 2. ^1H-NMR spectrum of dextran derivative with Boc-phe side group (DMSO-d^6 as solvent)

Figure 2 is the ^1H NMR spectrum of the dextran derivative with Boc-phe side groups. The peaks at 1.3 and 7.2 ppm are attributed to the proton of CH_3O– and phenyl in the Boc-L-phenylalanine side group. The absorptions at 3-5 ppm are for the protons in the dextran backbone, and the signals of the protons of CH_2 and CH in Boc-phe side groups overlapped with the signals of the protons on dextran backbone.

Figure 3 gives the ^1H NMR spectrum of dex-phe. The diminishing peak at 1.3 ppm of the

protons in Boc-protected groups (CH_3O) indicated that the diprotecting reaction processed successfully.



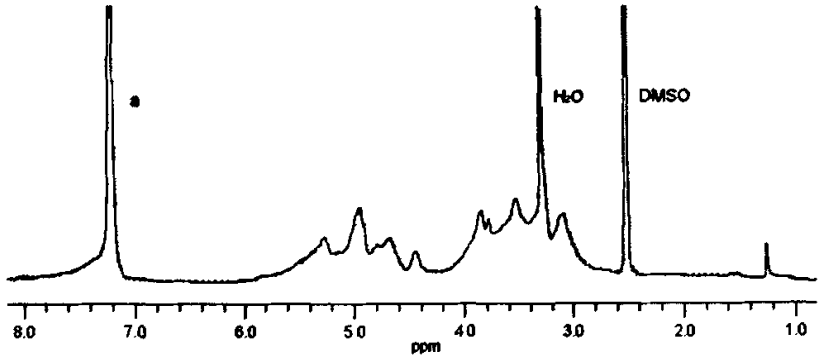


Figure 3. ^{1}H-NMR spectrum of dex-phe (DMSO-d^6 as solvent)

(dex-phe, SD 0.81, M_n 18200g/mol)

SD of the dex-phe was calculated from the nitrogen content which was determined by element analysis. The results in **Table 3** show that the SD values increase from 0.44 to 1.04 with the increase of [Boc-L-phe]/[dextran] molar ratio from 0.5 to 1.6, indicating that the SD of dex-phe can be controlled by the feed ratio of the Boc-L-phe to dextran.

Table 3. Element analysis results of dex-phe

Molar ratio of [Boc-L-phe]/[dextran]	N (A%)	SD
0.5	2.7	0.44
0.8	3.6	0.67
1.2	4.03	0.81
1.6	4.62	1.04

Table 4 gives the solubility of dex-phe in different solvents. The dex-phe can dissolve in the water and DMSO, partially dissolve in the DMF and acetone, but can't dissolve in the methanol and cyclohexane.

Table 4. Solubility test of dex-phe[a]

Solvent	Water	DMSO	DMF	Acetone	Methanol	Cyclohexane
Solubility	S	S	P	P	N	N

[a] SD 0.81

S, dissolve; P, partly dissolve; N, can't dissolve

The molecular weight and the molecular weight distributions of the dex-phe were measured by GPC and shown in **Table 5.** The M_n varies from 17300 to 18700 g/mol and M_w/M_n changes from 1.70 to 1.79, depending on the SD value of the dex-phe. Comparing with the dextran, the dex-phe has lower molecular weight values and broader molecular weight distribution, which is probably due to the partial degradation of the dextran during the reaction process.

Table 5. GPC results of dextran and dex-phe

Material	Molar ratio of [Boc-phe]/[dextran]	Yield[a] (A%)	M_n (g/mol)	M_w/M_n
dextran	-	-	23100	1.64
dex-phe	0.5	78.5	17500	1.70
dex-phe	0.8	81.2	17300	1.79
dex-phe	1.2	77.8	18200	1.73
dex-phe	1.6	76.5	18700	1.76

[a] yield was based on dextran

Formation and characterization of polyion complex particles

The dextran and dex-phe with a series PCL-COOH were dissolved in DMSO, and then water was added drop by drop to this solution. In **Table 6**, the precipitate appeared in the solution of the PCL-COOH and dextran indicates that the complex nanoparticles hasn't formed in this system. For the PCL-COOH with the molecular weight of 2300 and 3100 g/mol, adding water to the solutions of PCL-COOH and dex-phe in DMSO resulted in slight blue to white milky solution reveals the formation of complex nanoparticles. When adding

water to the solution of PCL-COOH with molecular weight of 5400 g/mol and dex-phe in DMSO, the resulting precipitate indicate the stability of the complex particles is relative to the molecular weight of PCL-COOH. That perhaps due to the higher molecular weight of the PCL-COOH destroys the balance of the hydrophobic and hydrophilic of the complex particles, and result in aggregation together of the particles and precipitating from the solution.

