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European Polymer Journal 36 (2000) 1703–1710

EUROPEAN POLYMER JOURNAL

Synthesis and characterization of novel kinds of polyethylene oxide drugs containing 5-fluorouracil and nitrogen mustard at one end and 4-amino-N-(2-pyrimidinyl) benzene sulfonamide at the other end

Sheng Chen, Zhaohua Huang, Junlian Huang*

Department of Macromolecular Science, Fudan University, The Open Laboratory of Molecular Engineering of Polymer, The Education Ministry of China, Shanghai 200433, People's Republic of China

Received 25 November 1998; received in revised form 1 September 1999; accepted 24 September 1999

Abstract

Using polyethylene oxide (PEO) with 4-amino-N-(2-pyrimidinyl) benzene sulfonamide (APBS) and hydroxyl at both ends as parent compounds, 5-fluorouracil (5-Fu) and nitrogen mustard (N-Mu) were successfully grafted on PEO end respectively by esterifying reaction of acyl chloride derivatives of 5-Fu and N-Mu with the hydroxyl end group, the yield measured by UV spectrometer and chemical analysis is 58% for 5-Fu and 41.3% for N-Mu respectively. The structure of these drugs are characterized by IR and NMR in detail.

Keywords: Polyethylene oxide; 4-amino-N-(2-pyrimidinyl) benzene sulfonamide; Polymeric drugs; Antitumor activity; 5-Fluorouracil; Nitrogen mustard

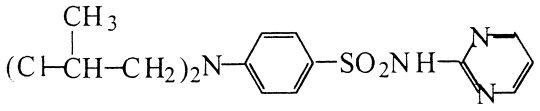
1. Introduction

5-Fluorouracil (5-Fu), an antimetabolite, and nitrogen mustard (N-Mu), a cytotoxin, have been widely used for a long time as antitumor agents. It is well known, however, they showed strong toxic side effects on living body. Thus, how to reduce or remove the toxicity of these small antitumor drugs is a very important subject for pharmacochemists. Many works have reported that it is possible to reduce the toxic side effects and prolong the duration of activity [1,2] using polymer as a carrier. At present, the targeting antitumor drugs are recognized as the most effective way to kill malignant tissue selectively and minimize the unfavorable side effects.

As early as 1950s Stevens [3] found that Sulfadiazine (SD), an antibiotic, and its homologues could be concentrated selectively in Yoshida Sarcoma growing in rats. Abel attempted to exploit its ability to concentrate in tumor cell by designing a cytotoxic derivative based on sulfadiazine, and sulfadiazine-mustard with the following structure was prepared, but it lost the ability to be taken up by those tumor cells which concentrate sulfadiazine (see Scheme 1) [4]. Recently, polyethylene oxide (PEO) with sulfadiazine and diethylenetriaminepentaacetic acid (DTPA) end groups were synthesized by us. After complexation with ^{153}Sm

* Corresponding author.

0014-3057/00/$ - see front matter
PII: S0014-3057(99)00243-8



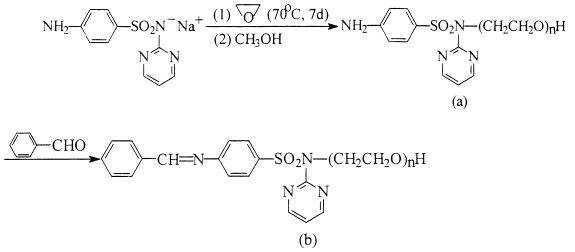
Scheme 1.

and ^{99}Tc it could be concentrated selectively in the tumor tissue (Sarcoma-180) [5], the ratio of concentration of the polymeric drug in tumor tissue to that in ordinary organs was about 2.3:1 or even 10:1. Since then, a series of polymeric drugs were prepared by us. The present article only focused on the synthesis and characterization of PEO with sulfadiazine and antitumor drugs (5-Fu and N-Mu) at both ends.

