Synthesis of Unsaturated Polyphosphoester as a Potential Injectable Tissue Engineering Scaffold Materials

Jin-Jun Qiu,[1,2] Cheng-Mei Liu,[1] Fen Hu,[1] Xiao-Dong Guo,[3] Qi-Xing Zheng[3]

[1]*Department of Chemistry, Huahzong University of Science and Technology, Wuhan 430074, China*
[2]*College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China*
[3]*Department of Orthopaedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*

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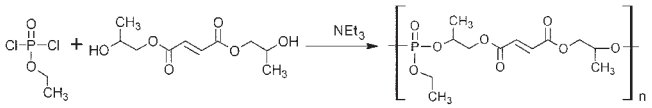
ABSTRACT: Polyphosphoester is a kind of biodegradable polymer with excellent biocompatibility and have been used in drug delivery, tissue engineering, and other bioapplications. A novel unsaturated polyphosphoester (UPPE) based on bis(1,2-propylene glycol) fumarate (BPGF) and ethyl dichlorophosphate was synthesized by polycondensation reaction, and crosslinkable double bonds was introduced into the resulting polymer through the fumarate groups. NMR results indicate that there are three possible bonding models in polyphosphoester because of three isomers of BPGF. The GPC results express that increase in polymerization time leads to high molecular weight of polyphosphester and narrow distribution of molecular weight. After 18 h of polymerization reaction, the molecular weight reached to 5956 g mol^{-1} and the polydispersity index was 1.12. The UPPE was soluble in *N*-vinyl pyrrolidone and easily crosslinked by free-radical polymerization. At the constant temperature (37°C), the maximum temperature due to heat release during crosslinking reaction varied from 41.1°C to 82.30°C and the setting time was between 1.95 and 10.28 min, according to different formulas. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 3095–3101, 2006

Key words: unsaturated polyphosphoester; bis(1,2-propylene glycol) fumarate; injectable; tissue engineering scaffold materials

INTRODUCTION

Current treatment for bone defects induced by injury or pathological change typically relies on autograft operation. This method raised the issue of function loss at the donor sites of the patient and shortage of graft material for extensive repair. To circumvent these problems, artificial materials have been developed to repair the bone loss and damage. There has been a long interest in the use of injectable materials because they can fill irregularly shaped defects and may allow bone augmentation, both with minimal surgical intervention. The ideal injectable materials would satisfy several important requirements[1]: it must first be compatible and degradable, have the correct mechanical properties, promotion of tissue formation, polymerization *in situ* in a timely fashion, and less exothermic avoidance detrimental effect to the surrounding tissue. Currently, the most commonly used injectable bone cement is poly(methyl methacrylate), but it is not degraded. Some other injectable biodegradable polymers include the polyanhydrides[2,3] and poly(propylene fumarate),[1,4–7]which have been investigated for use in bone repair. From the point of chemical structure, polyphosphoesters are analogs of nucleic and teichoic acids with excellent biodegradability and biocompatibility. The phosphoester bond in the polyphosphoester backbone can be cleaved by water and possibly enzymatic digestion under physiological conditions. The ultimate hydrolytic breakdown products of the polymers are phosphate, alcohol, and diol.[8,9] Polyphosphoesters are structurally versatile, manipulation of either the backbone or the side chain structure would readily alter their physicochemical properties. Polyphosphoesters have been investigated as biomaterials initially in drug delivery,[10–12] recently in gene carrier[13–15] and tissue engineering. Nerve guide conduit fabricated by using polyphosphoesters can be an effective aid for nerve regeneration.[16,17] The flexible P—O—C groups in the backbone result in polyphosphoesters commonly with low glass transition, which induced poor mechnical property in normal temperature. Therefore, this kind of polymers cannot satisfy the desirable feature of an injectable bone scaffolding materials. Mechanical property of linear polymer with low glass transition can be improved by crosslinking inter chains. Calcium treatment of polyphosphoester ionomers lead to significantly higher hardness and elastic modulus than those of the corresponding parent polyphosphoesters.[18,19] Calcium binding was evident from the increase in glass transition and melting tem-

Correspondence to: C.-M. Liu (liukui@mail.hust.edu.cn).



