Poly-α,β-(3-Hydroxypropyl)-DL-Aspartamide: A New Drug Carrier

GU-PING TANG,[1] K. J. ZHU,[2] Q. Q. CHEN[1]

[1] College of Pharmacy, Zhejiang University, Hangzhou Yanan Road 353, 310031 Zhejiang, People's Republic of China

[2] Department of Polymer Science and Engineering, Zhejiang University (310013), Hangzhou, Zhejiang, People's Republic of China

Received 15 September 1999; accepted 26 December 1999

ABSTRACT: Poly-α,β-(3-hydroxypropyl)-DL-aspartamide (PHPA) was synthesized by the ring-open reaction of polysuccinimide (PSI) and 3-hydroxypropylamine. The polymer was characterized by ^{1}H-NMR, ^{13}C-NMR, FTIR, and GPC. Mark–Houwink coefficients were obtained from viscometry and GPC measurements, $K = 5.53 \times 10^{-3}$ and $\alpha = 0.78$ in water. The acute toxicity of PHPA was examined and it revealed no death in ICR mice up to the dose treated of 15.3 kg/kg, and hematological parameters showed no significant difference between treated and control animals. The potential use of PHPA as a drug carrier was also investigated. In a typical case, a contraceptive drug, norethindrone (NET), was bonded to PHPA, and the drug sustained released as long as 120 days an *in vitro* test. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 2411–2417, 2000

Key words: Poly-α,β-(3-hydroxypropyl)-DL-aspartamide; drug polymer carrier; drug-sustained release

INTRODUCTION

In recent years, intensive studies of poly(amino acids) have been performed. Among them, poly(aspartic acids) have been paid more attention because of their wide usage and special properties, such as biocompatible, biodegradable, and nontoxic characters. In addition, a relatively simple route of synthesis with a high yield allows easy production in a large scale. As a potential plasma expander, poly-α,β-(2-hydroxyethyl)-DL-aspartamide (PHEA) has been studied since 1974.[1] Many model polymeric drugs have been reported, such as PHEA-L-dopa,[2] which was microencapsulated in alginate–chitosan microspheres in order to achieve drug release from a complex reservoir device. The pulmonary absorption kinetics of a single molecular weight distribution (MWD) of fluorophore-labeled-PHEA, a hydrophilic and biocompatible synthetic polypeptide, was studied in isolated perfused rat lung.[3] The hydrolysis of PHEA–KPN (ketoprofen), PHEA–NAP (naproxen), and PHEA–DFN (difunisal),[4–6] known as anti-inflammatory drugs, studied at pH 5.5 and 7.4, showed that intact free drug was released from the conjugate and it was demonstrated that esterase enzymes were able to cleave the ester bonds between drug molecules and the polymeric backbone. Poly(L-aspartic acid) derivaties–*cis*-plastin conjugates were prepared and *in vitro* cytotoxicity was studied.[7] In this communication, we report a new derivative of poly(aspartic acid), poly-α,β-(3-hydroxypropyl)-DL-aspartamide (PHPA). By changing the length of the side chain in the matrix, it is easy to conjugate the drug to the polymer. The preparation, characterization, and properties of PHPA are also reported. For evaluating the possible appli-

Correspondence to: G.-P. Tang (gllu@tiger.hzuniv.edu.cn).
Contract grant sponsor: National Natural Science Foundation of China.

Journal of Applied Polymer Science, Vol. 77, 2411–2417 (2000)

cation of this new polymer as a drug carrier, NET, a contraceptive drug, was conjugated to PHPA.

EXPERIMENTAL

Materials

DL-Aspartic acid and *N*,*N*′-dimethylformamide (DMF) were supplied by the Shanghai Chemical Co. (Shanghai, China); 3-hydroxypropylamine was purchased from the Sigma Chemical Co. (St. Louis, MO), NET was purchased from the Xianju Pharmaceutical Ltd. (Xianju, China) and recrystallized from ethanol. All reagents were analytical grade.