2. Experimental

2.1. Materials

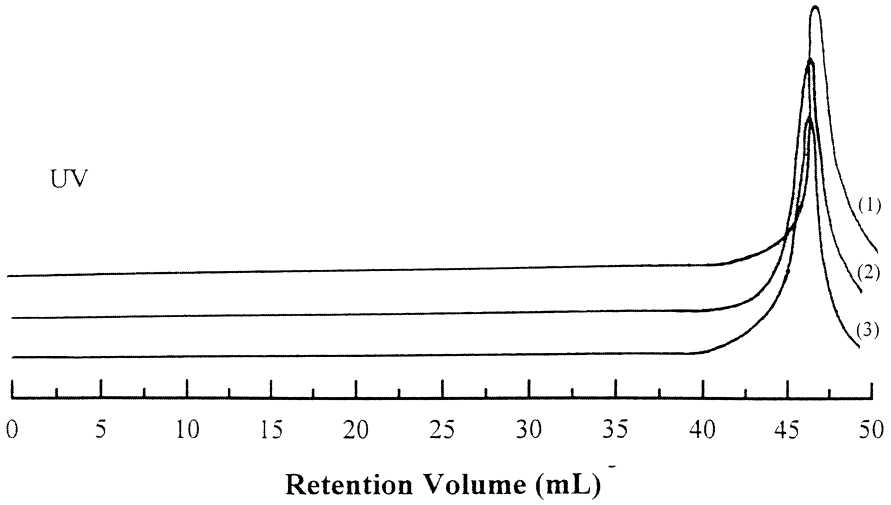
Sulfadiazine (Shanghai Sanwei Pharmaceutic Manufacturing Co.) was recrystallized twice from DMSO/ethanol (1/4 v/v); ethylene oxide (EO) (Shanghai Gaoqiao Chemical Engineering Factory) was dried for 2 days with calcium hydride, then distilled. Benzaldehyde (Jiangsu Yixing Auxiliary Factory) dried for 2 days with calcium hydride and distilled under reduced pressure in a stream of nitrogen. Bis(2-chloroethyl)amine hydrochloride was prepared by the reaction of diethanolamine with thionyl chloride according to the literature procedure [6]. Fuming sulfuric acid (50%) and diatomite were used as received. All solvents were purified by conventional methods.



Scheme 2.

2.2. Synthesis of PEO with 4-(N-benzylimido)-N-(2-pyrimidinyl) benzene sulfonamide (BPBS) and hydroxyl end groups (**b**)

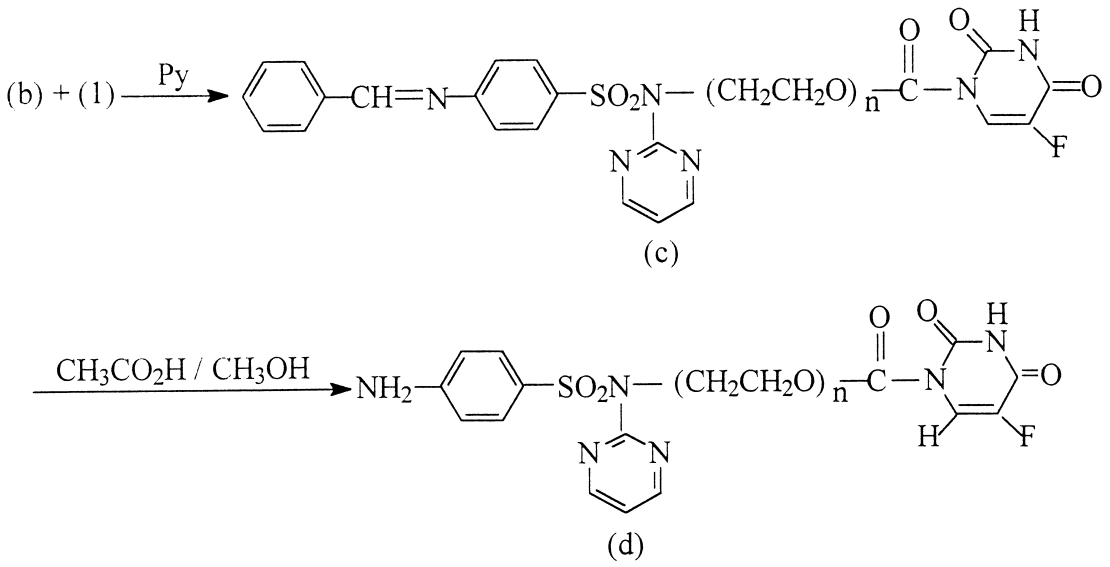
Ring-opening polymerization of EO was carried out in bulk and using sodium sulfadiazine as initiator and methanol as terminant, the whole process and the preparation of polymer (**b**) have been reported previously [7], and could be described briefly as follows (Scheme 2). The polymerized products with much narrow molecular weight distribution was confirmed by GPC (Fig. 1). All samples are slices crystals with a light yellow colour.