Scheme 1 Reaction scheme for the synthesis of unsaturated polyphosphoester.

peratures. Phosphoester linkage-containing hydrogels based on poly(ethyl glycol) were synthesized by photo-initiate crosslinking polymerization of the methacryloyl groups in the side chain or the end of the main chain of poly phosphoesters.[8,20] Here, a novel unsaturated polyphosphoester (UPPE) containing unsaturated double bonds in backbone based on bis(1,2-propylene glycol) fumarate (BPGF) and ethyl dichlorophosphate (EDP), which can be used as a potential injectable bone repair material, was synthesized. The crosslinking characteristic of the unsaturated was also investigated.

EXPERIMENTAL

Materials

All chemicals were obtained from Shanghai Chemical Reagents Company (Shanghai, China) unless described otherwise. Fumaric acid and benzoyl peroxide were purified by recrystalllization by standard method. Phosphorus trioxychloride was purified by distillation before use under Ar protection. Propylene oxide and dichloromethane was distilled from CaH_2. Ethyl ether, ethanol, and triethylamine was dried with sodium and distilled before use. 4-Methyl-2-pentanone, disodium hydrogenphosphate dodecahydrate, and pyridine were used directly. *N*-Dimethy-*p*-toluidine (DMT) was from Acros and *N*-vinyl pyrrolidone (NVP) was obtained from Merck company and purified by distillation.

Synthesis of bis(1,2-propylene glycol)fumarate (BPGF)

This compound was prepared according to a US patent[21] with a little modification. Into a well-dried three-neck flask were added 60 mL of 4-methyl-2-pentanone, 58.00 g (0.5 mol) of fumaric acid and 1.50 g of pyridine. The temperature was raised to 80–90°C with continuous stirring under Ar protection. Then 69.70 g (1.20 mol) of propylene oxide was added dropwise in 3 h. After the addition, the mixture was stirred at this temperature for another 0.5 h and the reaction was monitored by titrating the acid number of the mixture. When the acid number was no longer changed, the mixture was cooled to room temperature and washed with disodium hydrogenphosphate solution (7.8%, pH = 9). Then the mixture was washed with potassium chloride solution (5%) for two times. The crude product was dried with anhydrous magnesium sulfate overnight. The solvent was removed by rotary evaporation and pale yellow liquid was left as purified product with acid number 0.47. The yield was about 65.80%.

Synthesis of ethyl dichlorophosphate (EDP)

This intermediate was prepared by the reported method.[22] Into a well-dried 500 mL one-neck flask was added 180.00 g (1.17 mol) phosphorus trioxychloride and 210 mL dried ethyl ether. The flask was cooled outside with ice–water mixture bath. Then, 53.90 g (1.17 mol) of anhydrous ethanol was added dropwise under rapid stirring at 0°C. After the addition, let the temperature rise to room temperature slowly and stirred at this temperature for another 2 h. First, the solvent and unreacted ethanol was distilled and then the product was distilled under reduced pressure. Crude product (161.36 g) was obtained with a yield of 84.62%. Highly pure EDP for polymerization was obtained by further distillation under reduced pressure.

Preparation of unsaturated polyphosphoester (UPPE)

The polymerization reaction was shown in Scheme 1. All operation was done under Ar protection. Into a well-dried 500 mL three–neck flask, cooled outside with ice–water, was added 9.29 g (40 mmol) of BPGF in 150 mL dried dichlomethane and then 8.90 g (88 mmol) dried triethylamine with continuous rapid stirring. Then, 6.52 g (40 mmol) of ethy dichlorolphosphate dissolved in 20 mL dichlomethane was added dropwise into the mixture within about 1 h. After the addition was finished, the temperature was raised to refluxing temperature and the reaction was continued for a period of time. At the end of polymerization, the cooled mixture was washed thoroughly with diluted HCl and then sodium chloride solution to remove the byproducts. The cleaned mixture was dried by anhy-

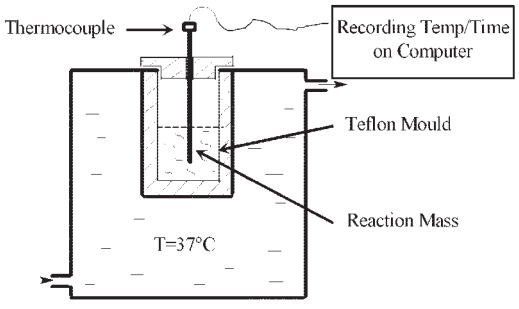


Figure 1 Schematic diagram of the Teflon mold designed to follow and record temperature–time parameters of the free-radical polymerization of UPPE formulations.

