Polycarbonateurethane Films Containing Complex of Copper(II) Catalyzed Generation of Nitric Oxide

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ABSTRACT: Nitric oxide (NO) is a well known potent antiplatelet agent, and its continuous release will effectively prevent the adhesion of platelets on artificial blood vessel walls. In this paper, polycarbonateurethane (PCU) with lipophilic Cu(II)-complex (Cu(II)-DTTCT) blending films were prepared and used as catalyst to generate NO from nitrite. The mechanical properties of PCU films blended with Cu(II)-DTTCT were characterized by tensile strength measurement. The tensile stress and Young's modulus of PCU films blending with Cu(II)-DTTCT increased, however, the elongation at break decreased compared with corresponding PCU films. The NO generation was investigated *in vitro* in the presence of $NaNO_2$ and ascorbic acid in PBS (pH = 7.4) at 37°C. The flux of NO generation was quantitatively measured by Griess assay. NO flux and velocity increased with the increase of $NaNO_2$ concentration, the concentration of ascorbic acid in PBS and the amount of Cu(II) in the films. The loss of Cu(II) from blending film surfaces was found during the *in vitro* NO generation experiments, which resulted in the decrease of NO flux in the second run. The PCU film could catalyze continually generation of NO for two days, which will provide a promising approach that enable endogenous NO generation on the surface of the medical devices. The generation of biologically active level of NO at the blood/polymer interface can reduce the risk of thrombosis on the implants. Polycarbonateurethane films with NO generation function may be used as high thromboresistant blood contacting materials or coating. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1712–1721, 2011

Key words: polycarbonateurethanes; lipophilic Cu(II)-complex; hemocompatibility; NO generation

INTRODUCTION

Polycarbonateurethane (PCU), a new type of polyurethane which provides biostability *in vivo*, relatively beneficial hemocompatibility and excellent mechanical properties, has been developed in recent years.[1,2] PCU has been used in various biomedical applications such as catheters, vascular grafts, blood bags, and artificial hearts.[3–6] However, platelet adhesion/activation can still occur when contacting with blood for extended periods, because PCU is not completely thromboresistant.[7–9] Many strategies have been developed to enhance blood compatibility during the last 20 years, the most significant three main strategies are bioinert or biopassive, biomimetic and bioactive approaches.[10–12]

Nitric oxide (NO) is a well-known inhibitor of platelet adhesion and activation. NO is continually released by the endothelium at a flux of $\sim 1 \times 10^{-10}$ mol cm^{-2} min^{-1} to inhibit the adhesion of platelet, as well as a potent inhibitor of smooth muscle cell proliferation.[13–15] Hence, polymers which can release or generate NO locally at their surface exhibit greatly enhanced thromboresistivity and have the potential to reduce neointimal hyperplasia caused by device damage to blood vessel walls.[16–19]

Therefore, the release or generation of NO from the surface or from nitrite is a promising approach to improve hemocompatibility of biomaterials.[20–22] Research activities focus on the circulation and reaction of NO *in vivo* is helpful to the comprehension of the complexity of reaction pathway to control NO release or generation *in vivo*.[23] Moreover, it is meaningful for the development of biomimetic materials which can release NO for a prolong time.[24–26] It has recently been suggested that NO-releasing PU materials may be one of the ideal materials for vascular grafts.[27] The NO-releasing materials were previously focused on how to create a limited NO storage, such as S-nitrosothiols and diazeniumdiolates, which limited the sustainable release of NO.[28–34] A new type of materials utilized nitroso mercaptan and nitrite in the blood circulation *in vivo* to give sustainable provider of NO. Several experiments had validated the feasibility of this approach, and the results showed good biocompatibility and can be long-term used in biomedical applications.[35–37] Puiu et al. synthesized

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Cu(II)-cyclen-polyurethane-based materials by attaching cyclen/Cu(II) moieties onto structurally modified polyurethane backbones, which catalyzed NO release in the presence of RSNOs.[22] Such NO generating polyurethane materials may potentially be used in a wide variety of long-term biomedical applications. Recently, Seabra et al. reported that NO released from biomaterials exerted a potent dose- and time-dependent antimicrobial activity against Staphylococcus aureus and a multidrug-resistant Pseudomonas aeruginosa strains.[19] These antibacterial and hemocompatible effects of NO released materials open a new perspective for the blood-contacting biomaterials, for avoiding their colonization with highly resistant bacteria.

It is well known that Cu(II) ion can oxidizes ascorbic acid to generate dehydroascorbate with concomitant generation of an unstable Cu(I) species, which can potentially serve to reduce nitrite to NO (based on redox potentials of these species).[38] Oh et al. demonstrated that copper/ascorbate/nitrite chemistry can indeed take place in solution at physiological pH, and further showed that copper(II) with appropriate ligands can serve as catalytic sites for this reaction, both in aqueous solution and within a hydrophobic polymeric film.[39] In addition, if the polymer phase is also doped with a lipophilic nitrite salt along with the copper(II) complex, significant levels of NO can be generated spontaneously at the interface when the films are in contact with fresh plasma or whole blood.

In this article, three PCUs were synthesized from polycarbonates with molecular weight of 1000, 1500, and 2000 g/mol by melting one-shot method. The lipophilic Cu(II)-complex, Cu(II)-dibenzo [e,k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene (Cu(II)-DTTCT) as a NO generating catalyst was introduced to PCU films by solution blending method to improve the hemocompatibility of PCUs. Furthermore, NO generation was investigated under various conditions. Leaching of Cu(II) from PCU films was also studied. The functionalized PCU by blend Cu(II)-DTTCT can continually catalyze NO generation which is potential for improving the hemocompatibility of blood-contacting surfaces. These biomaterials with NO releasing function may be used as thromboresistant blood contacting materials for artificial blood grafts, stents, and heart valves.