GPC spectrum of polymer (a), (b) and (c)

(1)Mn=1900,Mw/Mn=1.04;(2)Mn=4800,Mw/Mn=1.04; (3)Mn=10,000,Mw/Mn=1.10

Fig. 1. GPC diagrams of polymer (**a**) [(1):M_n = 1900; (2): 4800; (3): 10,000].



Scheme 3.

2.3. Synthesis of PEO with 4-amino-N-(2-pyrimidinyl) benzene sulfonamide and 5-Fu end groups (*d*)

The synthesis of desirable polymer (**d**) was divided into three steps as shown in Scheme 3. The first step is the preparation of 1-chloroformyl-5-fluorouracil (**1**): to a 500 ml three-neck flask containing 2.6 g (0.02 mol) of 5-Fu in 50 ml pyridine 4.0 g (0.04 mol) of phosgene in 40 ml toluene was added dropwise under stirring and cooled in an ice bath for 1 h, and then the mixture was stirred at room temperature for another 1 h. The excessive phosgene was driven away by a stream of nitrogen, the remainder (**1**) kept intact for the next reaction. The phosgene was synthesized according to the literature procedure [8]

The second is the synthesis of (**c**), and all three samples (M_n: 1900; 4800 and 10,000) are prepared using the same procedure which is described as follows: to above-mentioned three-neck flask 20.0 g (1.05×10^{-2} mol) of (**b**) ($M_n = 1900$) in 60 ml pyridine was added dropwise under stirring and cooled in an ice bath for 2 h, then the mixture was stirred at room temperature for 10 h. After the mixture was filtered and concentrated, the yellow solid was precipitated with ether. The polymer (**c**) could be purified by dissolution/precipitation with chloroform/ether in the yield of 75%. IR (cm^{-1}): 1135, 1105 and 1041(—CH_2OCH_2—), 1248(C—F), 1640(>C=N—), 1741(—COO—), 1712 and 1688 (—CO—N< for 5-Fu); ^1H NMR(δ: ppm): 7.50 (—CH=CF— for 5-Fu), 3.65 (—CH_2CH_2O—).

The last step is the hydrolysis of (**c**): 10.0 g (**c**) was dissolved in 60 ml of the mixture of acetic acid and methanol (1:2 v/v) under stirring and acidolized 24 h, and then precipitated with ether. The product (**d**) could also be purified by dissolution/precipitation with chloroform/ether in the yield of 85%. GPC confirmed that no chain cleavage of PEO in whole procedure was found.

For polymers (**c**) and (**d**), DSC showed that the sample with molecular weight 1900 is amorphous and the samples of 4800 and 10,000 are crystalline.

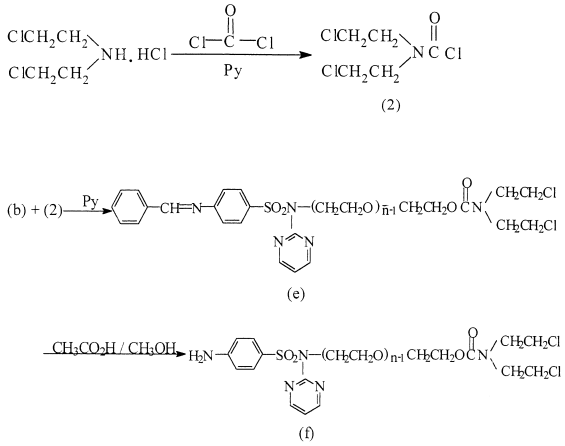
2.4. Synthesis of PEO with 4-amino-N-(2-pyrimidinyl) benzene sulfonamide and N-Mu end groups (*f*)

The synthesis procedure of objective polymer (**f**), as Scheme 4 indicated, is similar to the preparation of polymer (**d**). It is described in (Scheme 4).

