

Novel Blood-Compatible Waterborne Polyurethane using Chitosan as an Extender

Dan Xu, Zhen Meng, Ming Han, Kai Xi, Xudong Jia, Xuehai Yu, Qingmin Chen

Department of Polymer Science and Engineering, Nanjing University, Nanjing 210093, People's Republic of China

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ABSTRACT: A series of novel block polymers of polyurethane (PU) and chitosan have been prepared in two steps. The first step is the preparation of PU prepolymer, obtained from polytetramethylene oxide glycol (PTMO, $M_n = 1000$), isophorone diisocyanate (IPDI), and 2,2'-dimethylol propionic acid (DMPA), followed by ionizing PU prepolymer with triethylamine (TEA). The second step involves PU chain-extended by water-soluble chitosan of low molecular weight ($M_n = 5000$) by self-emulsion polymerization method. The sizes of the latex particles, morphology, and copolymer architecture have been characterized by

dynamic light scattering (DLS), general tensile test, infrared spectroscopy (IR), surface contact angle measurement, and transmission electron microscopy (TEM). Furthermore, it shows that the addition of chitosan remarkably increases anticoagulative property of PU elastomers confirmed by the recalcification time. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 109: 240–246, 2008

Key words: waterborne polyurethane; chitosan; self-emulsion; anticoagulative; blood-compatible

INTRODUCTION

Chitin,^{1–4} one of the most abundant naturally occurring polysaccharide, is a linear polymer consisting of *N*-acetyl-D-glucosamine units joined by $\beta(1,4)$ -glycosidic linkages. Its deacetylated derivative, chitosan, has active amino groups, exhibits much higher reactivity and water solubility, and has become the most applied chitin derivative.⁵ Chitosan possesses special biological properties, such as nontoxicity, heat stability, biocompatibility, resistance to corrosion, and unique properties of blood contact, which have been drawing wide attention.^{6–9} For more than 30 years, fundamental studies on chitosan have revealed its characteristics, such as complexation with metal ions, antibacterial and antifungal activities, tissue and cell compatibilities, and biodegradability by the enzymes chitinase, chitosanase, and lysozyme, etc.^{10–15}

On the other hand, polyurethane (PU) block copolymers have excellent abrasion resistance and the properties of both rubber and plastics. PUs are becoming more and more important as engineering materials.^{16–19} Their unique mechanical properties can be attributed to their specific microphase-separated morphology, which consists of hard-segment-rich and soft-segment-rich domains.²⁰ Interestingly, because of their unusual mechanical properties and blood compatibility, PUs have shown great potential

to be an important class of blood-contacting biomaterials.^{21,22} However, surface-induced thrombosis, protein fouling, and cytocompatibility are three serious consequences when using PUs as blood-contacting materials in hemodialysis.^{23,24} The most popular method for improving the anticoagulative is the modification of the materials themselves into antithrombogenic materials.²⁵

The synthesis of novel blood-compatible materials has been drawing wide attention. Currently, much effort has been applied to use natural material in the design of new biomaterial, and PU-modified chitosan has become a new frontier.^{21,26–28} Some advantages would be expected, such as increasing anticoagulant, excellent mechanical property, good thermal stability, low hydrophilicity, and so on. In their work, some use the swollen chitosan,²⁶ which is dispersed into DMF/glacial acetic acid mixtures, as an extender in the copolymerization because chitosan cannot be dissolved in organic solvent; others immobilize chitosan onto the surface of PU via various kinds of methods.^{21,27,28} The forms of the modified PU materials obtained by these methods are either gel or solid, so these PU materials are limited by processing difficulties.

In our current research, novel blood-compatible waterborne PU is synthesized by using water-soluble chitosan as an extender via self-emulsion polymerization method. Ordinarily chitosan can only be dissolved in acidic pH, and must be neutralized after reaction. Hence, water soluble chitosan bypassed the limit intrinsic to ordinary chitosan and provide a broader pH range for conducting a reaction in water.

Correspondence to: X. D. Jia (jiaxd@netra.nju.edu.cn.).

It is interesting note that PU-chitosan block copolymer emulsion, generated from water soluble chitosan, exhibited satisfactory freeze/thaw stability, and can be used widely due to its good processability. Particularly, this new structure still contained many active amido groups, which make it excellent candidate for further modification and wide application in chemistry and biochemistry.