EXPERIMENTAL SECTION

Materials and instrumentation

Materials

4,4′-Methylene diphenyl diisocyanate (MDI, Mitsui Chemicals Corporation, Japan) was filtrated by G3 tundish after melting, and then deposited in desiccators. Polycarbonate diols (PCN, Beijing Santomer Chemical, China) were dried at 80°C for 24 h. 1,4-Butanediol (BD) was distilled at reduced pressure before use. Other chemical reagents were used without further purification.

Instrumentation

The melting point of dried DTTCT was determined by RY-2 melting point thermometer (Tianjin Analytical Instrument Factory, China). ^{1}H-NMR was determined by Infinity Plus 300WB liquid NMR apparatus (Varian). Chemical elements and their status were determined by PHI-1600 X-ray photoelectron spectroscopy (XPS, Perkin Elmer). Surface chemistry of compounds was characterized by Bio-Rad FTS-6000 in transmission mode by Fourier transform infrared (FTIR) spectrometer. Gel permeation chromatography (GPC) was performed by Agilent1100 with a mixed D column (600 mm × 7.5 mm, Polymer Laboratories), a T60A dual detector (Viscotek GmbH) and a RI detector 8721 (ERC). THF was used as an eluent at a flow rate of 1.0 mL min^{-1} at room temperature. Narrow molecular weight distributed polystyrene standards were used for universal calibration. Molecular weight and polydispersity of PCUs were calculated by TriSEC GPC-Viscometry Module Software (Version 3.0, Viscotek GmbH). Mechanical properties were determined at room temperature by Testometric M350–10KN tensile testing apparatus. The deformation rate was 10 mm min^{-1}. The bone-shaped samples with dimensions of 60 mm × 6.5 mm (parallel area), a thickness of 0.5–0.7 mm and a free length of the clamped samples of 40 mm were used. All the results were the average of five parallel experiments.

Synthesis

Synthesis of polycarbonateurethanes

Melting one-shot method was used to synthesize polycarbonateurethanes. Polycarbonate diols, MDI and 1, 4-butanediol with molar ratio of PCN:MDI:BD = 1 : 2 : 1 were introduced into the reactor with agitation, and then the reactor was heated to 120–150°C, poured all of the output onto the polytetrafluoroethylene (PTFE) board, and put into oven with the protection of nitrogen at 110°C for 8 h. The synthetic reaction and ^{1}H-NMR of PCU2K are shown in Figure 1.

Synthesis of Cu(II) complex Cu-DTTCT

Cu(II) complex was synthesized according to the method described in literature.[39] Briefly, 0.1 mol benzil and 0.1 mol o-phenylenediamine were refluxed for 4 h in ethanol with a few drops of conc. HCl. After cooling, the reaction solution to room temperature and allowing the mixture to stand

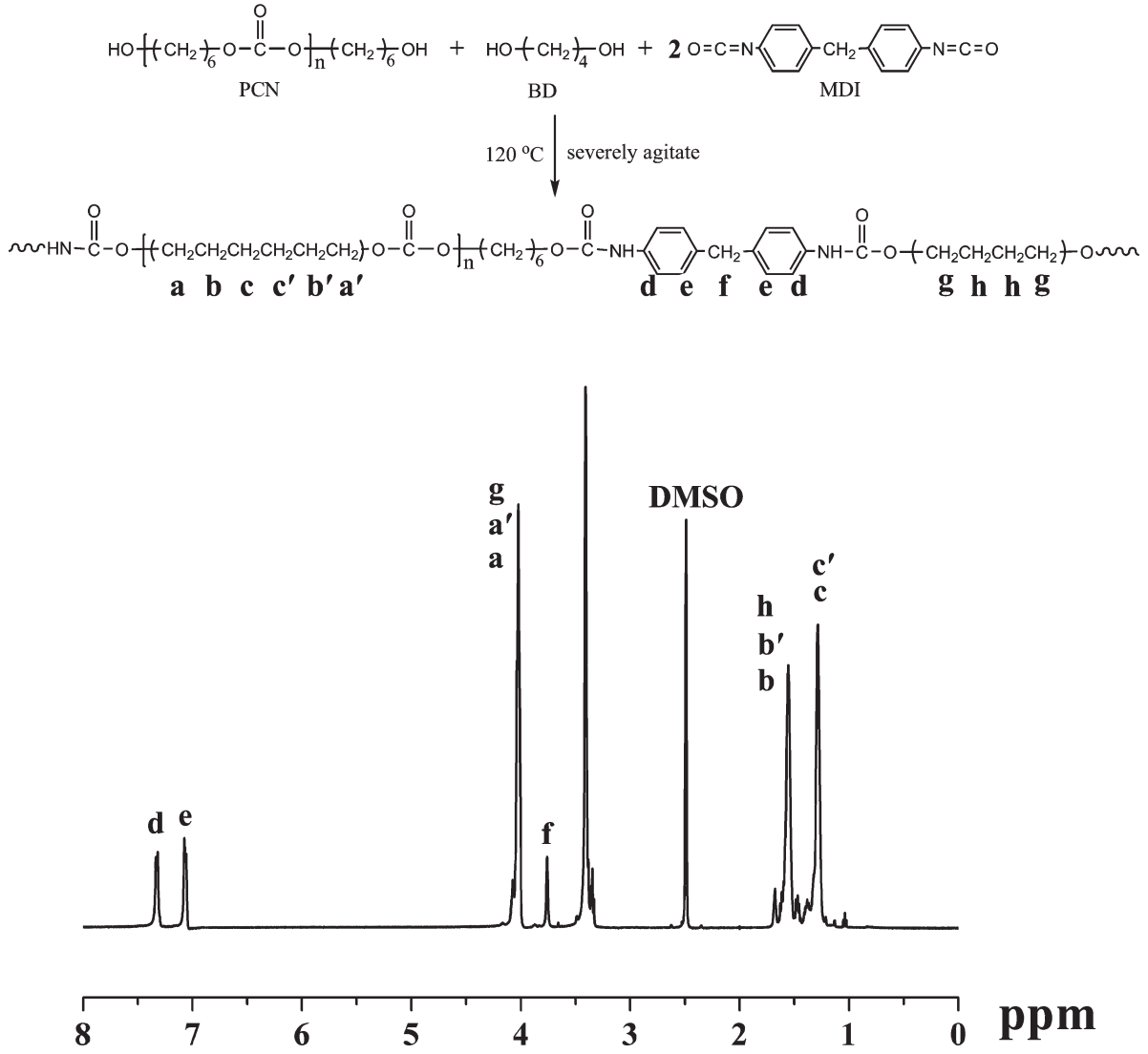

Figure 1 Scheme of synthesis of PCU by the melting one-shot method.