The yield of purified polymer (**e**) is 70%. IR (cm^{-1}): 667 (C—Cl), 1149, 1114 and 1061 (—CH_2OCH_2—), 1639 (>C=N—), 1737 (—COO); ^1H NMR(δ: ppm): 3.65 (—CH_2CH_2O), 4.08 (—CH_2— connected with ternary amine), 4.28 (—CH_2— connected with —Cl).

The polymer (**f**) was obtained by hydrolysis of (**e**) with the yield of 85% using the same method to prepare polymer (**d**).

DSC also showed for polymers (**e**) and (**f**), the sample with molecular weight 1900 is amorphous and the samples of molecular weight 4800 and 10,000 are crystalline.



Scheme 4.

2.5. Measurement and instruments

IR spectra were recorded by a Nicolet Magna-550 FTIR Spectrometer. ^{1}H-NMR spectra were scanned with a Bruker MSL-300 NMR Spectrometer with TMS as the internal standard and $CDCl_3$ as solvent. The molecular weight and molecular weight distribution were measured by a Shimadzu LC-3A high performance liquid chromatography equipped with microcomputer, using the monodistributed polystyrene as the standard sample and chloroform as the eluent. The UV spectra were obtained on a 756MC spectrophotometer (Shanghai Analytical Instruments Factory). DSC measurement was performed by a Perkin–Elmer TAC7/DX in nitrogen atmosphere with the heating rate of 10°C/min.

3. Results and discussion

3.1. Synthesis of objective polymer

Polymers (**d**) and (**f**) were synthesized by means of esterifying reaction of acyl chloride derivative of 5-Fu and N-Mu with polymer (**b**) in pyridine. All the objective polymers were structurally confirmed by ^{1}H-NMR and IR shown in Figs. 2–5.