In this article, a series of novel block polymers of PU and chitosan are prepared in two steps. The first step is the preparation of PU prepolymer, which is synthesized from PTMO ($M_n = 1000$), IPDI, and DMPA, followed by ionizing PU prepolymer by triethylamine (TEA). The second step is extending PU chain by water soluble chitosan ($M_n = 5000$) via a selfemulsion polymerization method. Films cast from the obtained block copolymer emulsions exhibited excellent mechanical properties and good anticoagulating character. The water swellability and hydrolytic properties of PU films were also investigated.

At present, anticoagulant property of blood contacting materials has been intensively investigated,^{21,22,25–28} but the problem of antibacterial and antifungal properties of these materials is not fully solved. This novel medical applicable material possibly has antibacterial and antifungal activities because of chitosan.²⁹

EXPERIMENT DETAILS

Materials

Chitosan (CS) from a shrimp shell was purchased from Yuhuan Ocean Biochemical (Taizhou, China). The degree of deacetylation was 90% (determined by liquid state ^1H NMR in acidic D_2O conditions³⁰) and its average molecular weight was $M_n = 5000$ (determined by GPC³¹).

Polytetramethylene oxide glycol (PTMO, $M_n = 1000$) was dried under vacuum, and isophrone diisocyanate (IPDI) and 2,2'-dimethylol propionic acid (DMPA) were used without further purification. These were all purchased from Adrich.

Acetone and triethylamine (TEA) were purchased from Shanghai Chemical (China) and purified by distillation.

Synthesis

The basic formulations are given in Table I. The copolymerization reaction was carried out under dry nitrogen atmosphere according to the general reaction scheme as shown in Figure 1. A typical procedure was described as below: PTMO ($M_n = 1000$, 10.0 g) and IPDI (6.66 g) were charged into a four-necked flask equipped with a mechanical stirrer, a thermometer, and a condenser. The solution was stirred at 80–85°C for 3 h with a stirring rate of 350–400 rpm. After

TABLE I
Content of Chitosan in the PU

Sample	Content of chitosan (g)	Ratio of $n_{\text{CS}}:n_{\text{PU}}$
A	0	–
B	1.62	1 : 2
C	3.24	1 : 1
D	6.48	1 : 0.5

n_{CS} is the molar number of amino group in chitosan, n_{PU} is the molar number of isocyanate group in IPDI.

DMPA (1.34 g) was added to the flask, the reaction was kept for another 2 h under the same condition. The PU prepolymer was then cooled to room temperature, dissolved in acetone (25 mL), and ionized with TEA (1.01 g). In the next step, CS (Table I) was dissolved in distilled water (100 mL), and the pH value of the solution was adjusted to 10. Following this, the CS solution was slowly dropped into PU prepolymer acetone solution and stirred at room temperature overnight.³² This was the selfemulsion polymerization process. A stable microemulsion with solids content of about 25% was finally cast into Teflon disk, and the disk was kept at room temperature for 3 days and then under vacuum for 1 week at 60°C to obtain the films of the polymers.

Characterization

Freeze-thaw stability of the emulsions

The freeze-thaw stability of the emulsions was measured as below: the emulsion (2 mL) was sealed in a weighing bottle and placed in an air oven at 90°C for 5 h. Then, it was immediately transferred to a refrigerator at 0°C for 5 h, which concluded a typical testing cycle. At least 10 testing cycles were performed for each sample to observe whether any system heterogeneity (e.g., phase separation or precipitation) could be detected in response to temperature fluctuations.

Hydrolytic stability of the samples

The samples were weighed (W_1) and kept in deionized water at 37°C for 48 days. Then the solution was completely dried and weighed (W_2). The hydrolytic weight loss was computed as follows:

Hydrolytic weight loss(HWL)

$$= (W_1 - W_2)/W_1 \times 100\%$$

The water-swelling ratio of the films was studied by the following procedures: a preweighed dry slab was immersed in deionized water at 25.0°C. After equilibrating for 24 h, the sample was blotted with