overnight, white crystalline product was separated by filtration, washed with cold ethanol to remove impurities, and then dried under vacuum. The synthesized DTTCT was dissolved in hot ethanolic solution and mixed in a 1 : 1 molar ratio with an ethanolic $CuCl_2$ solution; this mixture was refluxed for 2 h. After cooling to room temperature and standing overnight, the precipitated complex was filtered and washed with cold ethanol, and then dried under vacuum overnight. The synthetic scheme of Cu-DTTCT is shown in Figure 2.

Preparation of PCU films blending with Cu(II)-DTTCT

PCUs were dissolved in THF to prepare 10 wt % solutions, and then poured the solution onto the glass board and dried to obtain PCU films as references to blend films.

A calculated amount of PCUs and Cu(II)-DTTCT were dissolved in THF to prepare 10 wt % solution, and poured the solution onto the glass board (7.2 × 7.2 cm^2), which was aligned perfectly horizontal. The solution was evenly spread to ensure that film had uniform thickness. When the solvent was completely volatilized, the PCU film was peeled from the glass board and placed in the vacuum oven at 50°C for 24 h.

NO generation by catalysis of PCU film blending with Cu(II) complex

A calculated amount of ascorbic acid (Vc) and $NaNO_2$ of PBS solution (pH = 7.4) were mixed and introduced into a three-tube flask with a bubbling three-phase reactor having 100-mL PBS solution. Nitrogen was passed through the solution for 5 min to remove dissolved air. Two reactors were heated

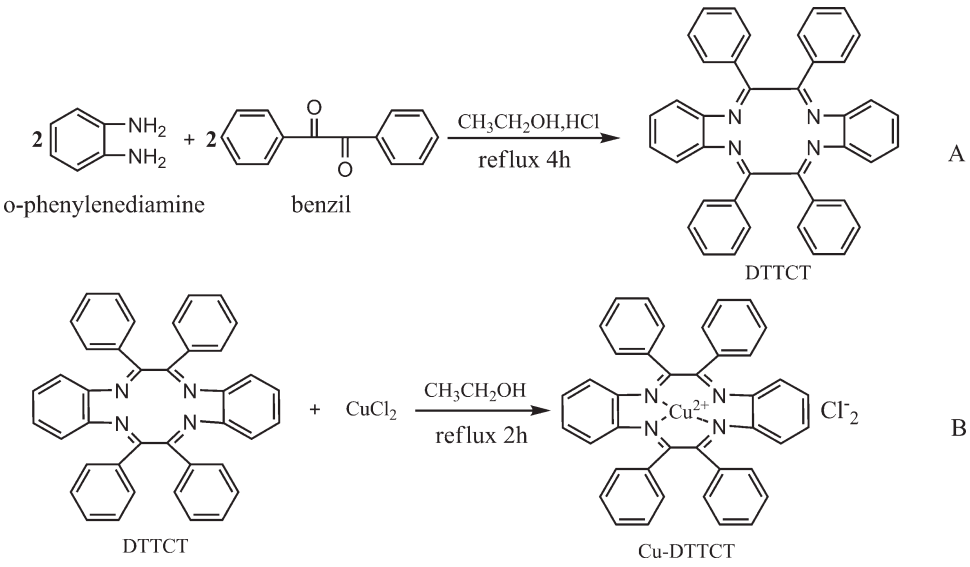

Figure 2 Scheme of synthesis of DTTCT (A) and Cu(II)-DTTCT (B).

to 37°C and maintained for a period. After PCU film blending with Cu(II) complex was added into the flask, 2-mL solution was taken out from the bubbling reactor from time to time, meanwhile the same volume of PBS solution was added into the flask to keep the volume of NO generation system invariably. The above 2-mL taken solution was mixed with 0.5-mL Griess reagent at room temperature above 20 min; its absorbency at 540 nm was tested by UV spectrophotometer of WFZ-26A. The mixture of 2-mL PBS solution and 0.5-mL Griess reagent was used as reference. The NO generation amount (Q) and NO average flux were calculated by the following formulas.

$$Q = C_n \times 100 + \sum_{i=1}^{n-1} C_i \times 2 \quad (1)$$

where C_i is the concentration of the solution after getting i times samples.

$$\text{NO flux} = \frac{Q}{M \times S \times t} \ (\text{mol cm}^{-2}\,\text{min}^{-1}) \quad (2)$$

where M is the $NaNO_2$ molar mass, S is the surface area of film, and t is the time.

Cytotoxicity test

Cell strain used in the experiment was 48–72 h exuberant growth of L-929 cells (fibroblast cells from mouse).

Cell inoculation: L-929 cells in the logarithmic phase were used to make 3×10^5 cell mL^{-1} of cell suspension. 10 mL of the above suspension was inoculated in Petri dishes to culture 24 h under condition of 37°C and 5% CO_2. And then the culture medium was discarded. The agar medium was poured into the original dishes under a suitable temperature.

Sample position: 0.1 mL extract from different test materials was dropped on a sterile filter paper, one for the negative control, positive control, and two test materials were placed on every Petri dish in which the cell had been inoculated, and then they were cultured under the condition of 37°C, 5% CO_2 and in dark for 24 h.

Staining: The filter papers in Petri dishes were all taken out. PBS was purchased to make neutral red dye, and its concentration was 1%. Ten milliliter of the dye was added to every dish. Then, they were observed under the microscope after 30 min.