Synthesis of PHPA

PHPA was prepared by modifying polysuccinimide (PSI) with 3-hydroxypropylamine in DMF. PSI was obtained by a condensation reaction of DL-aspartic acid in the presence of H_3PO_4 at 180°C.[1] Nine milliliters of 3-hydroxypropylamine (0.2 mol) was added dropwise to a continuously stirred solution of PSI (6 g, 2.0×10^{-4} mol) in 50 mL DMF. The reaction temperature was maintained in the range of 0–10°C and then kept for 4 h at room temperature. The mixture was added dropwise to 200 mL 1-butanol with stirring. A white product was precipitated, then filtered, washed with acetone until no base was contained in the product, and finally dried under a vacuum at room temperature. PHPA, 9.8 g, was obtained; yield 98%.

Synthesis of NET conjugated to PHPA (NET–PHPA)

NET (1 g, 3.4×10^{-3} mol) was dissolved in methylene dichloride (50 mL); 14 mL (12%) phosgene in benzene and 1 mL pyridine were carefully added to the solution with stirring. After 1–2 h, the reaction was completed as monitored by thin-layer chromatography (TLC.HF254) and NET chloroformate was found (Rf 0.09 for NET, and 0.32 for NET chloroformate, developed with a mixture solvent of benzene:ethyl acetate = 9:1 v/v). The reaction mixture was evaporated to dryness under reduced pressure. PHPA (1 g, 4.2×10^{-5} mol), dissolved in a mixture solution (5 mL DMF and 1.5 mL pyridine), was added to the NET chloroformate and allowed to react for 24 h at room temperature. The reaction product was precipitated from a 200 mL solvent mixture of butanol:ethyl acetate (v/v = 1:1). The precipitate was dissolved in 5 mL DMF and precipitated from another 200 mL of butanol/ethyl acetate solvent. To further assure removal of the noncoupled steroid, the precipitated polymer was extracted with 30 mL acetone for a period of 12 h. Finally, it was extracted with 30 mL water, isolated, and dried for 72 h in a vacuum at room temperature. The total yield of the polymer coupled with NET was 1.2 g.

Characterization

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Bruker AM-400 NMR spectrometer in $CDCl_3$ at 25°C. Sample tubes, 5-mm o.d., were used for the measurements.

The FTIR spectra were recorded on a Nicolet Inagana FTIR 750 at room temperature. Samples were pressed into KBr pellets.

The molecular weight of PHPA was determined by GPC using a Shimadzu LC-6A with a refractive index detector. The chromatography conditions were as follows: column Diol-150 Φ 7.9 × 250 mm; water as the mobile phase; a flow rate of 1 mL/min; column temperature 40°C; and glucosan standards. The intrinsic viscosity [η] was determined in water at 25 ± 0.1°C using an Ubbelohde viscometer. The outflow time for water was from 80 to 120 s.