Table 6. Adding water to the solution of PCL-COOH and Dex-Phe[a] (or dextran[b]) in DMSO

M_n of PCL-COOH (g/mol)	3100	2300	3100	5400
Polysaccharide	dextran	dex-phe	dex-phe	dex-phe
State	Precipitate	Slight blue solution	Slight blue to white solution	Precipitate

[a] dex-phe, M_n 18200 g/mol, SD 0.81; weight ratio of PCL-COOH to dex-phe was 1/2 Concentration of the solution, PCL-COOH 1mg/mL; dex-phe, 2mg/mL

[b] dextran, M_n 23100 g/mol; weight ratio of PCL-COOH to dextran was 1/2 Concentration of the solution, PCL-COOH 1mg/mL; dextran, 2mg/mL

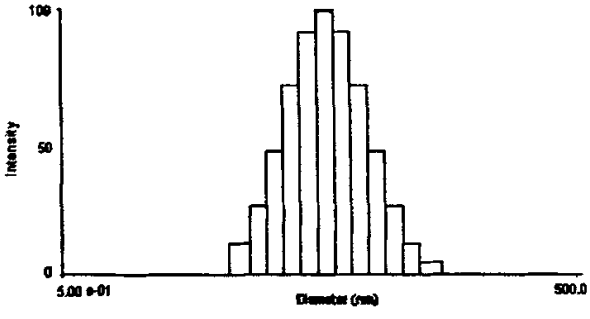


Figure 4. Size distribution of the particles in aqueous

(PCL-COOH, M_n 2300 g/mol; dex-phe, SD 0.81, M_n 18200 g/mol; weight ratio of PCL-COOH to dex-phe was 1/2, Concentration of the solution, PCL-COOH 1mg/mL; dex-phe, 2mg/mL)

The size distribution and hydrodynamic radius of complex particles were measured by dynamic light scattering (DLS) technique. The average hydrodynamic radius of particle (R_h)

of 169.5 nm was obtained, and the size distribution of the particle in aqueous is shown in **Figure 4.**

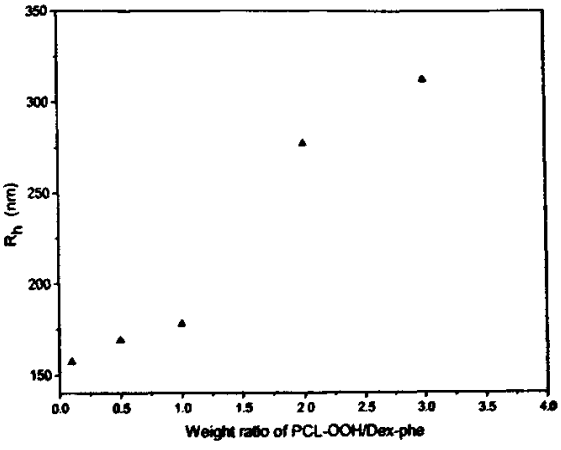


Figure 5. The relationship between the R_h of the nano particle and the weight ratio of PCL-COOH to dex-phe
(dex-phe, SD 0.81, M_n 18200 g/mol; PCL-COOH, M_n 2300 g/mol, Concentration of the solution, PCL-COOH 0.1-3.0 mg/mL; dex-phe, 1.0 mg/mL)

Figure 5 shows the relationship between the R_h of the complex particles and the weight ratio of PCL-COOH to dex-phe. R_h of the complex particles varied within 150-200nm at the weight ratio of PCL-COOH to dex-phe from 0.1 to 1. The R_h increases over 280 nm at the weight ratio of PCL-COOH to dex-phe above 2. The reason is probably that the decrease of the dex-phe content weakens the stability and causes the aggregating of the particles.