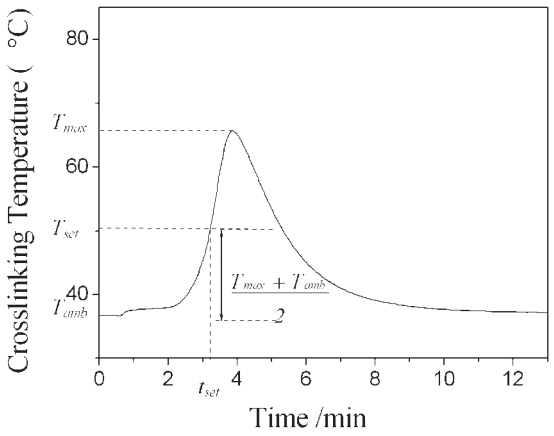


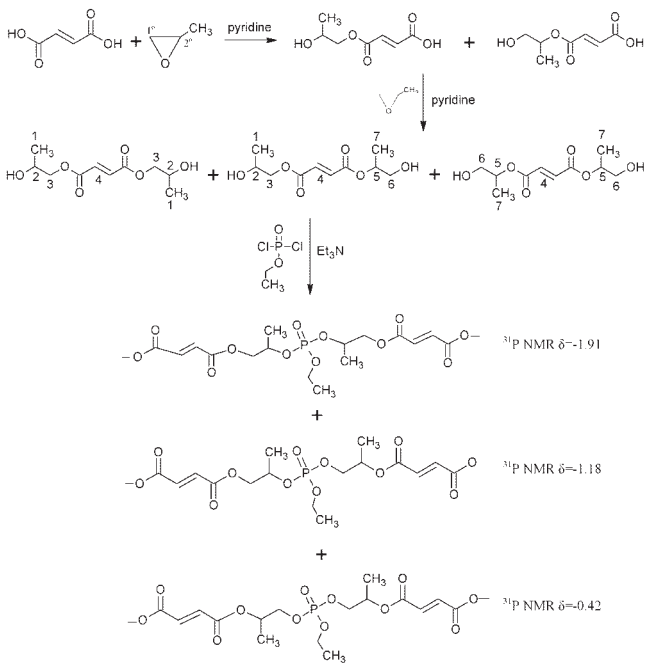
Figure 2 Representative temperature profiles for crosslinking formulation.

drous magnesium sulfate for 2 days and filtered. The filtrate was concentrated by rotary evaporator to remove most 4-methy-2-pentanone. Then, the mixture was precipitated into dichloromethane-ether for three times and dried under vacuum to remove any solvent and to obtain a yellow-sticky product. The molecular weight was measured by GPC.

Instrument and measurement

FTIR was recorded on EQUINOX-55 FTIR instrument. The NMR spectra were carried on the Varian Mercury Plus-400 system (^{1}H-NMR and^{13}C-NMR: $CDCl_3$ was used as a solvent and tetramethylsilane as an internal standard; ^{31}P NMR: $CDCl_3$ was used as a solvent and H_3PO_4 as an external standard). Molecular weight and its distribution were measured by gel permeation chromatography (GPC) and recorded on Agilent1100 high performance liquid chromatography system (Plgel MIXED-C type column, narrow distributed polystyrene from Waters as standard and tetrahydrofuran was used as an eluent at a rate of 1 mL/min).

