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SYNTHESIS OF MACROMOLECULAR PRODRUG OF 5-FLOUROURACIL

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= 국문초록 =

1-(Hydroxymethyl)-5-fluorouracil를 합성하고, 이것과 methacrylic anhydride를 이용하여 1-(methacryloyloxymethyl)-5-fluorouracil(MAOFU)를 합성하고, 또 이것을 cyclohexanone 용매 하에서 AIBN으로 60℃에서 methylmethacrylate와 공중합시켰다. Poly(MAOFU-co-MMA)의 흡광계수를 UV sphectrophotometer로 용매 THF를 사용하여 267.3nm의 파장에서 측정하였다. MAOFU와 MMA의 라디칼 공중합비를 Kelen-Tudos법으로 얻었다. 에탄올과 물을 용매로 하여 37℃에서 MAOFU와 poly(MAOFU)의 분해속도를 측정하였다.

INTRODUCTION

In recent years the use of the synthetic polymers as polymeric drug delivery systems has received increasing attention, several symposia being organized to discuss the current state-in-art of research into polymeric durgs, controlled release of bioactive materials,[9,14,23,24] applications of immobilized enzymes and proteins, and the general biomedical applications of polymers[17,21] The term "Polymeric drug"includes both polymers that contain a drug or chemotherapeutic unit as part of polymer backbone[2,19] and polymers which include the active units as pendant groups or as a terminal group on the polymer chain In the latter categories the designation "Polymeric drug carrier[5,6,7,20]" is more appropriate as the polymeric constituent serves simply as a drug carrier and is usually chosen because of its biological inactivity

Since the general idea of polymeric drugs was proposed by Ringsdorf[22], research on the design and preparation of them has become very active Some of the synthetic analogs of nucleic acids have already been found to have pharmaceutical activity Further, since some of the nucleic acid base derivatives such as 5-fluorouracil[18] and mercaptopurine[15], are highly pharmaceutically active, polymers

having such nucleic acid base derivatives have also become important as polymeric drugs The results of the present study suggest that N-acyloxymethylation is a potentially useful approach to obtain prodrugs of 5-fluorouracil The usefulness of this approach[1] which in the past has also been applied to various other NH-acidic drugs stems from the fact that by varying the acyl portion of the derivatives it is possible to control the rate of regeneration of drug. Some 1-acyloxymethyl derivatives of 5-fluorouracil have recently been shown to possess strong antitumor activity in mice[10] In view of the present results this activity may certainly be due to 5-fluorouracil formed upon enzymatic hydrolysis in vivo The present study deals with the synthesis of 1-(methacryloyloxymethyl)-5-fluorouracil similar to 1-acetoxymethyl-5-fluorouracil and its polymer

EXPERIMENTAL

Materials

5-Fluorouracil, aldehyde, paraformaldehyde, methanol, ethyl acetate, petroleum ether, acetic anhydride, sodium sulfate, benzene, ethanol, propionyl chloride, and methacrylic anhydride were purchased from Fluca AG, Switzerland and were used as received Methylmethacrylate was washed twice with aq. 5%NaOH(to remove inhibitor such as hydroquinone)and twice with water, dried with $MgSO_4$, and distilled from CaH_2 under nitrogen at reduced pressure The distillate was stored at low temperature and redistilled before use

Instruments

H-NMR spectra were run on a Varian 360A instrument using tetramethylsilane as zero reference. Ultraviolet spectral measurements were performed with a Simadzu 200A spectrophotometer equipped with a thermostatically controllled cell compartment, using 1cm quartz cells Infrared spectra were recorded on a Ferkin Elmer 1330 spectrophotometer

Synthesis of Monomer

1,3-Bis(acetoxymethyl)-5-fluorouracil

5-Fluorouracil(1.3g, 0 01mol)was dissolved in 4ml of a 37% aqueous solution of formaldehyde with pH being adjusted to 7 0 with sodium hydroxide. After 5h at room temperature the solution was lyophilized to give an oily residue to 1,3-dihydroxymethy1-5-fluorouracil This was subsequently dissolved in 10ml of dry pyridine and 3ml(0 03mole)of acetic anhydride was added while stirring After standing at room temperature for 2h the reaction solution was added to 100ml of the water The mixture was extracted twice with ether and the extracts were washed with diluted hydrochloric acid and water. The ether phase was dried over anhydrous sodium sulfate and evaporated in vacuum to give the title compound It was recrystallized from ether-petroleum ether, yielding 0 62g, mp 105-106°C.(Lit[3] mp 105°C)