Table 7. Zeta potential of the particles formed by dex-phe with different SD values

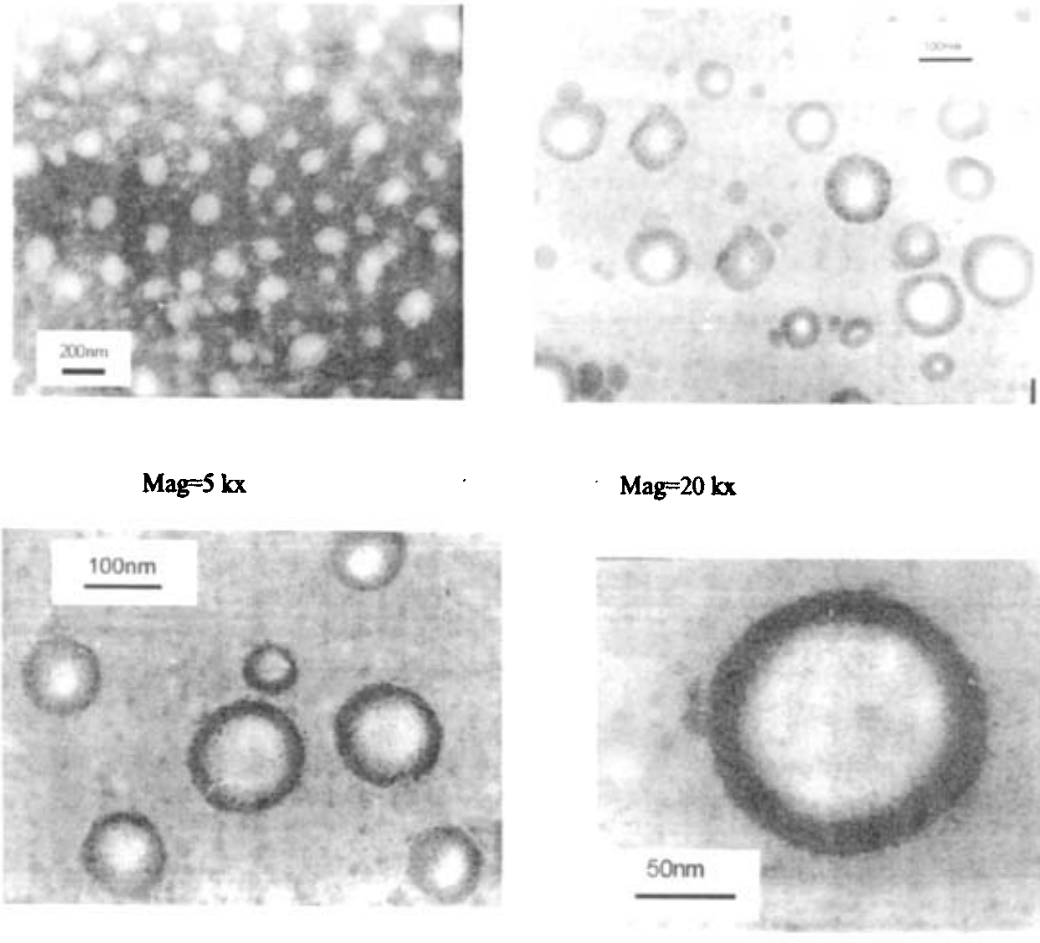
SD of dex-phe	0.44	0.67	0.81	1.04
Zeta-Potential (mv)	10.2	15.3	19.6	22.4

PCL-COOH, M_n 2300 g/mol; Weight ratio of PCL-COOH to dex-phe was 1/2

Zeta-potential measurements were conducted to determine the net charges on the particles surface (**Table 7**). The particles are positively charged and the zeta potential increased with the SD values of the dex-phe.

TEM image of the particles formed by dex-phe and PCL-COOH in aqueous solution is shown in **Figure 6.** The isolated particles take an approximately spherical shape with

diameter of 60-120 nm and the core–shell structure was observed. The white core consists of collapsed hydrophobic PCL-COOH chains and the black shell is formed by stained dex-phe.