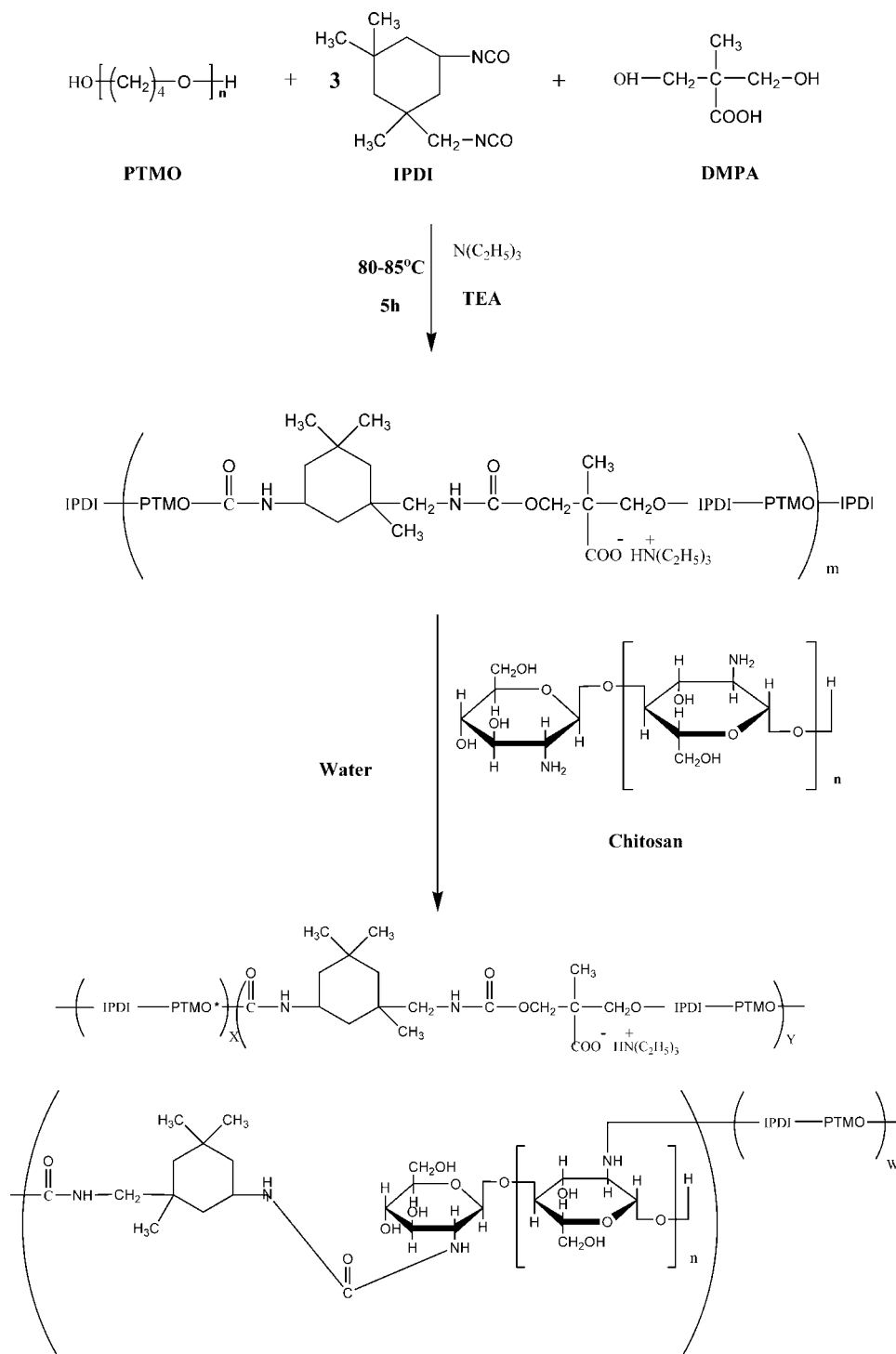


Figure 1 Synthesis of PU urea emulsion with chitosan as the chain extender.

laboratory tissue and weighed. The water swelling ratio (SR) was expressed as the weight percentage of water in the swollen sample

$$SR = (W_S - W_D) / W_D \times 100\%$$

where W_S is the weight of the swollen sample, and W_D is the weight of the dry sample.