RESULTS AND DISCUSSION

Synthesis and mechanical properties of PCUs

Three PCUs, nominated as PCU1K, PCU1.5K, and PCU2K, were synthesized with molar ratio of PCN:MDI:BD = 1 : 2 : 1 from PCNs with molecular weight of 1000, 1500, 2000 (Table I). The molecular weight of PCUs increased with the increase of molecular weight of PCN and decrease of the hard segment content. Because of high intermolecular forces of high molecular weight of PCU, the mechanical properties of films became better. Young's modulus, elongation at break, and tensile strength of PCUs increased significantly with the increase of molecular weight of PCN, because the PCN segments could form semicrystalline domains.

FTIR of PCU film

FTIR spectrum of PCU1K as an example is shown in Figure 3. As can be seen from the spectrum, the

TABLE I
Molecular Weight of PCUs Determined by GPC

Sample ID	Content of hard segment (wt %)	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w \times 10^{-3}$	$\overline{M}_z \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$
PCU1K	37.1	16.37	29.71	45.54	1.81
PCU1.5K	28.2	21.56	54.40	97.51	2.52
PCU2K	22.8	100.20	250.10	811.40	2.50

absorption at 3337.43 cm^{-1} corresponds to vibration of NH through the association of the hydrogen-bond, the absorptions at 2941.03 cm^{-1} and 2863.06 cm^{-1} correspond to CH_2 asymmetric stretching vibration and CH_2 symmetric stretching vibration, respectively. The band at 1704.16 cm^{-1} has to be attributed to the H-bonded carbonyl from urethane, whereas the band at 1745.57 cm^{-1} to the non-H bonded carbonyl from carbonate. The absorptions at 1596.65, 1263.08, 1078.52, 792.37 cm^{-1} correspond to vibration of C=C in phenyl, O—C=O asymmetric stretching vibration of PCN segments, C—O—C stretching vibration of carbonate groups, out-of-plane bending vibration of two neighboring hydrogen atoms in phenyl, respectively.

Characterization of DTTCT and Cu(II)-DTTCT

^{1}H-NMR of DTTCT

Figure 4 shows the ^{1}H-NMR spectrum of DTTCT in $CDCl_3$. The signals in the range 8.183–8.207 ppm (m, 1H, —C=CH—C, a) are characteristic of the protons at ortho-position of phenyl groups. The resonances in the range 7.774–7.799 ppm (m, 1H, —C=CH—C, e) are assigned to the protons at the ortho-position of phenyl groups near benzo groups. The chemical shift at 7.518–7.541 ppm (d, 1H, —C=CH—C, c) originates from the protons para-position of phenyl groups, at 7.321–7.396 ppm (m, 1H, —C=CH—C, b and d, or f) from the meta proton of phenyl groups, at 7.265 ppm (s, 1H, —C=CH—C, g) from the protons of benzo groups, respectively. The resonances of f-protons of benzo groups might be overlapped with meta-proton of phenyl groups.

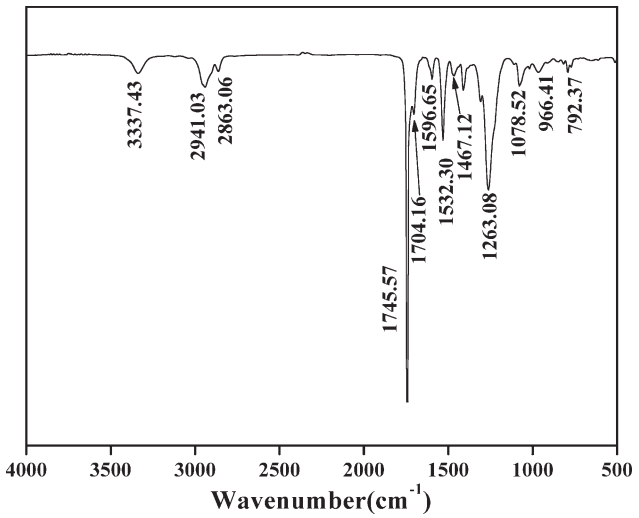

Figure 3 FT-IR spectrum of polycarbonateurethane PCU1K film.

XPS of Cu(II)-DTTCT

As can be seen from the XPS C1s spectrum curve of Cu(II)-DTTCT (Fig. 5), two peaks at 284.88 and 289.24 eV correspond to the carbons in benzo groups (C_6H_4—N=C), phenyl (C_6H_5—C=N) and —C=N—, respectively. The peak at 399.50 eV in the N1s spectrum corresponds to —C=N—. The peak at 934.48 eV in the Cu2p spectrum corresponds to Cu(II). There are two characteristic peaks at 197.89 and 199.48 eV corresponding to Cl2p, whereas the standard spectrum of $CuCl_2$ shows Cl2p at 200 eV.

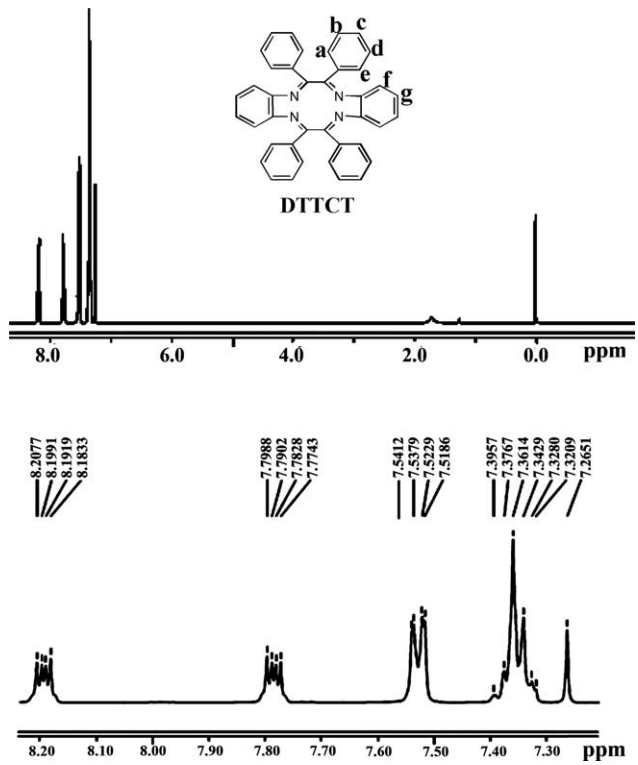

Figure 4 ^{1}H-NMR spectrum of DTTCT in $CDCl_3$.