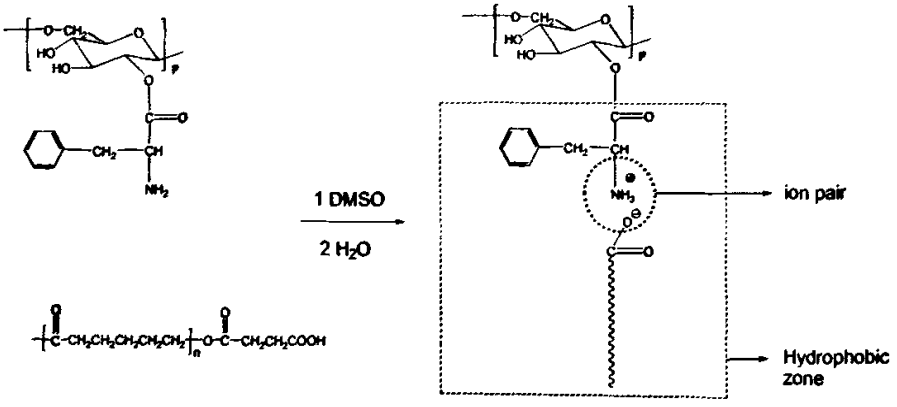
Mag=5 kx Mag=20 kx

Mag=40kx Mag=100 kx

Figure 6. TEM of polyion complex particles formed by dex-phe with PCL-COOH (PCL-COOH, M_n 2300 g/mol; dex-phe, SD 0.81, M_n 18200 g/mol; weight ratio of PCL-COOH to dextran was 1/2)

The simplified model of the complex particle formation is shown in **Scheme 3.** Water is the precipitant for PCL-COOH but solvent for dex-phe, when water is drop to the solution of dex-phe and PCL-COOH in DMSO, the hydrophobic L-phenylalanine side groups in dex-phe and PCL-COOH segments are aggregated together through the cooperative of hydrophobic interaction and electrostatic interaction to form the compact core. The ion pairs are embedded

within the hydrophobic core that avoided the dissociation of the ion pair in aqueous solution. The shell of the hydrophilic dextran segment surrounds the core to prevent the progressive aggregation of the core and stabilize the complex particles in the aqueous solution.



Scheme 3. The simplified model of formation of nano particle by the force of hydrophobic interaction and electrostatic interaction

Conclusions

In this paper, a series of dex-phe were synthesized and characterized. Polyion complex particles were formed by dropping water into the solution of dextran-phe with PCL-COOH in DMSO. The stability of the complex particles is related to the SD of dex-phe and the M_n of the PCL-COOH. TEM, DLS and Zeta-potential measurements demonstrate the complex particles take an approximately spherical morphology and core-shell structure. The driving force for the formation of the complex particles is hydrophobic interaction and electrostatic interaction between the dex-phe and PCL-COOH segments. The particles combine several key structural features that make these systems very beneficial for drug delivery. These are a hydrophobic ionic core, a hydrophilic shell, nanoscale size, the safety and biocompatibility of the L-leucine, dextran and poly (ε-caprolactone). Such polyion complex particles have the potential applications in pharmaceutical fields.

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Chapter 4

Synthesis and Characterization of Polyester Derivative Contained Glucose Ester Units

Introduction

Biodegradable aliphatic polyesters have been intensively studied and widely used in biomedical applications, including sutures, implants for bone fixation, scaffolds in tissue engineering, and carriers in drug delivery, etc. [1-3] However, hydrophobicity and lack of functional groups to which bioactive molecules (drugs, recognition agents, adhesion promoters. or probes) can be covalently attached are the sources of occasional shortcomings for some applications. [4] Recently, Some biodegradable polymers possessing functional or reactive groups such as amino, hydroxyl, carboxyl, thiol, etc. have been reported. [5-7]

Sugars is an essential component in organism nutrition, and contains multiple hydroxyl functional groups, synthetic sugar-containing polymers with well-defined structure have much potential for biocompatible and biodegradable polymeric materials. For example, Ouchi [8]reported the synthesis of poly(L-lactide) end-capped with lactose residue. Marcincinova[9] investigated the functionalized poly(lactic acid-*co*-glycolic acid)-based polyesters for biomedical and pharmaceutical applications. Uyama[10] reported a sugar-containing polyester synthesized by the polymerization of sorbitol and divinyl sebacate using *Candida antarctica* lipase as catalyst. Okada [11-13] reported a series of polyesters prepared from 1,4-dianhydro-D-glucitol and aliphatic dicarboxylic acids of the methylene chain length ranging from 2 to 10. Recently, Naoto Tsutsumi[14] reported a series of biodegradable network polyesters by the condensation of gluconolactone and citric acid.