For instance, the IR absorption peaks, such as 3059,

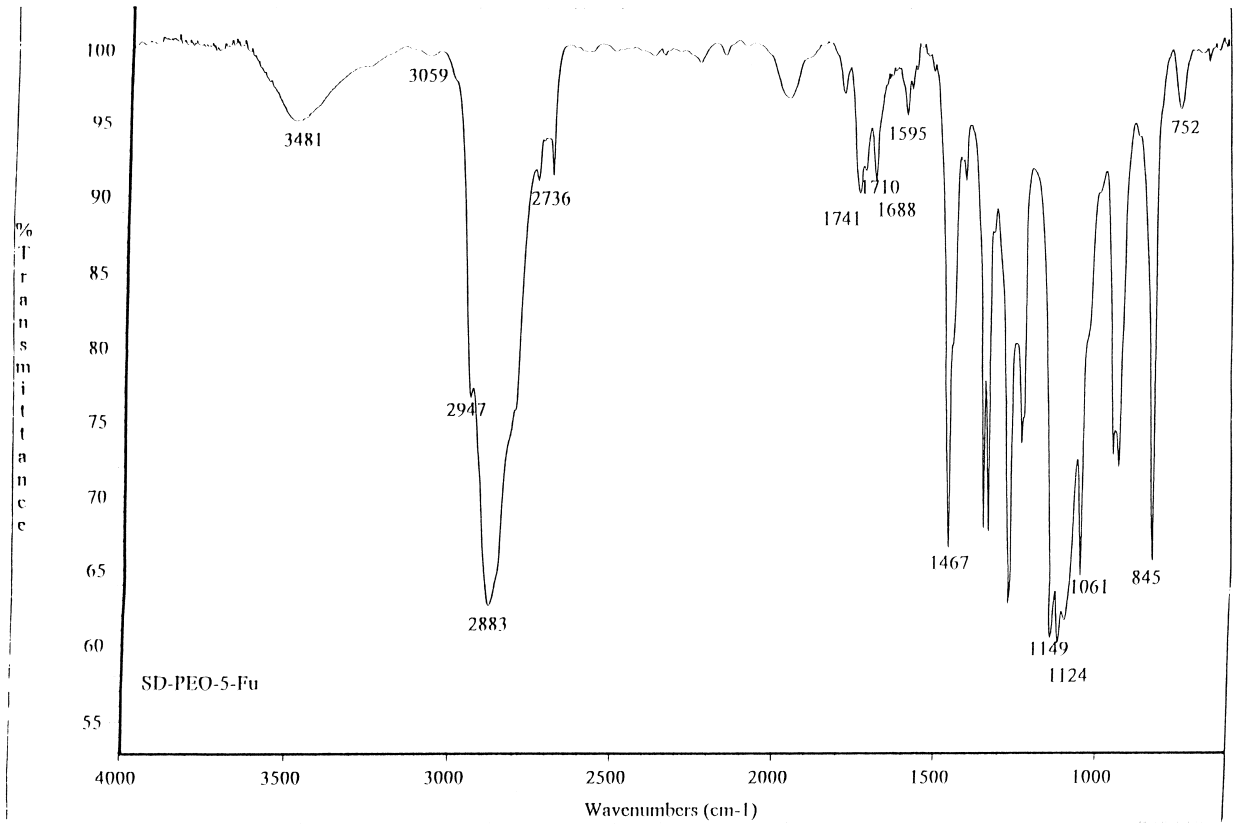


Fig. 2. IR spectrum of polymer (**d**).

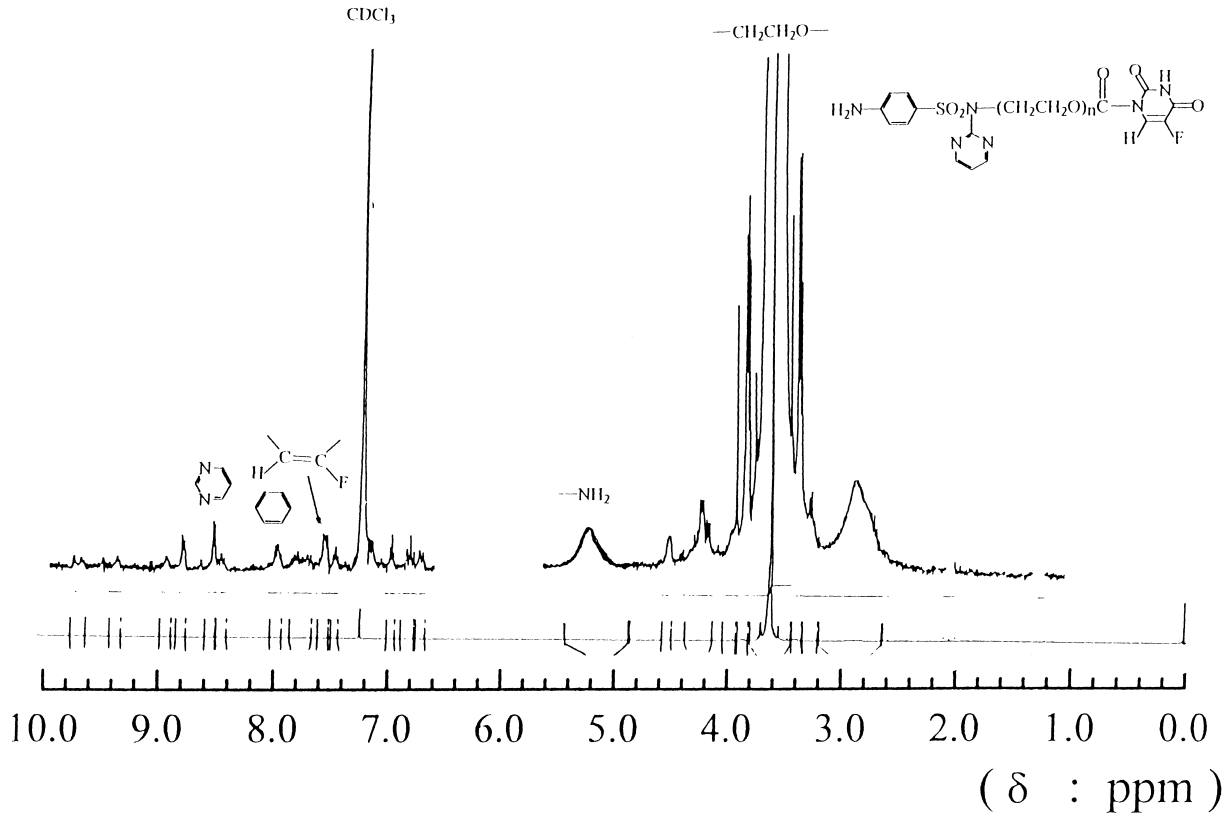


Fig. 3. ^{1}H NMR spectrum of polymer (**d**).