Evaluation of PHPA Property

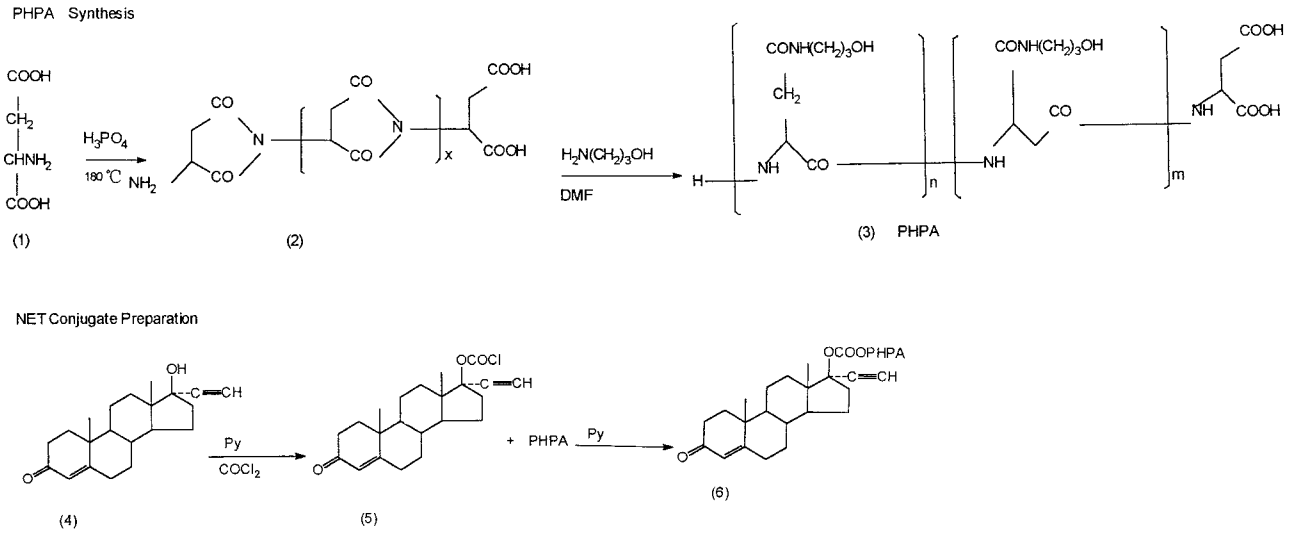
Stability of PHPA

Effect of Humidity on PHPA. Three aliquots of 30 mg PHPA were weighted in a small open flask and placed in a constant temperature (37 ± 0.1°C) oven and the humidity was kept at 70% (KCl solution) for 10 days. The samples were dissolved in distilled water and analyzed by GPC.

Effect of Light on PHPA. Three aliquots of 30 mg PHPA were weighted and placed in a box kept at 2000 LX of light to irradiate for 10 days. The samples were dissolved in distilled water and analyzed by GPC.

Effect of pH on PHPA. Eighteen aliquots of 10 mg PHPA were dispersed in 50 mL of the buffer solution, with varied pH from 2 to 12, then kept at 37 ± 0.1°C for 4 h. Each sample, after neutralization with 1 mol/L NaOH or 1 mol/L HCl, was analyzed by GPC.

Effect of Temperature on PHPA. Nine aliquots of 30 mg PHPA were placed with a thermostat and



Scheme 1 The routes of synthesis.

maintained at temperatures of 40 ± 0.1, 60 ± 0.1, and 80 ± 0.1°C for 10 days. Each sample was dissolved in distilled water and analyzed by GPC.

Laboratory Animals Test

ICR mice (18–25 g) were used for testing acute toxicity and hematologicalogy. The mice were kept at 25°C with a light/dark cycle of 12 h. All the mice were fed suitable diet pallets. A solution of PHPA dissolved in a sterile physiological saline was injected i.g. once only. Five different dosages of PHPA were used. Two groups of 50 mice each were used for the hematological studies. One group (as the control group) of 50 mice was treated i.p. with physiological saline (1 mL/100 g). The other group of 50 rats was implanted with PHPA (40 mg/per mouse; PHPA was pressed into a litter rod; Φ 3 × 5 mm). The experimental animals were killed at 7, 13, 20, 27, and 34 days. Each time there were 20 mice, including 10 mice