In this study, novel biodegradable polyester derivative containing glucose ester units was prepared by a three-step reaction from gluconolactone and maleic anhydride. The presence of free hydroxyl and double bond groups would provide the possibility for chemically

modification of the polyester chains via pendent groups and coupling biologically active molecules, that expend the group of known biomedical polyesters candidates.

Experiment

Materials

Gluconolactone (Alfa Aesar), 2, 2-dimethoxypropane (DMP) (Alfa Aesar) was distilled under reduced pressure prior to use. 732 type cation exchange resin (Shanghai huizhu resin Limited Co. China), maleic anhydride (Guangzhou reagent Co. China), and all other reagents were of analytical grade and used as received.

Measments

IR

The IR spectra were measured on a Nicolet Nexus 670 Fourier-transform infrared (FTIR) instrument.

^{1}H NMR

^{1}H NMR spectra were recorded on Mercury-Plus 300 (Varian, USA) spectrometer at 300 MHz, using tetramethysilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as a solvent. The concentration of the solution is 5mg/ml.

Gel permeation chromatography

Gel permeation chromatography (GPC) was used to determine the molecular weights and polydispersity of the PGM. GPC analysis was performed on a Waters HPLC system equipped with a 2690D separation module and a 2410 refractive index detector. Water was used as an eluent and the flow rate was 1.0 mL/min.

Differential scanning calorimetry

Differential scanning calorimetry (DSC-204, Netzsch, German) was used to determine the glass transition temperature (Tg) of PGM at a heating rate of 10℃ min^{-1}

Synthesis of 4,6-O-isopropylidene gluconolactone(PG)

27 ml acetone, 5.0950 g (0286 mol) of gluconolactone(G) and 4.80 ml (0.038 mol) of DMP

were added to a 100 ml two-neck flask equipped with a magnetic stirrer and thermometer. The flask was stirred in a ice water bath at a temperature of 15 ℃ for 15 min, and then 0.20 g p-toluenesulfonic acid monohydrate was added and stirring for another 0.5 hour. The mixture warmed slightly to room temperature for 20 hours to yield transparent solution.

50 % NaOH was added to the resulting solution with stirring until the pH=8. During this time, some white precipitate appeared in the solution. Stirring was maintained for 1 hour. The white gum was collected by vacuum filtration and washed three times with acetone.

The filtrate was concentrated by rotating evaporation to give the colorless viscous liquid PG. The crude PG was diluted with 30 ml chloroform and washed three times with water. The obtained chloroform solution were dried by anhydrous sodium sulfate and filtrated. The filtrate was condensed with rotating evaporation to give 4.33 g colorless PG syrup. (yield, 69.4%)

Thin-layer chromatography (TLC) was used to purify the product. The mixture of ethyl acetate- petroleum and ether (1/4, v/v) was as developing solvent, R_f= 0.53.

Synthesis of poly(4,6-O-isopropylidene gluconolactone-maleic) (PPGM)

A certain feed ratio of PG to maleic anhydride was added to a round-bottom flask with magnetic stirring and a nitrogen inlet tube. The mixture was flushed with a gentle stream of N_2 for several minutes with stirring, and heated to 60 ℃ in oil bath for 1 hour and under reducing pressure(<500Pa) for 4 hour. Then the temperature of the system increased to 140℃ for 6 hour under reducing pressure. Finally, the pressure of the system was lowered to 50 Pa for 2 hours at 140℃.

The viscosity of the system increased gradually with the reaction proceeding. The resulting product was cooled to room temperature and purified by precipitating two times using tetrahydrofuran (THF) as solvent and H_2O as the precipitator. The precipitate was filtrated and dried in vacuum at 50℃ for 24h.