1595, 1467 (phenyl and pyrimidinyl rings), 1741 (—COO—), 1710, 1688 (—CON— of 5-Fu), 1247 (C—F), 1124, 1100, 1061 cm^{-1} (—CH_2—O—CH_2—) in Fig. 2 and the proton chemical shift of 3.65 (—CH_2CH_2O—), 7.60 (—CH=C— of 5-Fu) and 7.2–8.8 ppm (phenyl and pyrimidinyl rings) in Fig. 3 evidenced existence of polymer (**d**).

In the acylation of 5-Fu, however, both 1—NH— and 3—NH— of acylimino of 5-Fu have reaction activity, but only 1—NH— acylation derivative of 5-Fu was obtained by controlling the proper feed ratio of 5-Fu and phosgene in the reaction [9]. The reaction intermediate (**1**) was very reactive, it may absorb the moisture and be hydrolyzed when it exposed in the air. Thus it is generally used to react with polymer (**b**) directly and could be easily removed from the final products by filtration due to its insolubility in chloroform.

Polymer (**f**) was synthesized early by the reaction of amine end group of polyethylene oxide with sulfadiazine at another end to N,N-dichloroethyl maleinamic acid (DCEMA) in the presence of dicyclohexylcarbodiimide. However this route is unsuccessful due to the instability of DECMA in which, the group of chloroethyl was rearranged to corresponding ternary ammonium salt with three-membered ring [10].

The present synthesis route for polymer (**f**) was quite successful. Figs. 4 and 5 showed the all structural information, such as IR absorption peaks of 3069, 1604, 1467 (phenyl and pyrimidinyl rings), 1737 (—COO—), 1648 (—NH_2), 1144, 1105, 1051 (—CH_2CH_2O—), 667 (C—Cl) cm^{-1} and proton chemical shift of 7.20–8.90 (phenyl and pyrimidinyl rings), 5.23–5.26 (—NH_2 conjugated with benzene ring and disappeared after exchanging with D_2O), 4.24 (—CH_2— connected with —Cl), 4.05 (—CH_2— connected with ternary amine), 3.65 (—CH_2CH_2O—) ppm, that is identical with the formula (**f**).

*3.2. Determination of the content of 5-Fu and N-Mu in polymer (**d**) and (**f**)*

The contents of 5-Fu in the polymer (**d**) was quite difficult to determine using the common measurement means as IR and NMR because of the smaller weight fraction of end group in the