3-Acetoxymethy1-5-fluorouracil

3-Acetoxymethy1-5-fluorouracil was prepared by alkaline hydrolysis of 1,3-bis (acetoxymethyl)-5-fluorouracil(1 0g)was dissolved in a mixture of 35ml of methanol and 35ml at which time the title compound was formed in maximum yield. Hydrochloric acid (2M) was added to give a pH of 6 and the reaction solution was concentrated in vacuum,

dried over anhydrous sodium sulfate, and evaporated in vacuum After column chromatography(silica gel, eluents toluene containing 1% of acetic aid with increasing amount of ethyl acetate)of the residue pure compound was isolated and recrystallized from ethyl acetate-petroleum ether, yield 0 23g mp 158-159°C(Lit[3] mp 158°C)

Chloromethyl propionate

To a chloroform solution of propionyl chloride in an ice bath was added one equivalent of paraformaldehyde and catalytic amount of anhydrous zinc chloride The mixture was stirred in the ice bath for several hours and then allowed to room temperature The cloudy solution that resulted was diluted with an equal volume of petroleum ether and filtered The filtrate was concentrated at room temperature on a rotary evaporator The concentrate that resulted was fractionally distilled to give propionyl chloride The product structure was confirmed by H-NMR and IR spectrum

H-NMR δ 5 72(CO-CH-Cl), 2 39(H-C-C=0) IR 1740cm^{-1}(C=0 stretching), 1260cm^{-1}(C-Cl stretching)

1-Propionyloxymethyl-5-fluorouracil (POMFU)

5-Fu(6 50g 0 05mol)was dissolved in 50ml of dimethylacetamide, then triethylamine (15 18g, 0 15mol)was added to the solution, The mixture was treated dropwise with chloromethyl propionate(8 00g, 0 065mol)over 30min The reaction mixture was stirred for 2h, allowed to stand overnight and then filtered to remove the precipitated triethylamine hydrochloride Then, dimethylacetamide was distilled from the filtrate and the residue was applied to a column packed with silica gel Elution with mixtures(8 2 to 8 1 in mixing ratio)of benzene and ethyl acetate gave POMFU This product was further recrystallized from benzene to afford pure 1-propionyl oxymethyl-5-Fluorouracil(7 93g, 73 1%), mp 105-106°C The product structure was confirmed by H-NMR and IR spectrum IR (KBr) 3430cm^{-1}(NH stretching), 1720cm^{-1} (C=0 stretching), 1020cm^{-1}(C-0 stretching), 1120cm^{-1}(C-F stretching) NMR(DMSO-d_6) δ 1 2(t, CH_3), 4 2(1 $COCH_2$), 4 5(t N-CH_2-), 8 1(d, C-H)Fig 1(IR spectrum), Fig 2 (NMR spectrum)

1-Hydroxymethyl-5-Fluorouacil(HMFU)

100ml of methanol and 12ml of 6N HCl were added to 2 16g of 1-propionyl methyl-5-fluorouracil(10 mmol) After additional refluxing for 3hr, the methanol was distilled off under vacuum to provide a crystalline product Recrystallization from ethanol gave 1 12g(70%)of the product mp 141~142°C The product structure was confirmed by H-NMR and IR spectrum IR 3520-3250cm^{-1} (OH stretching), 1820cm^{-1}(C=H stretching) 1025cm^{-1}(C-O stretching) NMR 8 1(=C-H d), 4 7(N-CH-), 3 5(O-H) Fig 3(IR spectrum) Fig 4(NMR spectrum)

1-(2′-Methacryloyloxymethyl)-5-Fluorouracil(MAOFU)

Methacrylic anhydride (1 54g, 0 01 mole) and sulfur as polymerization inhibitor were added to HMFU (1 60g, 0 01 mole)and stirred for 3hr at 90~100°C to give a clear solution The reaction mixture was cooled to 0°C to precipitate the product. The product was filtered and washed thoroughly with diethyl ether and recrystallized from ethanol Yield 1 19g(52 2%) mp 132~133°C The product structure was confirmed by H-NMR and IR spectrum

IR 3040cm^{-1}(=CH stretching), 1745cm^{-1}(-

C=0 stretching), 1660cm^{-1}(C=C stretching) 1022cm(C−0 stretching), 1120cm^{-1}(C−F stretching)
NMR(DMSO-d_6). δ 1 9(-CH_3), 4 8(N-CH_2), 5.7(CH_2, d), 8.5(=C-H) Fig 5(IR spectrum), Fig.6(NMR spectrum)

Homopolymerization

Monomer MAOFU(0 01M/1)was dissolved in cyclohexanone and AIBN(4 9x10M^{-4}/1) was added The solution was placed in a polymerization tube and degassed under vacuum. The polymerization was conducted at 60°C for 24h. The solution was poured into excess n-hexane with stirring and the precipitated product was filtered. The product was dried under at room temperature.