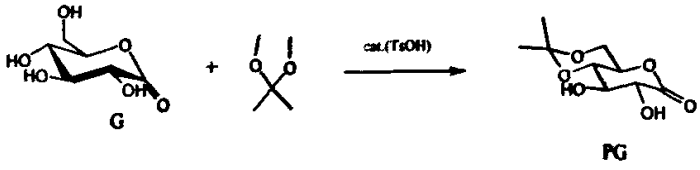
The obtained PPGM could be dissolved in the chloroform, methanol, THF and DMSO but didn't be dissolve in water.

Synthesis of poly (gluconolactone-maleic diester) (PGM)

The equivalent volume of 60% of acetate acid was added dropwise to a solution of PPGM in THF at room temperature with stirring. After 12 hours at room temperature, the majority of the solvent was removed on a rotary evaporator under reduced pressure. Then 0.2 mol/L NaOH was added until the pH of the solution is 7, and the solution was treated with the cation exchange resin to remove the Na^+. After filtrated, the filtrate was precipitated two times with H_2O as the solvent and acetone as the precipitator. The precipitate was filtrated and dried in vacuum at 35℃ for 24h.

Results and Discussion

Protection of 4, 6- hydroxyl groups in D-gluconic acid-d-lactone (gluconolactone, GL)



Scheme 1 Synthesis of PG

As the gluconolactone with four free hydroxyl groups, the successful preparation of linear oligomer with active function from gluconolactone required an efficient method for the selective protection of two hydroxyl groups in the gluconolactone molecule. The general ways for the selective protection and de-protection 4,6- hydroxyl group in the saccharine molecule are isopropylidenation and benzylation reaction. In the experiment, selective protection of 4-and 6- hydroxyl group of gluconolactone was carried out with 2, 2-dimethoxy propanes in the presence of p-toluenesulfonic acid catalyst at room temperature to yield 4,6-O-isopropylidene gluconolactone (PG)[15]. The process is shown in Scheme 1.

Figure1 shows the ^1H NMR spectrum of PG. The peak at 1.35 ppm is attributed to the proton of methylene in the isopropylidinene. The weak peaks at 4.33, 4.21, 4.07, 3.98, 3.83, 3.70 ppm were due to the protons in the gluconolactone molecule.

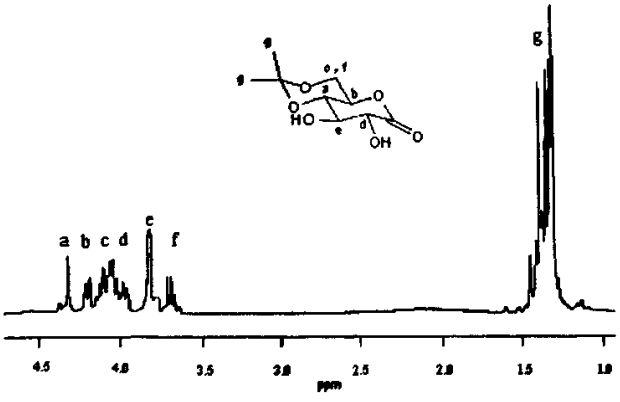


Figure 1. ^{1}H NMR spectrum of PG ($CDCl_3$ as solvent)