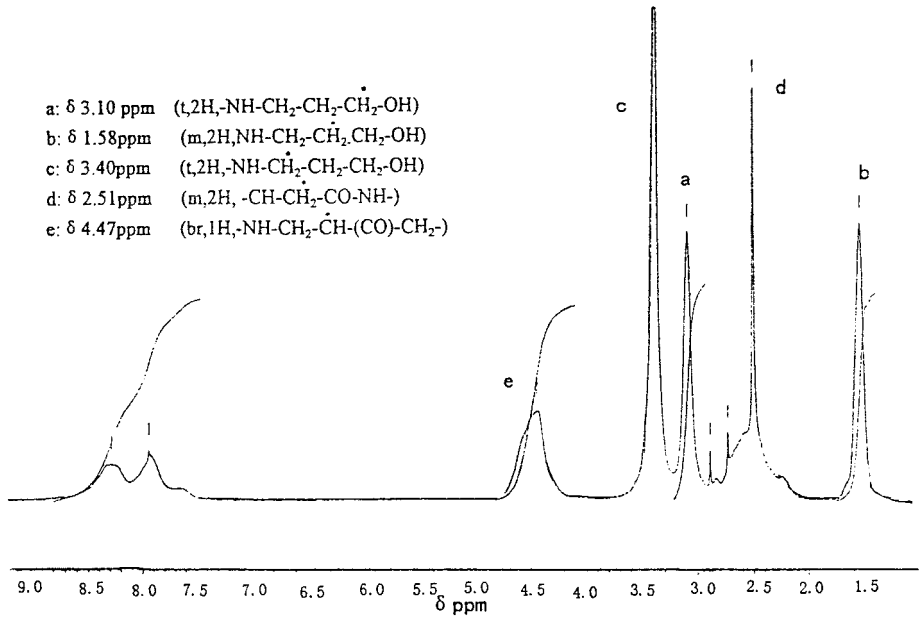


Figure 1 ^{1}H-NMR spectrum and assignment for PHPA measured in $CDCl_3$ at 25°C: (a) 1.58 ppm; (b) 2.51 ppm; (c) 3.10 ppm; (d) 3.40 ppm; (e) 4.47 ppm.

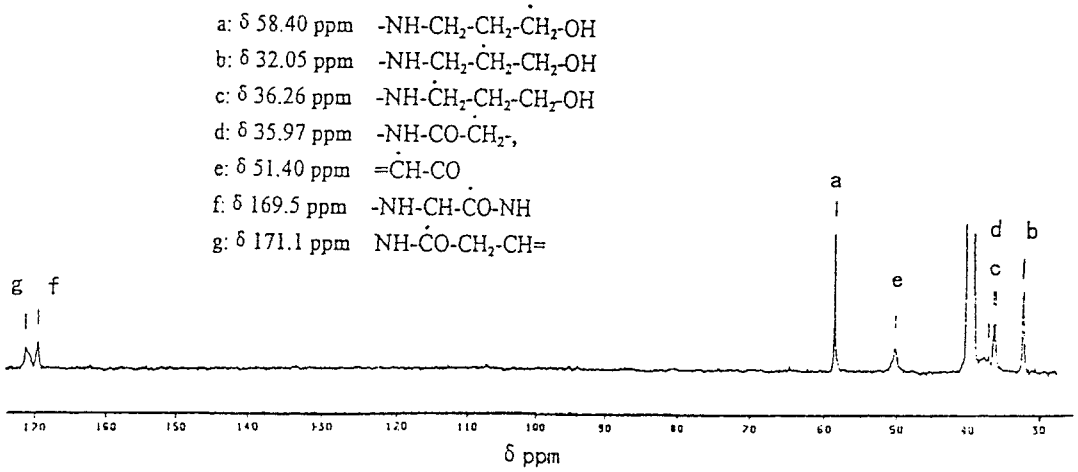


Figure 2 ^{13}C-NMR spectrum and assignment for PHPA measure in $CDCl_3$ at 25°C: (a) 32.05 ppm; (b) 35.87 ppm; (c) 36.26 ppm; (d) 50.09; (e) 58.40 ppm; (f) 169.5 ppm; (g) 171.1 ppm.

in the control group and 10 mice in the treated group.

Analysis of NET and NET–PHPA

An appropriate retention time for the NET determination was found when a reversed-phase C18 column was eluted at room temperature with CH_3OH:H_2O in the ratio 70:30 (v/v). The flow rate was 1.0 mL/min and the elute was monitored at 254 nm. Quantification of NET was done by measurements of the peak area in relation to that of the authentic sample of the drug using a calibration curve. The straight-line equation (in the concentration rage of 0.5–5.0 μg/mL) was $A = -2.470 \times 10^{-3} + 2.372 \times 10^{4}C$ ($n = 5$, $r = 0.966$). The method allows us to determine the drug amount released from NET–PHPA by all the performed hydrolysis procedures.

RESULTS AND DISCUSSION

Synthesis

The reaction of PSI with 3-hydroxypropylamine leads to a polymer, PHPA, and the conjugate formation of NET–PHPA is shown in Scheme 1. Due to an exothermic reaction when 3-hydroxypropylamine was added, it is necessary to use ice bath, keeping the reaction temperature range of 0–10°C. Excess amine should be washed with acetone and removed completely. The experiment also showed that PHPA is well soluble in water and DMF, but is insoluble in those solvents, such as methanol, ethanol, and methylene dichloride, which have low dielectric constants.