Copolymerization of MAOFU and MMA

MAOFU(0 01M/1), AIBN(4.9x10M^{-4}7/1) and dry cyclohexanone were charged into a polymerization tube The solution was degassed with three freeze-thaw cycles. The tube was then sealed and heated at 60°C for 48h. The solution was poured into excess n-hexane with vigorous stirring The precipitate was filtered, dried in vacuum at room temperature for 48h, and weighed. Fig 7

Analysis of copolymers

Poly(MAOFU), poly(MMA), and poly (MAOFU-co-MMA) were dissolved in tetrahydrofuran. The copolymer composition was determined from UV spectrum at 267.3nm

Hydrolysis of MAOFU and homo(MAOFU)

The rates of formation of hydroxymethyl-5-fluorouracil were studied under pseudo-first order conditions, using ethanol-water(1 · 1) mixture. The solution was kept in water bath at 37°C

RESULT and DISCUSSION

Determination of monomer reactivity ratios

When two monomers such as M_1 and M_2 copolymerize, there are two kinds of free radicals which form the growing ends of the polymer molecules[15] Each kind of radical may react with either kind of monomer molecule

$$M_1 + M_1 \xrightarrow{k_{11}} M_1 \quad (1)$$

$$M_1 + M_2 \xrightarrow{k_{12}} M_2 \quad (2)$$

$$M_2 + M_2 \xrightarrow{k_{22}} M_2 \quad (3)$$

$$M_2 + M_1 \xrightarrow{k_{21}} M_1 \quad (4)$$

A kinetic analysis of copolymerization behavior results in the copolymer composition equation

$$\frac{d[M_1]}{d[M_2]} = \frac{[M_1]}{[M_2]} \frac{r_1[M_1]+[M_2]}{[M_2]+r_2[M_2]} \quad \cdots\cdots (5)$$

where the rectivity ratios are defined as:

$$r_1 = \frac{K_{11}}{K_{12}}, \quad r_2 = \frac{k_{22}}{k_{21}},$$

where the reactivity ratios are defined as $[M_1]$ and $[M_2]$ are the mole composition of the monomer feed, and $d[M_1]$ and $d[M_2]$ the mole composition of the copolymer formed instantaneously. $[M_1]$ will be used to represent MAOFU and $[M_2]$ will be MMA
Various methods have been proprosed to obtain the "best" r_1 and r_2 pair
(1) According to the Fineman and Ross method[4], equation (5) is rewritten as

$$\frac{X(Y-1)}{Y} = r_1 \frac{X^2}{Y} - r_2$$

where $X=[M_1]/[M_2]$ and $Y=m_1/m_2$
A plot of X^2/Y gives a straight line whose slope is r_1 and intercept r_2

(2) For the Mayo and Lewis method (or the intersection method[16]). equation (5)is rewritten

$$r_2=\frac{[M_1]}{[M_2]}\left[\frac{m_2}{m_1}\left(1+\frac{[M_1]}{[M_2]}r_1\right)-1\right]=ar_1+b$$

where a and b are parameters computable from $[M_1]$, $[M_2]$, ml and m_2 Plotting r_2 versus rl produces a straight line Each experiment produces a straight line, the intersection of which provides r_1 and r_2

(3) Kelen and Tudos[12] showed that the disadvantage of the Fineman and Ross method can be ablished by using the following graphically evaluable linear equation

$$\frac{G}{\alpha+F}=(r_1+\frac{r_2}{\alpha})\frac{F}{\alpha+F}-\frac{r_2}{\alpha} \quad \cdots\cdots \quad \cdots\cdots \cdot (6)$$

where α is an arbitrary positive constant By introducing

$$\eta=\frac{G}{\alpha+F} \text{ and } \xi=\frac{F}{\alpha+F} \quad \cdots\cdots\cdots\cdots (7)$$

equation (6) can be written

$$\eta=(r_1+\frac{r_2}{\alpha})\xi-\frac{r_2}{\alpha} \qquad (0<\xi<1)$$

Plotting against, a straight line is obtained which on extrapolation to $\xi=0$ and $\xi=1$ gives $-r_2/\alpha$ and r_1(as interceps), respectively. The experimental data can be distributed symmetrically along the line when α is obtained by $\alpha=\sqrt{Fm\cdot Fn}$, where Fm and F_M are the lowest and highest F values, respectively To determine r_1 and r_2 values, the data from Table 1 was used to draw Kelen-Tudős plot