Dynamic lighting scattering

The diameter D_p of the latex particles was measured by dynamic lighting scattering (DLS) using 90 plus particle size analyzer (Brookhaven). The index of the analyzer was as follows: temperature = 25.0 °C; suspension = aqueous; viscosity = 0.890 cp; Ref. index fluid = 1.330; angle = 90.00; wave-

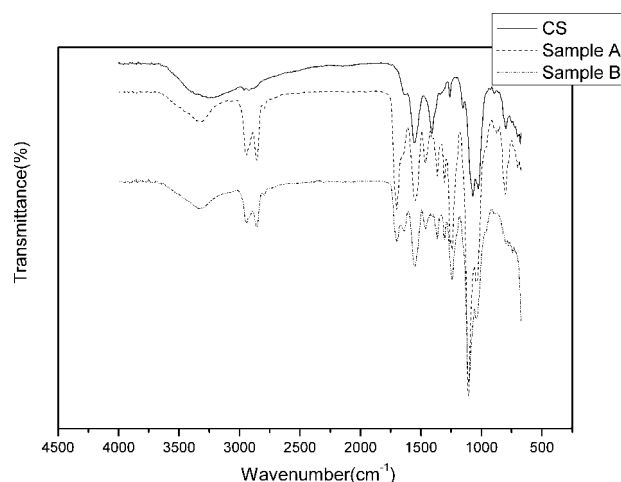


Figure 2 IR spectra of CS, Samples A and C.

length = 660.0 nm. The latex sample was diluted by deionized water.

Transmission electron microscopy analysis

JEM-1005, (JEOL, Japan). In a typical experiment, the latex sample was diluted, and one drop of the colloidal dispersion was put to carbon film supported by a copper grid.

General tensile test

The mechanical properties were determined on a table model Instron Series IX automated materials testing system with interface type of 4200. An ASTM 1708 standard die was used for the samples and they were dried under a vacuum for a minimum of 48 h before testing. The index was as follows: sample rate: 2.0 pts/s; crosshead speed: 20.0 mm/min; full scale load range: 0.50 kN; humidity: 60%; temperature: 15°C.

Infrared spectroscopy

Infrared spectra were recorded with a Nicolet 5DX Fourier transform infrared spectrometer using membrane as samples.

Surface contact angle measurement

The sample was measured by CAM 200 (KSV Instrument, Finland) at room temperature. The data was collected 1 min after the 6.5 μL drop of double-distilled water had been placed on the surface of the film. At least 10 measurements were used and the average contact angle was then calculated.

Determination of the blood coagulation time

Pieces of clean ground slides were coated with emulsions of the samples. After being dried, 0.2 mL of fresh rabbit blood was added onto each piece of the slides, followed by adding a 20 μL solution of CaCl_2 (0.2 mol/L) onto each slide. A series of ground slides was put into distilled water (50 mL). After the hemoglobin was diffused into the water, the solution was tested by a spectrophotometer at 540 nm. The results were compared with those of the same ground slide without coatings.

Determination of the recalcification time

A series of dried tubes was coated with emulsions and dried. Fresh rabbit blood (0.1 mL) containing sodium citrate and a solution of CaCl_2 (0.025 mol/L, 20.1 mL) were added into each tube. The time of the emergence of a milky white flocculate was recorded. The results were compared with those of blank tubes and the tubes coated with silicon oil.

RESULTS AND DISCUSSION

Infrared spectroscopy

It is expected that the copolymerization of PU with CS would occur by the formation of urea ($-\text{NHCONH}-$) groups due to the reaction between the $-\text{NH}_2$ groups from CS with the $-\text{NCO}$ groups from PU prepolymers, considering the higher reactivity of $-\text{NH}_2$ in relation to the $-\text{OH}$ groups of CS.²¹ As shown in Figure 2, in comparison with PU, PU chain-extended by CS has an additional adsorption band appearing around 1680 cm^{-1} ($-\text{NH}$ angular deformation), which is expected to be associated with the intramolecular hydrogen bonding between $-\text{C}=\text{O}$ and $-\text{NH}_2$ groups in CS. On the other hand, the bands of 1750 and 1450 cm^{-1} appear in both of the spectra of PU and PU chain-extended by CS. The band around 1750 cm^{-1} is attributed to $-\text{C}=\text{O}$ axial deformation of the primary ester group in the molecule, and the band around 1450 cm^{-1} corresponds to the characteristic vibration of ureide. Hence, absorbance around 1680 cm^{-1} band can be used to follow the formation of CS in PU, and the results from IR spectra show that PU and CS had polymerized.