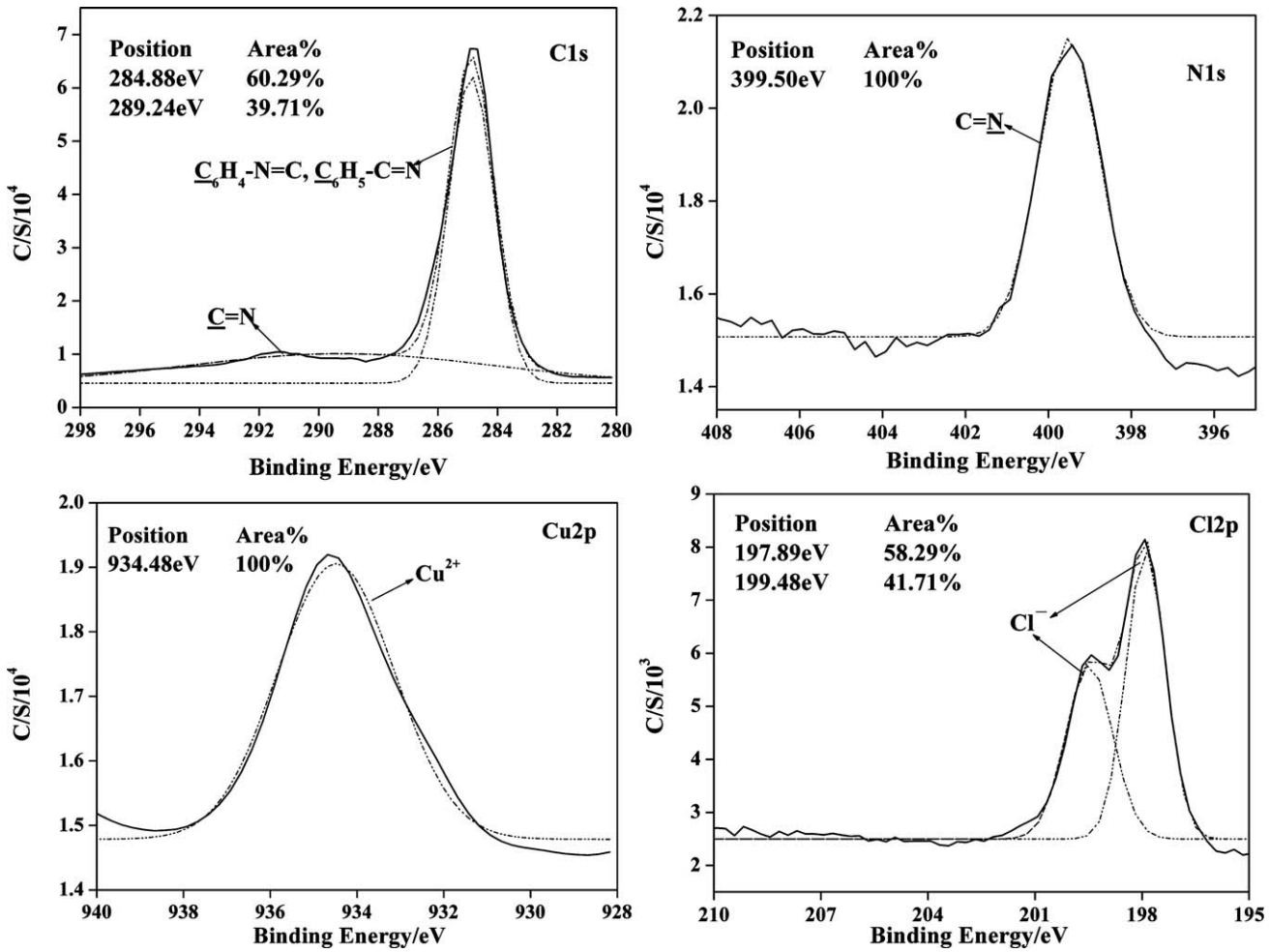

Figure 5 C1s, N1s, Cu2p and Cl2p core-level spectra of Cu(II)-DTTCT.

Mechanical properties of PCU films and blend films with Cu(II) complex

The mechanical properties of PCU films and blend films with Cu(II) complex were determined by tensile test, and the results were listed in Table II. The tensile strength and Young's modulus of blend films increased, whereas elongation at break decreased compared with corresponding PCU films. Cu(II) complex having a regular structure tended easily to form crystalline phase in the films. The crystalline microstructure in blend films resulted in high tensile strength and low elongation at break of PCU. Moreover, phenyl, benzo groups, 1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene and Cu(II) in Cu(II)-DTTCT might interact with polyurethane chains via van der Waals force. This interaction effects also increased the tensile strength of PCU films. PCU2K films showed higher elongation at break and tensile strength than the PCU1K films because PCU2K has high molecular weight.

TABLE II
Mechanical Properties of PCU Films and PCU Blend Films with Cu(II)-DTTCT[a]

Sample ID		Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
PCU1K	Original PCU films	3.06	140	2.71
	PCU films blending with Cu(II) complex (Cu (II) 0.096 wt %)	6.45	77	2.96
PCU1.5K	Original PCU films	22.22	670	5.41
	PCU films blending with Cu(II) complex (Cu (II) 0.096 wt %)	30.95	410	6.35
PCU2K	Original PCU films	35.30	680	9.07
	PCU films blending with Cu(II) complex (Cu (II) 0.096 wt %)	43.21	500	13.76

[a] Original PCU films and PCU films blending with Cu(II) complex (Cu (II) 0.096 wt %)