From the extinction coefficient(E*)of the copolymer samples at 267 3nm, the weight fraction of poly(MAOFU), x, could then be calculated from the following equation E*= 0 0385+1 16x ……(8)

$d[M_1]/d[M_2]$ in equation(5)correspond to x in equation (8) The reactivity ratios for the copolymerization of MAOFU(r_1) and MMA (r_2)are 0 27 and 1 54 respectively This shows that MMA radicals are more stable than MAOFU radical and that this reactivity is due to steric hinderance offered by pendant group of MAOFU

Hydrolysis of MAOFU and poly(MAOFU)

All rate studies were carried out at 37°C The reactions were generally followed by direct UV-spectrophotometry by recording the absorbance changes accompaning the hydrolysis at 270nm where the absorption of MAOFU and hydroxymethyl differed maximally Pseudo-first-order rate constant[5] was calculated from the slopes of linear plots of log(A∞-At)against time where A∞ and At are the absorbance readings at infinity and time, t, respectively K for MAOFU is 6 42x 10^4/sec K for poly(MAOFU)is 7 4x10^4/sec

Table 1. Determination of monomer reactivity ratios for the copolymerization of MAOFU and MMA

Exp No.	$X=\frac{M1}{M2}$	$Y=\frac{m1}{m2}$	X^2	Y−1	$F=\frac{X^2}{Y}$	$G=\frac{X(Y-1)}{Y}$	$\alpha+F$	$7=\frac{G}{\alpha+F}$	$\xi=\frac{F}{\alpha+F}$
1	0 47	0. 27	0. 22	−0. 73	0. 1	−1. 27	2. 79	−0. 46	0. 29
2	0. 71	0. 37	0. 50	−0 63	1. 38	−1. 11	3 36	−0 33	0. 41
3	1. 0	0. 46	1. 0	−0. 54	2. 17	−1. 17	4. 15	−0. 28	0. 52
4	1 50	0 71	2. 25	−0. 29	3 17	−0. 64	5. 15	−0. 12	0. 62
5	2. 15	0. 94	4 54	−0. 06	4. 83	−0. 14	6 81	−0 02	0. 71

CONCLUSION

MAOFU was synthesized by reaction of 1-(hydroxymethyl)-5-fluorouracil with methacryl anhydride. First, the polymerization of MAOFU and copolymerization of MAOFU with MMA were carried out in cyclohexane at 60°C, using AIBN as an initiator. The specific absoptivity of poly (MAOFU-co-MMA) was measured by UV spectrophotometric means at 267 3nm in THF. The calibration curve was obtained changing concentration ratios of both homopolymers and the following equation was derived

$k=0.0385+1\ 16x$

The monomer reactivity ratios, r1 and r2 were determined by Kelen-Tüdős method. MAOFU(M1)-MMA(M2). $r1=0\ 269$ and $r2=1.54$.

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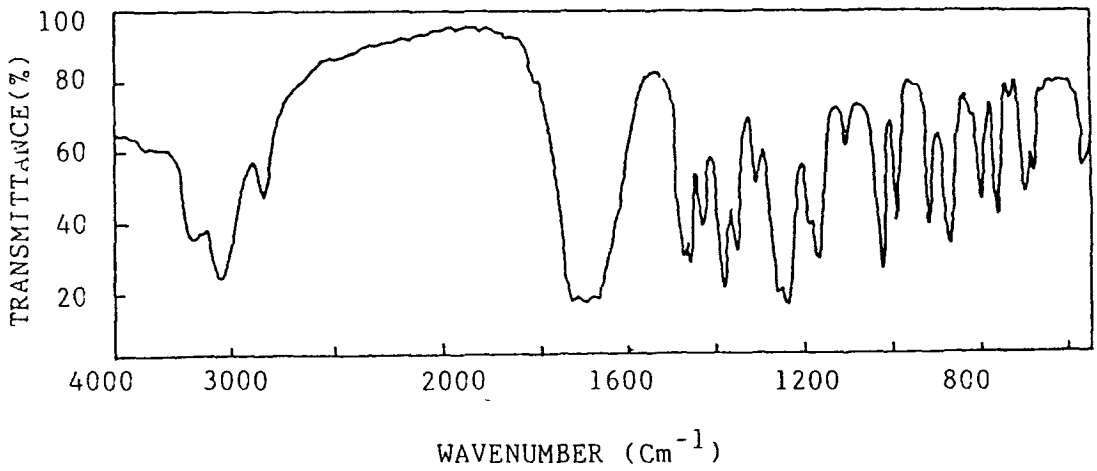