Characterization

The FTIR spectrum revealed bands at 3100 cm^{-1} (OH), 1650 cm^{-1} (amide I), and 1540 cm^{-1} (amide II). ^{1}H-NMR and ^{13}C-NMR spectra of PHPA is shown in Figures 1 and 2, respectively. Extract ^{1}H-NMR ($CDCL_3$): δ 1.58 (m, 2H, NH—CH_2—CH_2—CH_2—OH), δ 2.51 (m, 2H, —CH—CH_2—CO—NH—), δ 3.10 (t, 2H, —NH—CH_2—CH_2—CH_2OH), δ 3.40 (t, 2H, —NH—CH_2—CH_2—CH_2—OH), δ 4.47, [br, —NH—CH_2—CH—(CO)—CH_2—]. In the ^{13}C-NMR spectrum of PHPA, the peaks at 58.4, 32.05, and 36.26 ppm correspond to —NH—CH_2, CH_2, and CH_2—OH, re-

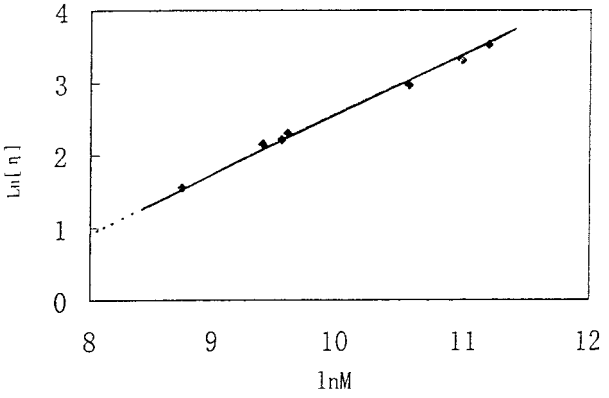


Figure 3 Intrinsic viscosity of PHPA in water at 25°C. [$\eta = 5.53 \pm 10^{-3} M^{0.87}$.

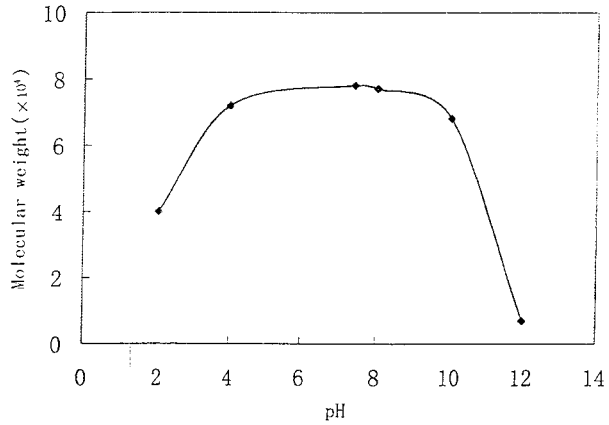


Figure 4 Effect of pH on PHPA stability.

spectively; 50.09 ppm, to ═CH—CO—; 171.1 and 169.5 ppm, to NH—CO—CH_2—CH═ and —NH—CH—CO—NH—, respectively, and 35.97 ppm, to —NH—CO—CH_2—.

Mark–Houwink Equation

Mark–Houwink coefficients are important parameters for polymer materials. The values of K and α for PHPA in water were determined by the intrinsic viscosities and GPC of seven samples. The results were expressed as reduced viscosities:

$$\eta = (\eta - \eta_0)/\eta_0 C$$

Figure 3 shows the experimental points for water and a fit curve that corresponds to the equation. A standard curve of glucosan standards, which have a molecular weight range from 5000 to 150,000, was derived from the experimental condition: $\ln M = 5.974 - 0.138\, t_R$ ($r = 0.9954$, $n = 5$). A very linear relation was observed between the $\ln[\eta]$ and M_n. The parameters of K and α were obtained: $K = 5.53 \times 10^{-3}$ and $\alpha = 0.78$, and the Mark–Houwink equation was also obtained:

$$[\eta] = 5.53 \times 10^{-3} M^{0.78} \quad (n = 7\ r = 0.999)$$

Stability of PHPA

The stability of PHPA was evaluated *in vitro*. Figure 4 indicates the stability of PHPA in different pH buffer solutions. It can be seen that in the range of pH 5–10, the buffer solution of PHPA is stable, but in acid or base, it is in some degree of degradation, especially at pH 12. The ester linkage may be labile in the base solution and be easily cloven. The fragments of PHPA were measured by GPC. It shows that small molecular weight fragments appeared. The effect of temperature on PHPA is obvious. Figure 5 shows that as temperature increases from 25 to 80°C, the molecular weight of PHPA increased. Because of the active hydroxyls in the spacers of the polymer, they can be further condensed. Furthermore, the effect of humidity and light on PHPA were also investigated in the experimental condition. It had no remarkable effect on the molecular weight of PHPA in the experimental condition.

Toxicity Studies

No death was recorded in the mice treated i.g. with the PHPA solution up to a dose of 15.3 kg/kg.

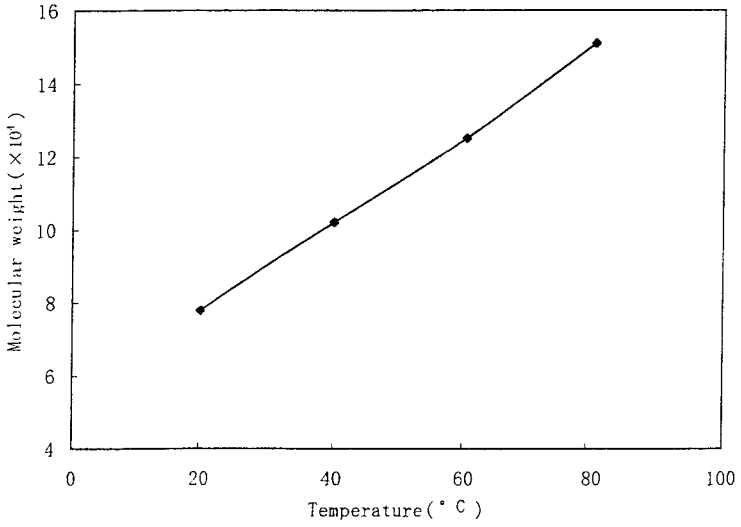


Figure 5 Effect of temperature on PHPA stability.

Table I Hematological Parameters in Mice Treated for 6, 13, 20, 28, and 34 Days with PHPA (a Rod, Implant in Back of Mice, 40 mg) or Physiological Saline (1 mL/kg, i.p.) (mean ± SE)

Treatment (Day)		WBC (no./cm^3) × 10^3	Cr (μmol/L)	SGPT (μ/L)	Hg (g/1000 mL)
6	Saline	10.3 ± 0.1	—	29.55 ± 0.90	13.1 ± 0.3
	PHPA	11.2 ± 1.3	—	26.17 ± 5.20	13.1 ± 0.3
13	Saline	11.2 ± 0.5	27.0 ± 2.8	43.33 ± 6.98	13.9 ± 0.4
	PHPA	10.9 ± 0.2	26.7 ± 0.4	36.22 ± 2.30	13.0 ± 0.3
20	Saline	15.8 ± 1.0	29.1 ± 0.8	36.46 ± 6.44	12.5 ± 0.4
	PHPA	13.2 ± 2.0	27.1 ± 0.2	43.44 ± 2.81	13.6 ± 0.4
27	Saline	15.4 ± 1.2	36.4 ± 1.0	35.92 ± 3.12	13.1 ± 0.1
	PHPA	15.0 ± 0.6	36.9 ± 4.1	41.28 ± 0.86	13.6 ± 0.5
34	Saline	12.0 ± 1.8	28.9 ± 0.3	32.87 ± 7.74	13.6 ± 0.4
	PHPA	14.6 ± 1.5	15.8 ± 2.4	40.25 ± 8.34	13.4 ± 0.4

Under the experimental condition, it was not possible to determine the LD_{50} in mice, as it was clearly greater than 15.3 kg/kg. The results of the hematological studies are shown in Table I. As we can see, the treatment of the animals with the PHPA solution did not cause any change in hemoglobin levels. No differences between the number of red cells and white cells of treated and control animals were found. At same time, glutamic pyruvic transaminse (SGPT) and creatinuria (Cr) were also found to be no different from those in the control animals.