Water swellability and CS loss

PU can undergo several types of degradation: mechanical degradation, biofouling, hydrolysis, oxidation, and so on. In polyether-based PU, the ether linkages are easy to oxidize and calcify, while the ester linkages in polyester-based PU were facilitated by an attack of water.²⁰

TABLE II
Water-Swelling Ratios and Chitosan Loss of PU

Sample	RS (%; 24 h)	HWL (%; 48 days)
A	54.6	0.04
B	44.7	4.52
C	21.3	8.61
D	10.9	9.90

In our current research, for PTMO-based water-borne PU, the water-SR after 24 h changed from 54.6 to 10.9% (Table II) when the CS content increased from 0 to 25.4% (wt %). Hence, the water-SR can be adjusted in a very broad range by changing the CS content. This ratio changes could be attributed to the degree of crosslinking by CS. The amino groups in CS serve as both chain extenders and crosslink points. Consequently, higher crosslinking can make the films more difficult to swell in water. It can also be noted from Table II that all the samples display a modest weight loss after 48 days, which means PU and CS had polymerized well. The hydrolytic weight loss ratio after 48 days is changed from 0.04 to 9.90% when the CS content is increased from 0 to 25.4%. It can be explained that with the addition of CS, the PU forms more crosslinking points, which makes CS more difficult to react with PU prepolymers due to the steric effect. Although the hydrolytic weight loss happens, most of CS react with PU prepolymers.

Emulsion properties and morphology

Table III shows the diameters of the particles which are synthesized by PU and CS with different equivalents. The sizes of the latex particles increase along with the CS content. With the addition of CS, the PU latex particles get more CS enwrapped by copolymerization and the PU forms more crosslinking points, so the diameter of the latex particle

TABLE III
DLS of PU Chain-Extended by Chitosan

Sample	Effective diameter (nm)	Polydispersity
A	68.4	0.216
B	104.7	0.198
C	187.7	0.354
D	272.3	0.465

increased correspondingly. And the polydispersities of the latex particles increase in the order of $A \approx B < C < D$, similar to the particle size increase, $A < B < C < D$.

The results shown in Figure 3 are consistent with the diameters of the particles from DSL. Though the size of the latex particles increased, the emulsions are stable because these polymers have hydrophilic groups such as CS and carboxylic anions.

All the emulsions exhibited satisfactory stability in the whole range of the testing temperature and the transparency of the emulsions increased in the order of $D < C < B < A$. Generally, the transparency reflects the particle size of the emulsion and the concentration of solubilizing groups. The solubilizing groups in this system were carboxylic anions and CS, and the content of carboxylate anions was equally designed. The ionic groups increase in the order of $A < B < C < D$, and the sizes of the latex particles are in the same order.

Mechanical properties

The results of general tensile test (Table IV) demonstrated that samples showed excellent mechanical property. With the increase of the CS content, Young's modulus increases greatly, however, the elongation of strain decreased sharply. It may be attributed to the addition of CS, latex particles formed many crosslinking points. But Young's mod-

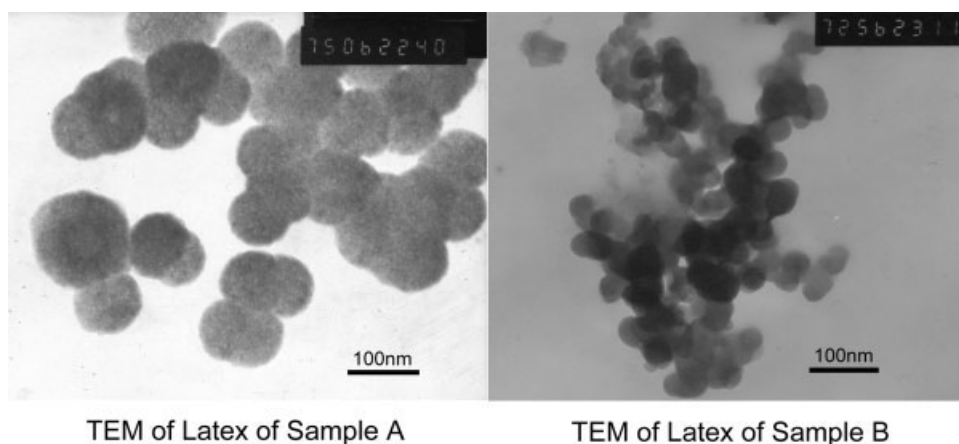


Figure 3 TEM of latex containing PU.