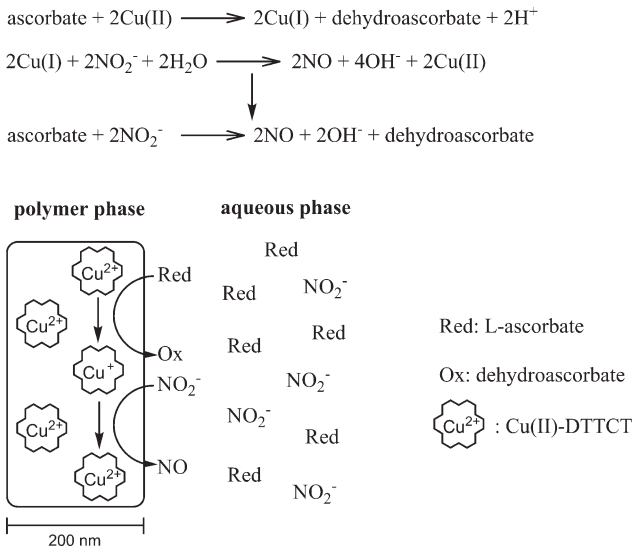

Figure 6 Schematic representation of catalytic NO generation at the interface of PCU films doped lipophilic Cu(II) complex when bathed in a solution containing nitrite and ascorbate.

NO generation by catalysis of PCU film containing Cu(II) complex *in vitro*

NO generation through catalysis of PCU1K blending with Cu(II) complex *in vitro* was investigated in a PBS solution (pH = 7.4) of $NaNO_2$ and ascorbic acid as release medium at 37°C. Reactions between Cu(II) complex and other medium are shown in Figure 6.[36] Endogenous ascorbate in fresh whole blood could provide the electrons required to continually reduce the Cu(II) to Cu(I), thereby yielding an effective catalytic generation of NO. This chemistry can take place directly in aqueous phases with an appropriately Cu(II) species (Cu(II)-DTTCT) at PCU film surface. This is likely attributed to very slow kinetics for this reaction. The process of reactions is as follows: ascorbate reduces Cu(II) to Cu(I) rapidly, while it is oxidized to dehydroascorbate.[40] Cu(I) can then reduce nitrite to NO at physiological pH, generating NO and Cu(II). Those two steps are thermodynamically and kinetically favored, but controlled by diffusion.

Influence of the concentration of $NaNO_2$ on NO generation

Although the concentration of ascorbic acid was 2.5 mmol/L and the content of Cu(II) in polymeric film was 0.096 wt %, NO generation experiments with five different concentrations of $NaNO_2$ were investigated with respect to the dependence of the NO flux on reaction time. Based on the fact that concentration of $NaNO_2$ in body was very low, the concentrations from 1 to 5 mmol/L $NaNO_2$ were selected for the study. The influence of the concentration of $NaNO_2$ on kinetic curve of NO release was shown in Figure 7.

It can be found that the generation velocity of NO is high in initial, especially at 30–60 min. After 120 min, NO flux increased gradually (Fig. 7). This might be resulted from high catalytic activity of non-embedded Cu(II) on the surface in the initial stage. The amount and the velocity of NO generation increased with increasing concentration of $NaNO_2$. The NO_2^- diffused into PCU films and interacted with Cu(I) to produce more NO when concentration of $NaNO_2$ was high.

Influence of the concentration of Vc on NO generation

NO generation experiments were performed with 2.0 mmol/L $NaNO_2$ and Cu content in film is about 0.096 wt %, and 0.5, 1.5, 2.5, and 3.5 mmol/L of ascorbic acid. The kinetic curve of NO generation shows that the velocity of NO generation is high in initial stage especially in first 180 min (Fig. 8). The reason is similar to above discussion in the effect of concentration of $NaNO_2$ on NO-release.

When the concentration of Vc increased from 0.5 to 3.5 mmol/L, the amount and velocity of NO generation increased. Vc molecules can diffuse to the surface and into the films when concentration of Vc in PBS was high. The opportunity of collision between Cu(II) and Vc increased with the increase of the concentration of Vc. The output of Cu(I) caused high amount and velocity of release of NO relatively.

Influence of amount of Cu(II) in polymeric film on NO generation

When the concentrations of $NaNO_2$ and Vc were 2.0 mmol/L and Vc 2.5 mmol/L, respectively, the

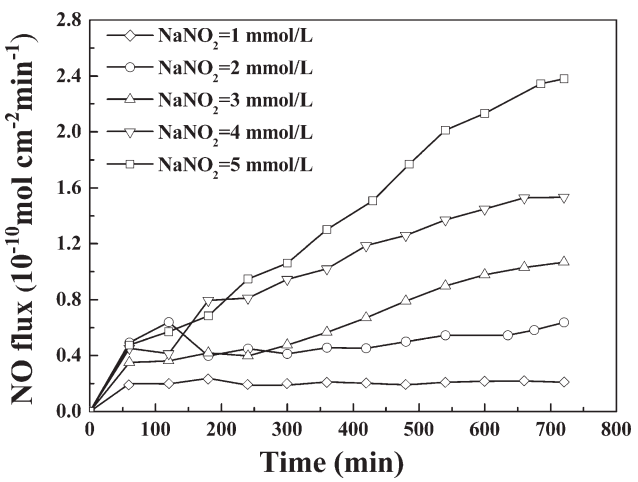

Figure 7 Kinetics curves of the NO releasing with various nitrite concentrations [Reaction conditions: PBS pH 7.4, 37°C, Vc, 2.5 mmol/L; Cu(II) in PCU1K film, 0.01 mmol (0.096 wt%)].

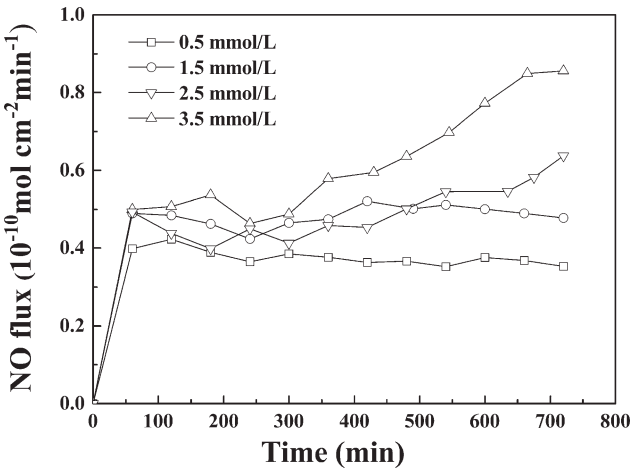

Figure 8 Kinetics curves of the NO releasing with various ascorbate acid concentrations [Reaction conditions: PBS pH 7.4, 37°C, $NaNO_2$, 2.0 mmol/L; Cu(II) in PCU1K film, 0.01 mmol (0.096 wt%)].

influence of the amount of Cu(II) in the blend films on NO generation was investigated.