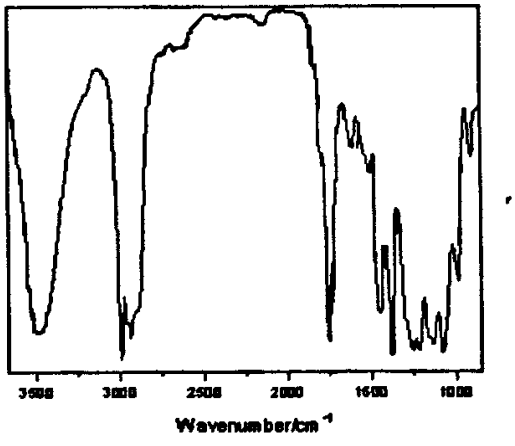


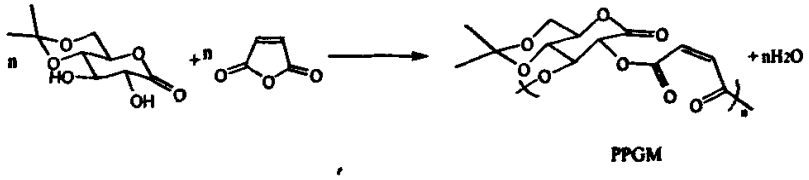
Figure 2. FT-IR spectrum of PG

Figure 2 shows the FT-IR spectrum of PG. The bands at 1382 and 1371 cm^{-1} are the characteristic adsorption of isopropylidinene group. The bands at 1439、2989、2936 cm^{-1} correspond to the C-H bond absorption of the isopropylidinene. The adsorption at 1753 cm^{-1} corresponds to vibration of C=O double bonds and the region from 3450 to 3600 cm^{-1} is the adsorption of O-H stretching shake.

Purity of the monomer is very important in the condensation polymerization. In the experiment, the crude PG is purified by silica gel column chromatography using ethyl acetate-petroleum and ether (1/4, v/v) as the eluent. The fraction in the middle band is collected and removed the solvent with rotating evaporation method.

Synthesis and characterization of PPGM

The reaction of maleic anhydrate with PG was performed by melt polycondensation and reaction route was shown in scheme 2. At the first step, the reaction temperature of the reaction system was kept at 60 ℃ in N_2 atmosphere for 1 hour and then in reducing pressure for another 4 hours. During this stage, the viscosity of the system increases gradually. At the second step, the temperature was raised to 140℃in reducing pressure to remove the water produced in the reaction and yield high molecular weight product.



Scheme 2. Polycondensation of PG and maleic anhydrade

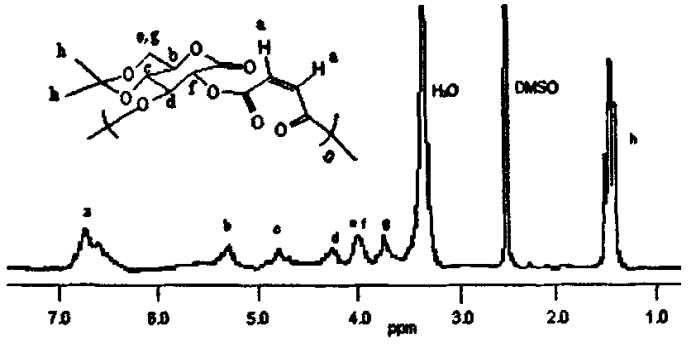
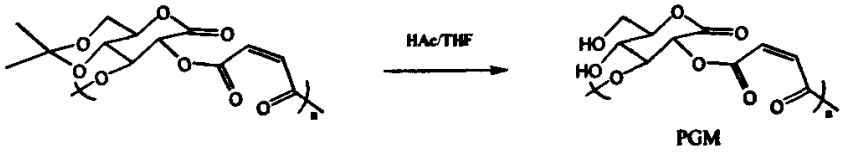


Figure 3. ^1H NMR spectrum of PPGM (d_6-DMSO as solvent)

Figure 3 shows the ^{1}H NMR spectrum of PPGM. The peaks at 6.85 and 6.41 ppm are assigned to protons in double bond of the maleic acetate structure. Peaks at 5.22, 4.80, 4.23, 4.01 and 3.74 ppm are for methylene and methenyl protons of gluconic acid unit. Peaks at 1.34 ppm correspond to the methyl protons of the isopropylidinene group.

Synthesis and characterization of the PGM

Removal of the methoxy propylidene from the PG was shown in **Scheme 3.** The reaction takes place in THF in the presence of the acetic acid at room temperature. The PGM could be dissolved in water but hardly dissolved in organic solvents. This is perhaps due to the high molecule polarity from hydroxyl groups in the PGM.