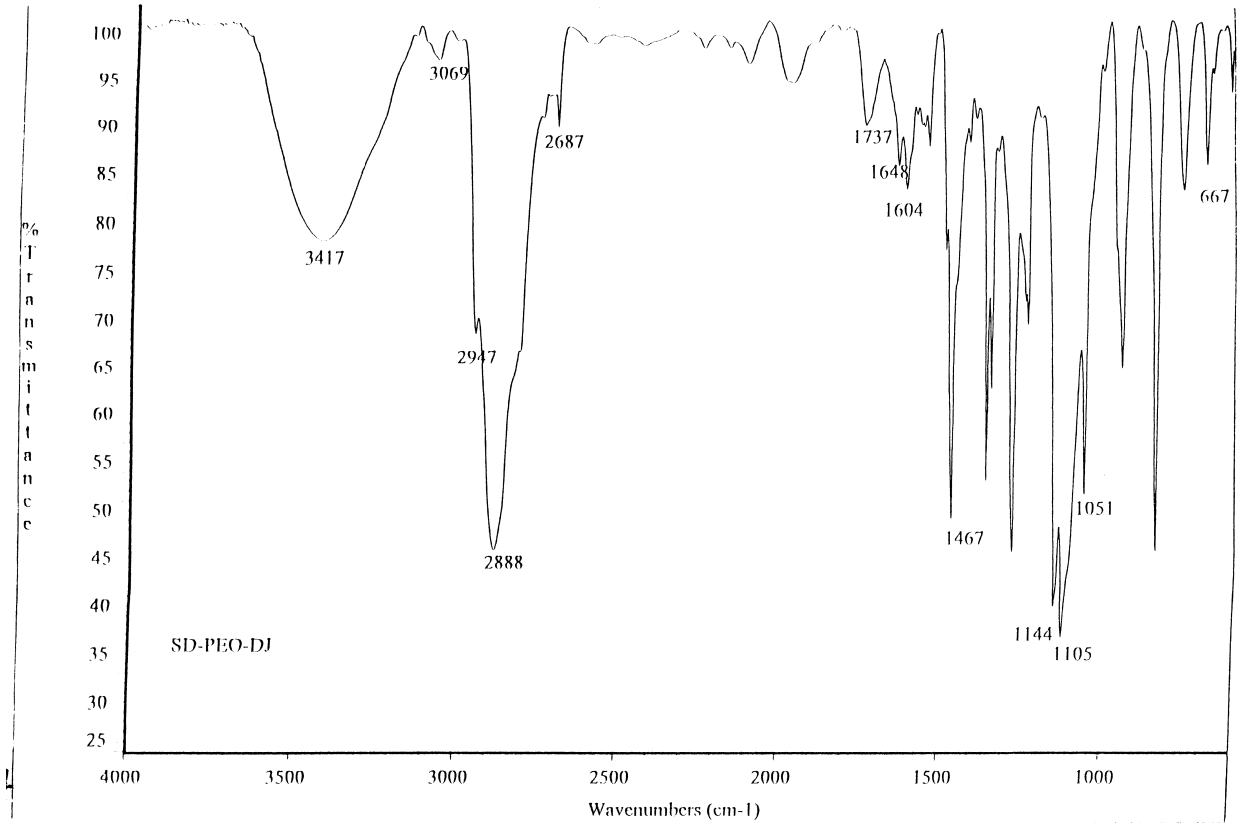


Fig. 4. IR spectrum of polymer (**f**).

whole PEO polymer chains. Zhuo et al. [11] reported that amidating bonds in the derivatives of 5-Fu could be completely hydrolyzed by refluxing in 5 N NaOH aqueous solution for 10 h to give free 5-Fu, so the content of 5-Fu could be determined by UV spectra. However, it was found that in our system both SD and 5-Fu for polymer (**d**) have the absorption at 220 ($\pi \rightarrow \pi^*$) and 280 nm ($n \rightarrow \pi^*$) shown in Fig. 6, so 5-Fu contents could only be derived from the UV spectra of the mixture of SD and 5-Fu contingent upon no interaction of SD to 5-Fu in the solution. Fortunately, it was confirmed that the absorption of the mixture of SD and 5-Fu with a certain concentration is finely equal to the sum of the absorption of corresponding SD and 5-Fu with the same concentration, so the 5-Fu contents would be proportional to the absorption of the mixture when the concentration of SD in the mixture is fixed. Thus, 5-Fu contents could be obtained by comparing the standard curve of absorbance vs concentration of mixture of SD and 5-Fu. Table 1 listed the contents of 5-Fu in polymer (**d**) with different molecular weights.

Table 1
5-Fu and N-Mu contents in polymer (**d**)

		Contents (mol %)		Weight (mg) per gram of polymer (**d**)	
Molecular weight (M_n)	Molecular weight distribution	5-Fu	N-Mu	5-Fu	N-Mu
1900	1.04	58.0	41.3	35.0	28.3
4800	1.04	55.2	40.0	13.9	11.4
10,000	1.10	50.5	38.8	6.46	5.45