The amount of Cu in the blood is about 11–24 μmol/L and most of them are coordinating with protein or other ligands. Based on this, the films were prepared by blending of 5–20 μmol Cu(II)-DTTCT with 0.66 g PCU to obtain the PCU film with Cu(II) contents of 0.048, 0.096, and 0.192 wt %. It can be found from Figure 9 that the amount and velocity of NO generation increased with increasing amount of Cu(II) in the tests. According to the reaction scheme in Figure 6, NO is generated by the reduction of $NaNO_2$ in the present of Cu(I) as catalyst. This means that the amount of Cu(I) and $NaNO_2$ influences directly on the velocity of NO generation. The Cu(II) can be reduced to form Cu(I) when more Cu(II)-DTTCT complex existed in the blend films. This increases the velocity of NO generation *in vitro*. Although Vc can act as a reductant and $NaNO_2$ as an oxidant, Vc can't reduce $NaNO_2$ directly unless by means of the reaction from Cu(II) to Cu(I) or other catalysts. The result shows that there was no NO generation without Cu(II). So Cu(II) can be regard as a catalyst in this reaction.

Influence of PCUs on NO generation

PCU1K and PCU2K were synthesized with similar feed ratio (PCN:MDI:BD = 1 : 2 : 1, molar ratio) from PCN with the molecular weight of 1000 and 2000 as the soft segment. These PCUs blended with Cu(II)-DTTCT (Cu(II) content 0.096 wt %) were used to investigated the influence of the soft segment of PCUs on the NO generation.

As shown in Figure 10, the amount and velocity of NO generation was higher with PCU1K blend film than with PCU2K blend film. PCU1K prepared from the PCN1000 contained higher content of hard segment (37.1%) due to similar molar feed ratio than PCU2K (hard segment 22.8%). The hard segment having urethane groups are more hydrophilic than soft segment polycarbonate. The hydrophilic microphase is favorable for water adsorption and water molecule diffusion into it. This effect makes Vc and/or $NaNO_2$ easily diffuse into the PCU films and react with Cu(II). The number average molecular weight of PCU1K (M_n 16370) is significantly lower than that of PCU2K (M_n 100200). Some of the Cu(II)-DTTC might be embedded tightly in PCU2K and lose the activity because of strong interaction or fixation. Both high hydrophobicity and molecular weight of PCU2K resulted in the lower NO generation.

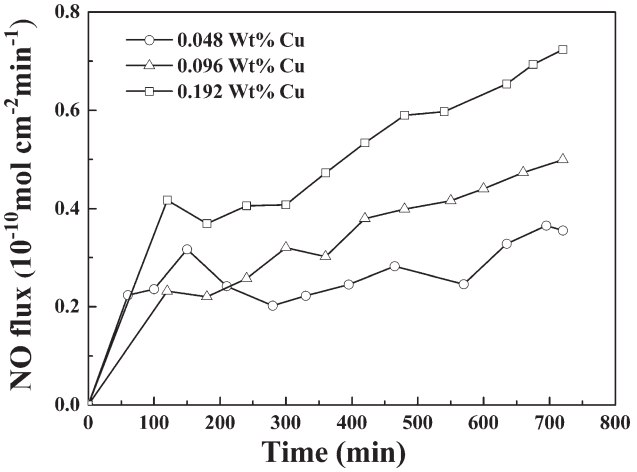

Figure 9 Kinetics curves of the NO releasing with various content of Cu(II) (Reaction conditions: PBS pH 7.4, 37°C, $NaNO_2$, 2.0 mmol/L, Vc, 2.5 mmol/L, PCU1K blend film with 0.005, 0.010 and 0.020 mmol Cu in 0.66 g PCU1K).

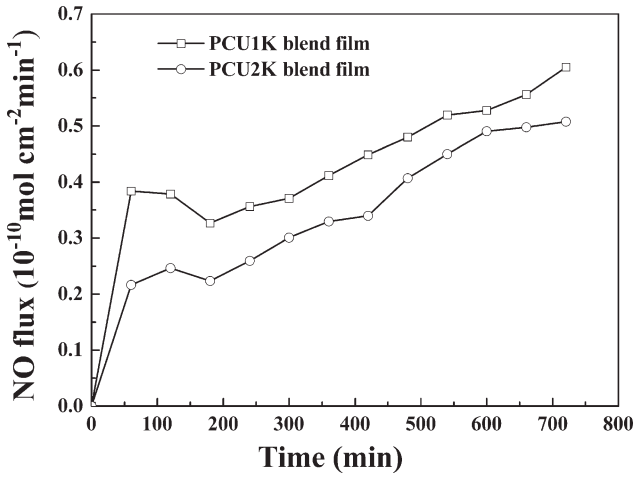

Figure 10 Effect of molecular weight of PCN in PCU on NO releasing (Reaction conditions: PBS pH 7.4, 37°C, $NaNO_2$, 2.0 mmol/L, Vc, 2.5 mmol/L, Cu(II), 0.01mmol (0.096 wt%) in PCU1K and PCU2K blend films).