Scheme 3. Synthesis of the PGM

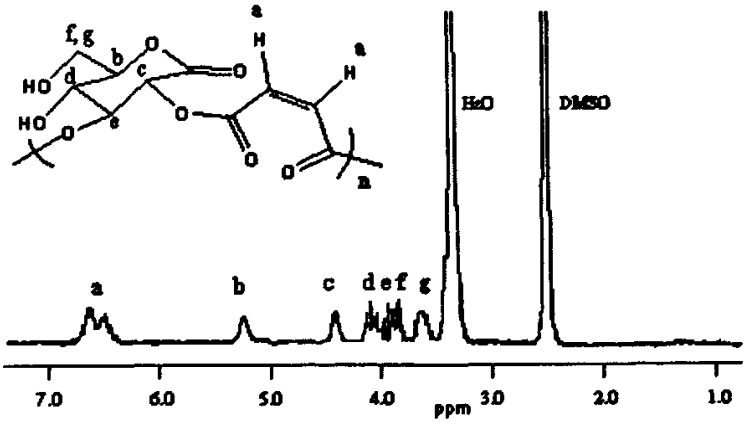


Figure 4. ^{1}H NMR spectrum of PGM (in d_6-DMSO)

The ^{1}H NMR spectrum of PGM was shown in **Figure 4.** The peaks at 6.82 and 6.49 ppm were assigned to the protons in double bond of the maleic acetate structure. The signals at 5.20, 4.25, 4.10, 3.90 and 3.63 ppm were assigned to methylene and methenyl protons of gluconic acid unit. Comparing with the ^{1}H NMR spectrum of PPGM (**Figure 3**), the peaks at 1.34 ppm of the methyl protons in isopropylidinene group disappeared.

Table 1. GPC and DSC results of PGM

[maleic anhydrade]/[PPGM] [a]	M_n [b] (g/mol)	M_w [b] (g/mol)	M_w/M_n [b]	Tg [c] ℃
1.0/1.2	1100	2200	2.0	-39
1.0/1.1	2600	4900	1.9	-28
1.0/1.0	4100	7800	1.9	-21

[a] Molar ratio; [b]Determined by GPC; [c] Determined by DSC, 10℃/min

Table 1 gave the GPC and DSC results of the PGM. The M_n of PGM was restricted from 1100 to 2200 g/mol by different feed ratio of maleic anhydrade to PPGM. The values of M_w/M_n of different polymers are close each other and about 1.9-2.00. The glass transition temperature (Tg) increased from -39℃ to -21℃ with the M_n of the polymer from 900 to 4100g/mol.

Table 2 gave the solubility of the PGM in different solvents. It can be found that the solubility of the PGM was related to the polarity of the solvent.

Table 2. Solubility of the PGM

Solvent	Water	Pyridine	DMF	DMSO	Methanol	Acetone	THF
Solubility	S	P	P	S	N	N	N

S soluble; N nonsoluble; P partially soluble

DMF = N,N'-dmethylformamide DMSO=dmethyl siphoxide; THF= tetrahydrofuran

Conclusion

PGM, a novel biodegradable polyester derivative containing glucose ester units, was prepared by a three-step reaction from D-gluconic acid-d-lactone and maleic anhydride. The polymer were characterized by GPC, ^{1}H NMR, FTIR and DSC. The M_n of the PGM are in the

range of 1100 to 4100 g/mol with the M_w/M_n of 1.9-2.00. The glass transition temperature of the PGM increases from -39℃ to -21℃ with the M_n from 1100 to 4100 g/mol. The presence of free hydroxyl and double bond groups in the PGM would provide the possibility for chemically modification of the polymer chain and coupling biologically active molecules, that expands the group of known biomedical polyesters candidates.

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个人简历

2005 年 6 月博士毕业于武汉大学，博士论文期间在武汉大学生物医用高分子教育部重点实验室进行了天然氨基酸引发下脂肪族环酯单体的开环聚合反应研究。2006 年 9 月进入中山大学化学博士后流动站从事有关含糖链两亲性脂肪族聚酯的研究工作。在站期间，参加了国家自然科学基金“直链淀粉的两亲化嵌段改性及相关应用基础研究”（20676155），中科院纤维素重点实验室访问学者基金（批准号 LCLC-2007-02）“脂肪族聚酯链段两亲性纤维素衍生物的研究”等课题的研究工作。相关研究结果已发表 SCI 论文两篇，待发表论文三篇。期间两次参加国内学术会议并作口头报告。

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