The temperature profiles throughout the crosslinking process were recorded as the recommended method and instrument (Fig. 1) as in Ref. 23. The temperature was measured using a calibrated Pt-100 thermocouple and all data were recorded simultaneously on computer. The procedure was as follows: 1.0 g UPPE was mixed with half amount of total NVP



Scheme 2 Three isomers of BPGF and three bonding models in polyphosphoester.

required at room temperature to get a clear solution with continuous stirring, then desired amount of DMT was added; BPO was dissolved in another half of NVP. These two parts were preheated in water-bath to 37°C, then mixed rapidly in a cylindrical Teflon mold 10 mm in diameter and 15 mm in height, which was suspended in a water-bath ((37 ± 0.5)°C) at time zero. Figure 2 shows the representative temperature curve during the crosslinking process. The viscosity of virgin form of different formulas in Table IV but without adding initiator BPO was determined by rotation viscometer(NDJ-1) at room temperature.

Compressive testing of UPPE/NVP was conducted using a mechanical testing system (Model WDW-20), following the guidelines set in ISO 5833 : 2002(E). The UPPE was dissolved in the appropriate amount of NVP. The DMT was added, followed by the BPO, which was dissolved in the remaining NVP. The mixture was placed into the Teflon molds 6 mm in diameter and 12 mm in height. After a 24-h period, the cylinders were removed. Samples were compressed at a crosshead speed of 1 mm/min until failure while

TABLE I
FTIR Spectra Data of Monomer and Polymer

FTIR (γ/cm^{-1})	—OH	—CH_2—, —CH_3	C═O	C═C	C—O—C	P—O	C—O	P═O
BPGF	3404	2800–3000	1723	1642	1165, 1299	–	–	–
EDP	–	2800–3000	–	–	–	1030	1103	1295
UPPE	3427	2800–3000	1724	1645	1161, 1299	1031	1120	1262

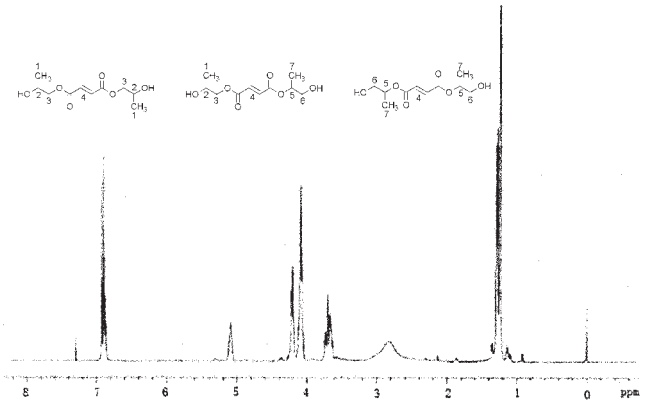


Figure 3 ^{1}H NMR spectrum of BPGF.

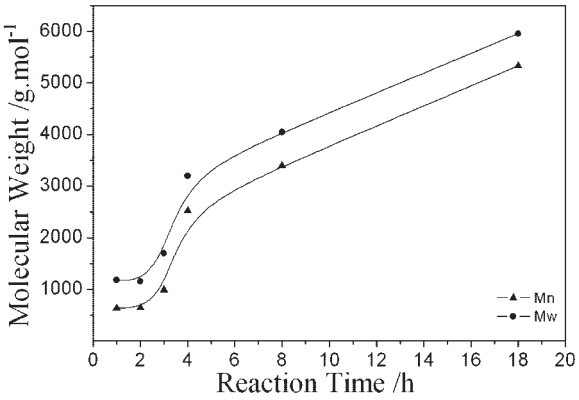


Figure 4 The effect of reaction time on molecular weight.

stress and strain were monitored. The initial slope of the stress–strain curve determined the elastic modulus of the sample.

RESULTS AND DISCUSSION

Synthesis and structure characterization of BPGF and EDP

The FTIR spectra data of BPGF, EDP, and UPPE were listed in Table I. Comparing the spectra of BPGF and UPPE, some new peaks appeared in polymer spectrum: 1031 cm^{-1} was due to P—O bond vibration, 1120 cm^{-1} due to C—O vibration, and 1262cm^{-1} due to P═O vibration.