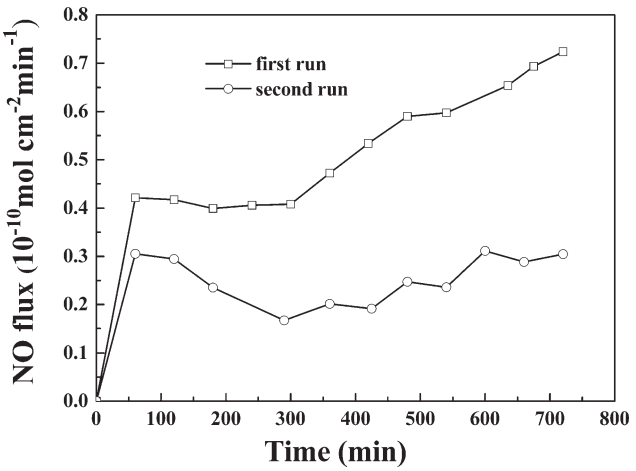

Figure 11 Comparison of NO releasing between first and second run in the same medium. [Reaction conditions: PBS pH 7.4, 37°C, $NaNO_2$, 2.0 mmol/L, Vc, 2.5 mmol/L, Cu(II), 0.01mmol in PCU1K (0.096 wt %)]

Loss of Cu(II) during NO generation *in vitro*

NO generation ability was investigated with the PCU1K film blending with Cu(II)-DTTCT for twice run under similar conditions. After NO generation experiment for 720 min in first run, the blend film was removed and dried, and then used in the second run. NO generation at second run was found obviously lower than at first run (Fig. 11). Both the velocity and the amount of NO decreased significantly. The possible reason might be the loss of Cu(II), especially loose Cu(II) on the surface can easily dissolve in PBS solution. To approve this hypothesis, the films blended with Cu-DTTCT were dipped into distilled water, PBS solution, PBS solution with Vc and $NaNO_2$ for about 24 h, respectively, and then the color of the filtrate was observed. Only PBS solution with Vc and $NaNO_2$ changed its color to light green, which indicates the dissolution of Cu-DTTCT from the film and loss of Cu(II).

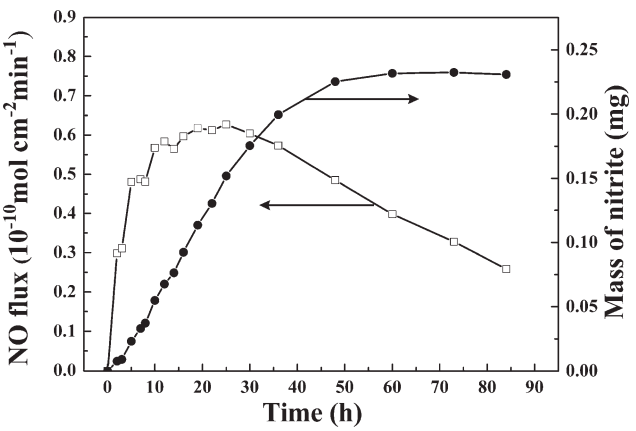

Figure 12 NO releasing of PCU1K films blending with Cu(II)-DTTCT [Reaction conditions: $NaNO_2$, 2.0 mmol/L, Vc, 2.5 mmol/L, Cu(II), 0.01mmol (0.096 wt %)].

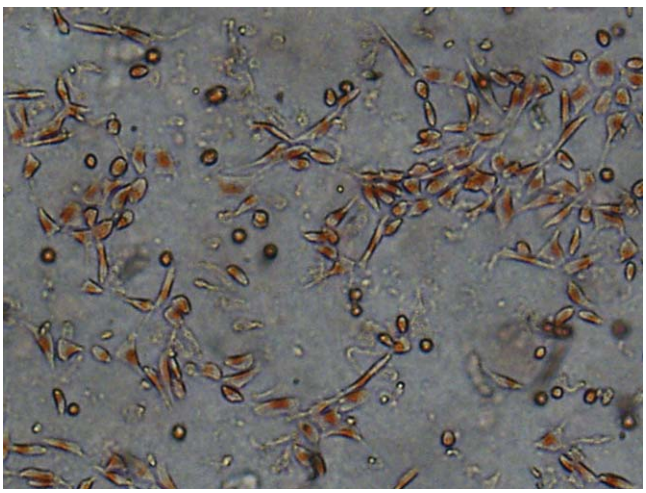

Figure 13 Results of cytotoxicity test of synthesized PCU1K films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Test of maximum generation time

The maximum NO generation time was investigated *in vitro* with the PCU1K film coating from THF solution with Cu(II)-DTTCT. The result showed that this film can catalyze NO release in PBS medium for about 2 d (Fig. 12), this result is similar to our previous studies on NO releasing from PCU film after being grafted with L-CySNO.[41] After 3 d, NO did not release any more. Such functionalized PCU films blending with Cu(II)-DTTCT have potential application for improving the hemocompatibility of blood contacting surfaces of biomedical implants. The result showed that the generation of biologically active levels of NO at the polymer surface could mimic normal endothelial cells which line on blood vessels, and a concomitant reduced risk of thrombosis on polyurethane biomaterials.

The cytotoxicity tests

Cytotoxicity tests of biomaterials are usually the first screening tool for biocompatibility required by the FDA for approval of a biomedical device. The cytotoxicity test of the synthesized PCU was carried out using a standard *in vitro* 929 cell culture test. Figure 13 showed that 929 cells grew well on PCU surface. The high cell viability proves that the PCU material has better biocompatibility (Fig. 13).

CONCLUSIONS

The lipophilic Cu(II)-complex(Cu(II)-DTTCT) as a NO generating catalyst was introduced to polycar-

bonateurethane films by solution blending method. PCU films with Cu(II) complex showed higher tensile strength and lower elongation at break compared with blank PCU films. The velocity of NO generation increased with the increase of the concentrations of $NaNO_2$ and Vc in PBS, and the amount of Cu(II) in the films. The amount and the velocity of NO generation were high when PCU was prepared from the soft segment with molecular weight of 1000. The ability of catalytic NO generation decreased at second run because of the loss of Cu(II) from the PCU blend films. The further investigations will be carried out to decrease or avoid the loss of Cu(II) during NO generation. The PCU film can gradually generate NO in PBS medium for about two days, which indicates the materials have potential application for blood contacting implants, such as artificial blood vessel.

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