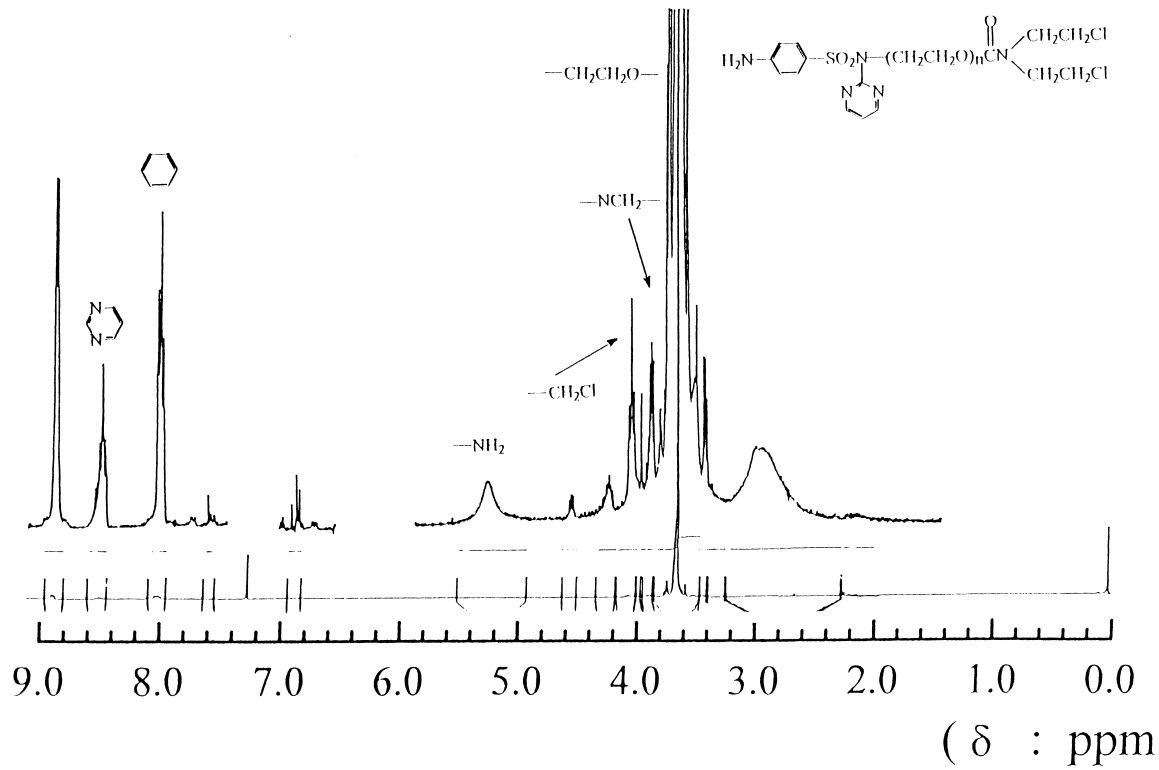


Fig. 5. ^{1}H NMR spectrum of polymer (**f**).

The content of N-Mu in polymer (**f**) was determined by titrating the silver nitrate solution with standard ammonium sulfocyanate solution after the polymer (**f**) was perfectly hydrolyzed in the 1 N NaOH and acid-treated with nitric acid, and then excessive silver nitrate solution was added. The N-Mu content shown in Table 1 could be calculated by following the formula:

$$P_{\mathrm{N-Mu}}\,(\mathrm{mg/g\ polymer}) = \frac{M_{\mathrm{n-Mu}} \times (V_1C_1 - V_2C_2)}{W_{\mathrm{polymer}}}.$$

here P is mustard contents in polymer, $M_{\mathrm{N-Mu}}$ is the molecular weight of mustard, V_1, V_2 are the total volume of $AgNO_3$ solution and consumed volume of NH_4SCN solution, and C_1, C_2 as the concentrations of $AgNO_3$ and NH_4SCN respectively.

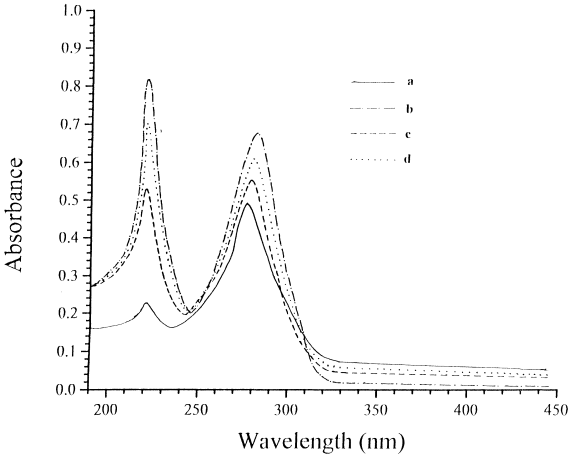


Fig. 6. UV spectra of SD (a), 5-Fu (b), the mixture of SD and 5-Fu (c) and polymer (d) (concentration: 1×10^{-4} mol/l, solvent: 1.0 N NaOH).

Acknowledgements

We appreciate the financial support from the Natural Science Foundation of China and the Shanghai Development Foundation of Science and Technology.

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