Fig. 1. IR sepctrum of POMFU (Solid Phase, KBr)

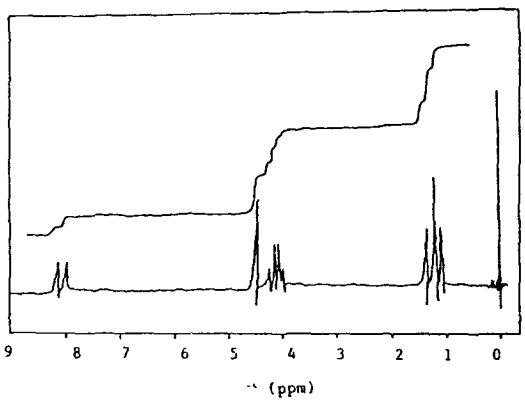

Fig. 2. NMR spectrum of POMFU (Solid Phase, KBr)

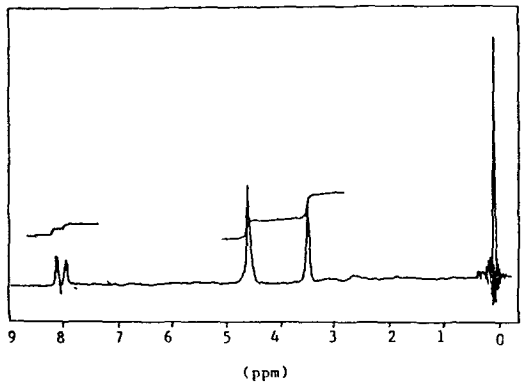

Fig. 4. NMR spectrum of HMFU (DMSO-d6)

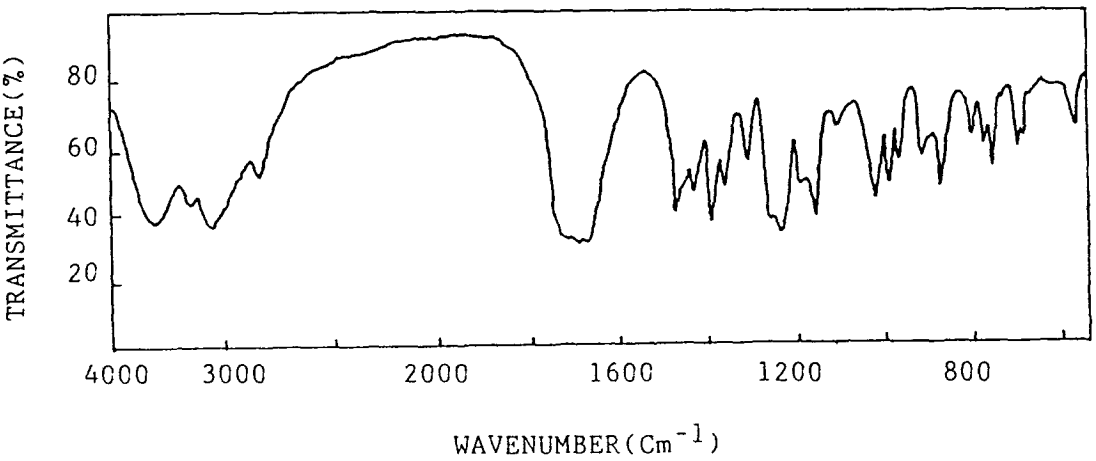

Fig. 3. IR spectrum of HMFU (Solid Phase, KBr)