According to the reaction mechanism, when fumaric acid reacted with propylene oxide, two isomers were first obtained because of different ring-opening format (site 1°or site 2°). Then, two monoesters were continued to react with propylene oxide to form three isomers. This reaction mechanism was summarized in Scheme 2. The ^{1}H NMR results of BPGF (Fig. 3 and Table II) also proved that the product consist of three isomers. At the end of reaction, the average acid number of reaction mixture was 7.58, and it indicated that there are monoesters of fumarate left. This monoester will affect the polycondensation reaction and need to be removed from the product. The monoester was washed away by disodium hydrogen phosphate solution.

TABLE II
^{1}H NMR Data of BPGF

Functional group	Figure label	Proton (ppm)
Methyl	1,7	1.13–1.36
Propyl methine of 2° alcohol	2	4.05–4.09
Propyl methylene of 2° alcohol	3	4.16–4.26
Olefinic bonds	4	6.86–6.94
Propyl methine of 1° alcohol	5	5.08–5.12
Propyl methylene of 1° alcohol	6	3.63–3.74

Preparation of UPPE

The UPPE was prepared by polycondensation reaction of BPGF and EDP. The purity of monomers was a key factor for the success of polymerization. Because EDP was very reactive, the polymerization temperature must be kept at low temperature at beginning to avoid any side reaction. After addition of the EDP, the temperature was raised to refluxing temperature to increase the molecular weight. The molecular weight increased with prolonging polymerization time (Fig. 4). In the first 4 h, the molecular weight increased rapidly and then became very slow. After 3 h of polymerization, some low molecular weight component still existed in the reaction mixture (Fig. 5). The peak of M_w = 176 g mol^{-1} belongs to EDP and the peak of M_w = 297 g mol^{-1} belongs to BPGF. The main products in this time are oligomers with M_w = 743 g mol^{-1} and M_w = 1140 g mol^{-1}. After 4 h of polymerization reaction, there were nearly no monomers left in reaction mixture but the molecular weight of polymer was still low. When the polymerization time reached 18 h, the molecular weight of polymer reached 5956 g mol^{-1}. The polymerization reaction is better to be completed in 20 h to avoid any crosslinking product.

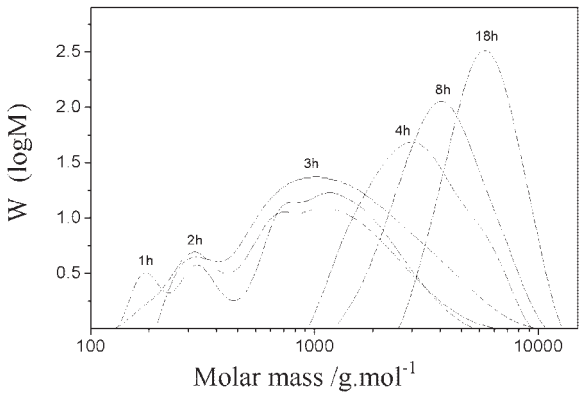


Figure 5 Molecular weight distribution of UPPE.

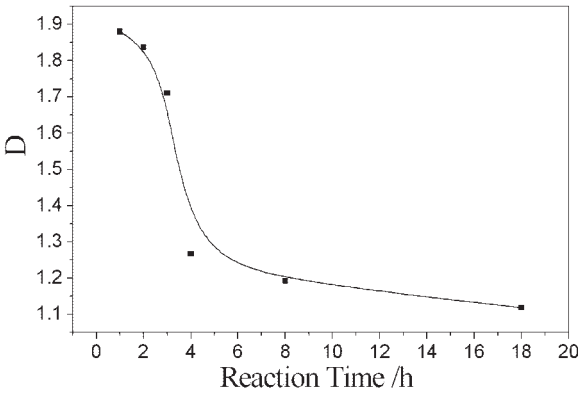


Figure 6 The effect of reaction time on the dispersion degree.

The polydispersity index decreased with increasing the polymerization time. At the beginning, the molecular weight distribution was wide and turned narrow with prolonging polymerization time (Fig. 6). After 18 h of polymerization, the polydispersity index was 1.12.