Release of NET

The NET–PHPA conjugate was characterized by FTIR: 3000 cm^{-1} (—OH), 1654 cm^{-1} (amide I), 1540 cm^{-1} (amide II), and 1730 cm^{-1} (—COO). The band of 1730 cm^{-1} (—COO) showed that NET was linked to PHPA by carbonate bone. The amount of NET bounded to the polymer, evaluated by alkaline hydrolysis followed by HPLC, and its results indicated that NET loading was found to be about 37.1% (w/w).

As a potential candidate for a controlled delivery system, PHPA is suitable for the conjugation with drugs because of active hydroxyl groups in the side chain. NET, one of the contraceptive steroids, as a model drug, was covalently coupled to PHPA through 17-carbonate.

To obtain preliminary information about the potential use of the NET–PHPA as a drug-delivery system, an *in vitro* hydrolysis study was performed, subjecting the conjugate to hydrolysis in the buffer solution at pH 7.4 (tris-HCl). The drug-release profiles depicted in Figure 6 show that a NET sustained release was observed in 3 months.

CONCLUSIONS

PHPA can be synthesized by the ring-opening reaction of PSI with 3-hydroxypropylamino. It is stable in the conditions of light, humidity, and a neutral buffer solution, but obviously degrades in an acid or base solution and also condensation appears at high temperature. The model drug, NET, is conjugated to the polymer, and a sustained release rate is observed. Biocompatible, biodegradable, and nontoxic characteristics make it possible for it to be a useful material in protein-delivery systems.

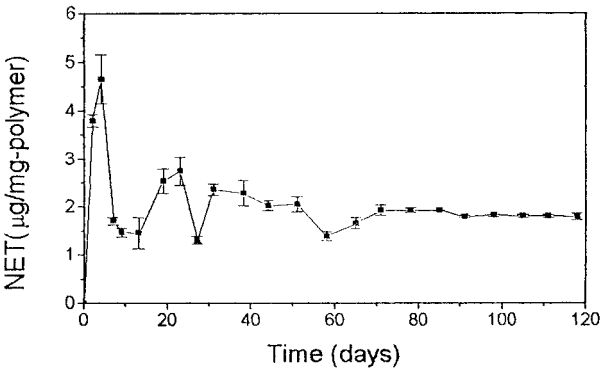


Figure 6 NET release from PHPA polymer matrix at 37°C in tris-HCl (pH 7.4) buffer solution. Drug load: 37.1 wt %; particle size: 83.65 ± 31.83 μm; n = 3.

We are indebted to the National Natural Science Foundation of China for their support of this work.

REFERENCES

1. Antoni, G.; Neri, P.; Sclavo, I. S. V. T. Biopolymers 1974, 13, 1721–1729.
2. Filipovic-Grcic, J.; Maysinger, D.; Zorc, B. Int J Pharm 1995, 116, 39–44.
3. Byron, P. R.; Sun, Z.; Katayama, H. Pharm Res 1994, 11, 221–225.
4. Giammona, G.; Tomarchio, V.; Pitarresi, G. Polymer 1997, 38, 3315–3323.
5. Giammona, G.; Pitarresi, G.; Tomarchio, V. J Controll Rel 1996, 41, 195–203.
6. Giammona, G.; Cavallaro, G.; Fontana, G. Eur J Pharm Sci 1996, 4, 273–282.
7. Lu, Z.-R.; Yu, J.-H.; Zhuo, R.-X.; Wang, X.-L.; Yang, F.-H. Chem J Chin Univ 1998, 19, 817–821.