TABLE IV
General of Tensile Test of PU Chain-Extended by Chitosan

Sample	% Strain at Peak (%)	Young's modulus (MPa)	Stress at break (Mpa)
A	825.333	41.769	30.865
B	847.333	18.573	28.371
C	165.200	111.881	17.993
D	108.567	149.558	15.373

ulus decreased unexpectedly for sample B. It is speculated that the construct of PU chain-extended by water was in order, and when the content of CS was 1.62 g, crosslinking points were few, but the well-defined construct was destroyed. This may explain why Young's modulus of sample B is smaller than that of sample A.

Surface contact angle measurement of the films

The results from Table V show that the samples become more hydrophilic after the addition of CS. As a result, the contact angles decrease from 80.1 to 49.1 when the CS content is increased from 0 to 25.4% (wt %). It is ascribed to the introduction of hydrophilic amino groups and hydroxyl groups in CS.

Blood compatibility

Blood compatibility was evaluated by the free hemoglobin concentration in water and the recalcification time. The relationship between the test times and the $A_{540\text{nm}}$ of the hemoglobin solution is shown in Figure 4. The higher absorption intensity represents the greater free hemoglobin concentration and indicates better blood compatibility. According to Figure 4, the four samples obviously have an anticoagulant character. The blood compatibility increases in the order of $A < B < D < C$. The recalcification times of the blank tube and the tubes coated with silicon oil and with the samples were also measured. The recalcification time of the blank tube is 32.7 s and the recalcification time of tube coated with silicon oil is 51.3 s. The recalcification time ratios of the tube coated with the samples to the blank tube and to the

TABLE V
The Static Contact Angle of PU Chain-Extended by Chitosan

Sample	The static contact angle (°)
A	80.1
B	68.4
C	59.2
D	49.1

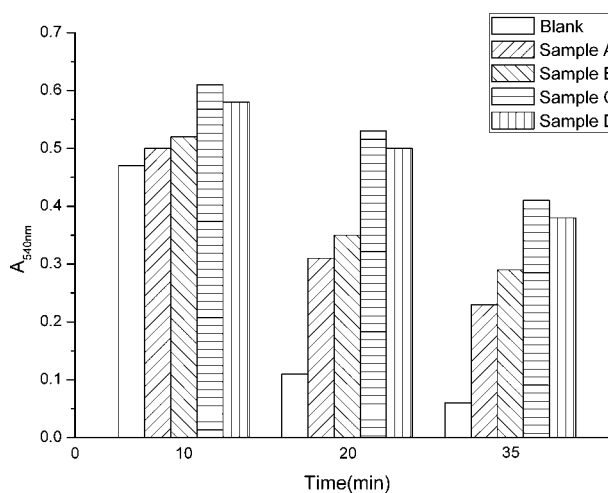


Figure 4 Relationships between test times and $A_{540\text{nm}}$ of hemoglobin solution.

tube coated with silicon oil are shown in Table VI. It is shown that the blood compatibility also increased in the order of $A < B < D < C$, which is consistent with the result of the hemoglobin method. It can also be seen that the blood compatibility of sample C and sample D are better than those of silicon oil. The better blood compatibility of PU may be associated with the addition of CS.

CONCLUSION

A series of novel blood-compatible waterborne PU materials were synthesized by copolymerization, in which water soluble CS was employed as an extender. The structure and properties had been extensively characterized. These novel modified PU materials exhibited excellent mechanical property, good thermal stability, and good anticoagulant. These polymers showed great potential in blood contacting devices. Furthermore, by varying the content of CS, a series of hydrophobic films with tensile elongation for 108–847% and Young's Modulus from 18 to 149 MPa could be obtained. Though these films had

TABLE VI
The Result of Anticoagulant Test of PU Chain-Extended by Chitosan

Sample	The recalcification time (s)	$t_{\text{sample}}/t_{\text{black}}$	$t_{\text{sample}}/t_{\text{silicon oil}}$
Black assay	32.7 ± 2.49	–	0.64
Silicon oil	51.3 ± 7.01	1.57	–
A	45.1 ± 4.86	1.38	0.88
B	46.4 ± 5.57	1.42	0.90
C	$78.1 \pm 13.9^*$	2.39	1.52
D	$73.6 \pm 8.11^*$	2.25	1.43

*: anticoagulant properties of sample C and sample D are good.

hydrophilic groups, they were not soluble in water due to crosslinking. Based on mechanical properties and blood compatibility of the materials, samples C and D are better candidates for bioapplications.

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