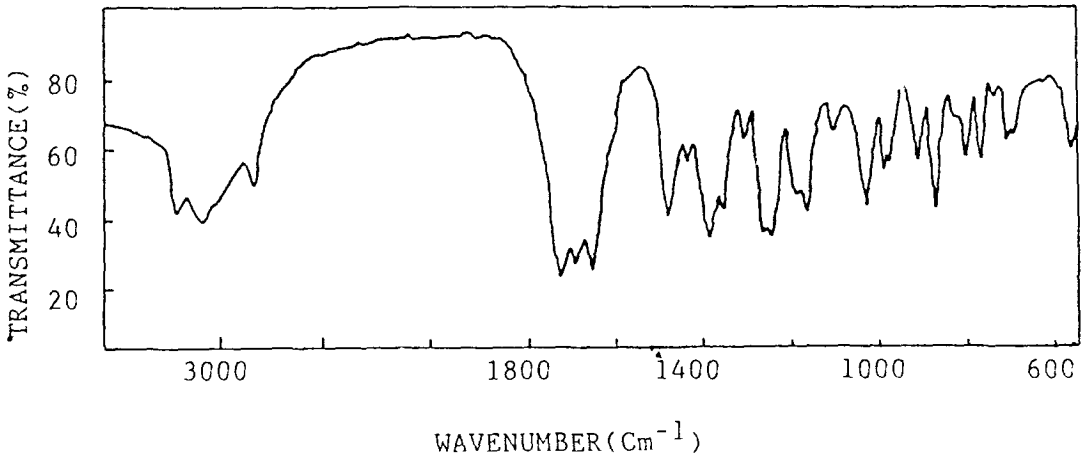

Fig. 5. IR spectrum of MAOFU(Solid Phase, KBr)

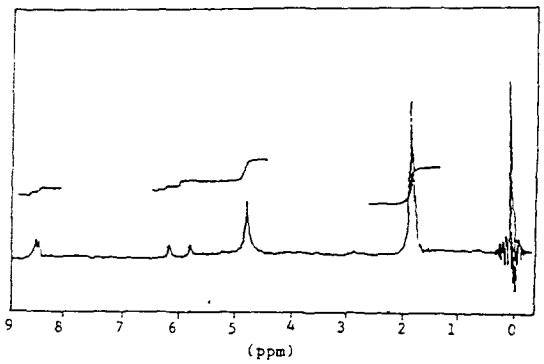

Fig. 6. NMR spectrum of MAOFU (DMSO-d6)

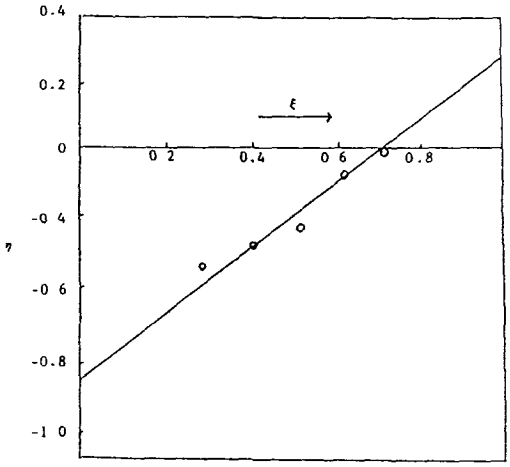

Fig. 8. Kelen-Tudos plot for the copolymerization of MAOFU and MMA at 60°C

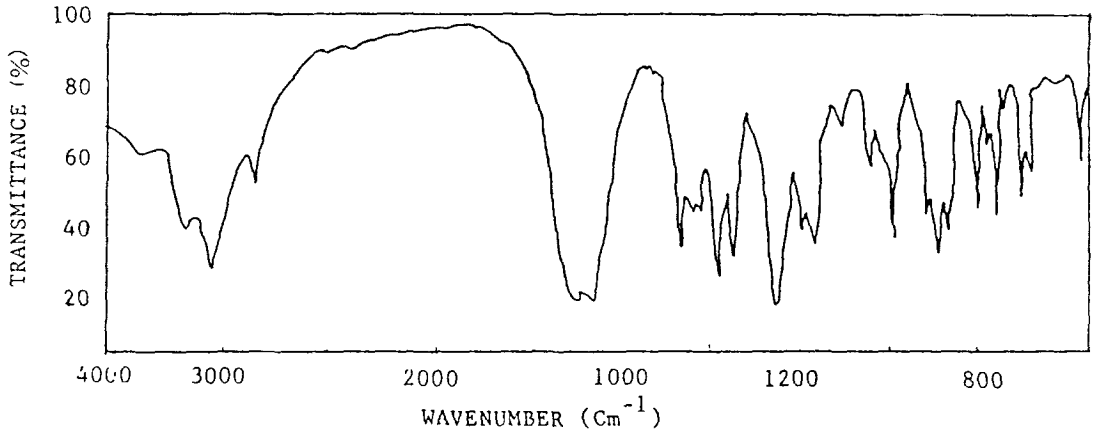

Fig. 7. IR spectrum of poly (MAOFU-co-MMA)(Solid Phase, KBr)