Characterization of UPPE

FTIR spectrum of polymer was shown in Figure 7. Comparing with the spectrum of BPGF three new peaks appeared because of phosphor–related bonds; 1031, 1120, and 1262 cm^{-1} were due to P—O, C—O, and P═O stretch vibration, respectively.

The NMR (^1H, ^{13}C, ^{31}P) spectra of polyphosphoester was shown in Figure 8. From the ^{31}P NMR spectrum, we found three characteristic peaks in it (−0.42, −1.18, −1.91 ppm). It means that there are three kinds of phosphor atoms located in the main chain of polyphosphoester. The result was consistent very well with the fact that all three isomers of BPGF were random, chemically bonded into polymer chains. Chaubal[24] et al. obtained the same result as they studied the chemical structure of a polymer resulting from poly(DL-lactide) and EDP, they also got three peaks (−1.6, −1.9, −2.4 ppm) in ^{31}P spectrum of polymer. They studied this phenomenon by

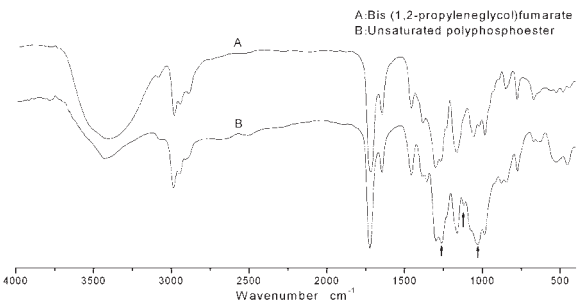


Figure 7 FTIR spectra of BPGF and polyphosphoester.

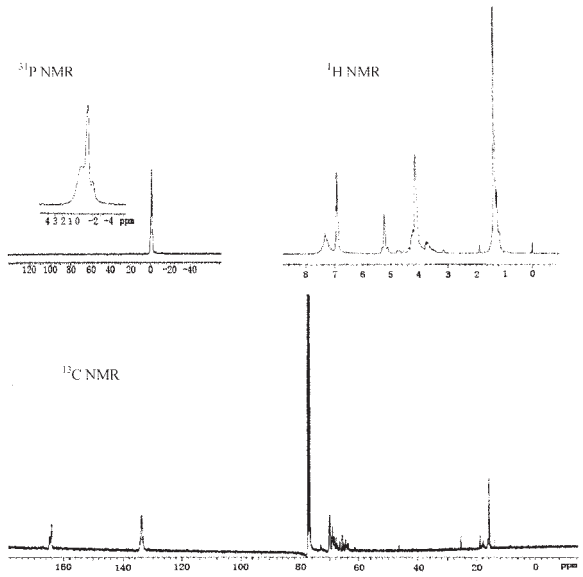


Figure 8 NMR Spectra of UPPE.

model compound and concluded that these results were due to different bonding sequence of P-O bond. ^1H NMR and ^{13}C NMR results gave the same results. Comparing the ^1H NMR and ^{13}C NMR spectra of BPGF, all the typical peaks appeared almost at the same position. The chemical shift data are collected in Table III.

Crosslinking reaction

Except excellent biocompatibility, degradability, low viscosity, and desired mechanical strength,[1] ideal injectable tissue engineering scaffold materials must have appropriate setting time and acceptable maximum setting temperature. Biocompatibility and degradability of polyphosphoester have been extensively studied.[14,16,19] The UPPE used in this research was pale yellow liquid with low molecular weight and becomes a semisolid with a molecular weight of 5956 g mol^{-1}. At present, the most widely used injectable bone repairing materials was based on poly(methyl methacrylate),[23] whose setting time was between 4.5 and 12.5 min and maximum curing temperature was 97°C. Such high curing temperature will cause the necrosis of surrounding tissue.[25] Moreover, the use of nondegradable materials may elicit a long-time inflammatory response to fragmentation and particulate formation. According to ISO5833:2002(E), the setting time (T_{set}) was defined as the time period from starting temperature (T_{amb}) to curing temperature (T_{set}) (Fig. 2). The curing temperature was defined as:

$$T_{set} = \frac{T_{amb} + T_{max}}{2}$$

TABLE III
NMR Data of UPPE

Functional group	Figure label	Proton (ppm)	Carbon (ppm)	Phosphorous (ppm)
Methyl	1, 7	1.19–1.32	15.81–16.20	–
Propyl methine of 2° alcohol	2	4.10–4.20	65.64	–
Propyl methylene of 2° alcohol	3	4.22–4.25	69.97	–
Olefinic bonds	4	6.87–6.89	133.23–134.10	–
Propyl methine of 1° alcohol	5	5.09–5.25	72.89	–
Propyl methylene of 1° alcohol	6	3.69–3.76	64.19	–
Carbonyl	–	–	164.03–164.68	–
Phosphate	–	–	–	−0.42, −1.18, −1.91

TABLE IV
Crosslinking Reaction Character of UPPE-NVP

Sample code	M_w (g mol^{-1})	UPPE (g)	NVP (g)	DMT (g)	BPO (g)	T_{max} (°C)	Setting time (min)	Compressive strength (Mpa)	Compressive modulus (Mpa)	Viscosity (Pa s)
1	1720	1.00	0.80	0.0053	0.0050	82.30	1.95	11.04	101.91	12.50
2	1720	1.00	0.40	0.0011	0.0010	41.14	10.28	5.92	7.69	33.31
3	5559	1.00	0.80	0.0036	0.0030	77.45	2.97	18.43	497.20	13.01
4	5956	1.00	0.60	0.0010	0.0010	59.1	7.40	35.52	717.70	76.24

The formula and crosslinking characters of polymer were listed in Table IV. NVP was used as crosslinking reagent. All the mixture based on UPPE and NVP are liquid and can be easily injected into target tissue. The maximum curing temperature were between 41.1 and 82.30 °C (Fig. 9) and lower than that of resulting from PMMA. It can decrease the damage of surrounding tissue. The setting time varied between 1.95 and 10.28 min. Such long operation time can facilitate the injection operation. The compressive strength was between 5.92 and 35.52 Mpa, and the compressive modulus varied from 7.69 to 717.70 Mpa. The compressive strength and compressive modulus of human trabecular bone were close to 5 and 50 Mpa, respectively.[26] The values of PMMA were 46 and 1147 Mpa[27] are far in excess of the natural trabecular bone and might cause stress shielding of the bone surrounding the implant site. In this research, the highest compressive strength and compressive modulus were 35.52 and 717.70 Mpa, respectively. The highest values are lower than those of PMMA and close to natural trabecular bone. By adjusting the ratio of components, an ideal crosslinking matrix with equal compressive properties with natural bone can be obtained.

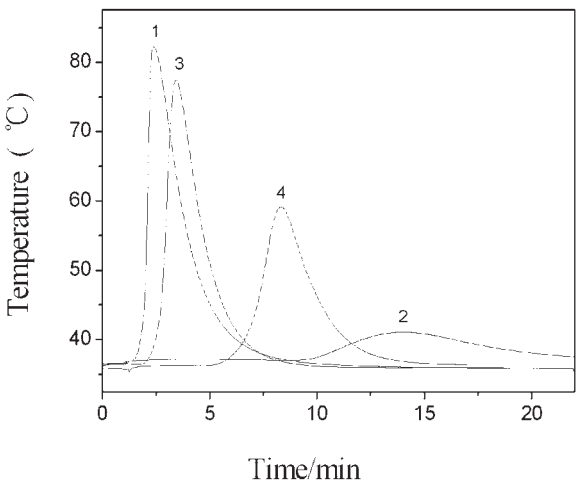


Figure 9 Crosslinking reaction temperature curve of UPPE-NVP.

CONCLUSIONS

A novel UPPE with double bond in the backbone was prepared by condensation polymerization. The polyphosphoester can form crosslinking matrix with mechanical properties close to natural trabecular bone. The setting time, maximum temperature, compressive strength, and compressive modulus of polyphosphoester can be adjusted by changing the amount of UPPE, crosslinker(*N*-vinyl pyrrolidone), and initiator. From the results obtained from this research, we can conclude that UPPE is a potential injectable tissue engineering scaffold materials with excellent operation properties.

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