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SYNTHESIS OF POLYMERS CONTAINING 2-HYDROXYEHYL-5-FLUOROURACIL

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= 국문초록 =

2-chloroethylmethacrylate를 합성하고, 이것과 5-Fluorouracil을 이용하여 1-(methacryloyloxyethyl)-5-fluorouracil (MAOEFU)을 합성하고, 또 이것을 ethanol 용매 하에서 AIBN으로 60℃에서 methacrylic acid와 공중합시켰다. Poly(MAOEFU-co-MA)의 흡광계수를 UV sphectrophotometer를 사용하여 THF속에서 268.5nm의 파장을 이용하여 측정하였다. MAOEFU와 MA의 라디칼 공중합에 대한 공중합비를 Kelen-Tudos법을 이용하여 얻었다. 물과 에탄올을 용매로 하여 37℃에서 MAOEFU와 poly(MAOEFU)의 분해속도를 측정하였다.

INTRODUCTION

The use of polymeric materials in biomedical applications such as artificial skin[3,22], breast implants[19], soft contact lenses[1], urethral catheters[15], and carriers for therapeutic agents[4,14,20,21] has been known for many years Recently, polymers have found a new use in medicine in the field of chemotherapy[2] This use has been either in the area of slow or controlled release polymers[16], in which a drug is embedded in a polymeric matrix, or in the area of chemotherapeutic polymers or polymeric drugs In this latter case, the therapeutic agent is attached more or less permanently to a polymer chain A chemotherapeutic polymer contains the drug unit fixed on or in the polymer chain These could be prepared by direct polymerization of a related monomeric species or by modifying a preformed polymer The basis hope in these polymeric drugs is to (1) increase the specificity of action, (2) reduce toxic side effects and (3) permit a longer duration of drug action The fact that polymeric materials can have powerful physiological effects is well established

As far as the type of bond between the drug and the matrix is concerned, ester bonds or iminic bonds are usually prefered because as a rule, they are easily cleaved in the body fluids. Ferruti's[7,8,9] work on functional drug-binding matrices was firstly concerned with the synthesis of polymeric imidazolides and benzo triazolides. To this purpose, a series of high molecular weight polymers with a polyvinylic backbone have been synthesized. Other activated derivatives of polymeric acids have been synthesized by Ringsdorf[12] and his group. The present paper deals with the synthesis of methacryloyl type polymers with 2-hydroxyethyl-5-fluorouracil which is pharmaceutically active[10].

EXPERIMENTAL

A. Material

5-Fluorouracil, hexamethyldisilazane, 2-bromoethyl acetate, 2-chloroethanol, DMF, and methacryloyl chloride were purchased from Aldrich Company and was used as received. Methacrylic acid was dried with anhydrous calsium chloride and distilled under vacuum [b p 72°C(14mmHg)]. Triethylamine was dried with Linde type 4A molecular sieve and then distilled under reduced pressure [b p 14°C(30mmHg)]. Buffer substances and all other chemicals or solvents used were of reagent grade.

B. Instruments

Infrared spectrum was taken on a Perkin-Elmer 1330 spectrophotometer using potassium bromide pellet. H-NMR spectra were recorded on a Varian 360A instrument using tetramethylsilane as zero reference. Ultraviolet spectral measurements were performed with a Simadzu 200A spectrophotometer equipped with a thermostatically controlled cell compartment, using 1cm quarz cells.

C. Preparation of monomer and polymers

1. Synthesis of 2, 4-bis(trimethylsilyloxy)-5-fluoropyrimidine.

A suspension of 6.5g(0.05mole) of 5-fluorouracil in 25ml (0.12mol)of hexamethyldisilazane was refluxed for 3h when a clear solution resulted. Distillation at atmospheric pressure removed some material boiling at 85-96°C. The residue was then distilled at 114-116°C/14mmHg to give the product (Lit[6]. 115°C/14mmHg)

2. Synthesis of 1-(2′-hydroxyethyl)-5-fluorouracil

To 2.7g(10mmol) of 2, 4-bis(trimethylsilyloxy)-5-fluoropyrimidine was added 3.4g(20mmol) of 2-bromoethyl acetate, and the solution was stirred at 60°C for 10 days. 100ml of methanol and 100ml of 6N HCl were added to the reaction mixture. After additional refluxing for 1h, the methanol was distilled off under vacuum to provide a crystalline product. Recrystallization from ethanol gave 0.54g(31%) of the product(colorless cubes, mp158-159°C). The UV and NMR date agreed with those in the literature[10].
NMR: δ 2.6(CH), 4.2(NCH CH-), 7.8(H-6)
UV: 0.1M HCl 274nm $\log \varepsilon = 3.94$ 0.1M NaOH 272nm $\log \varepsilon = 3.80$

3. Synthesis of 2-chloroethyl methacrylate

To a three-necked round bottom flask equipped with a reflux condenser and a stirrer was added 2-chloroethanol of 1ml(0.012mol) and N, N-dimethylformamide of 30ml and the mixture was dissolved at room temperature. Then, triethylamine of 0.01mol was added to the reaction mixture. After refluxing for 2h with stirring, the reaction mixture was allowed to stand at room temperature. It was then treated dropwise with methacryloyl chloride of 0 01mol over 1 5h(ice bath cooling)and filtered to remove triethylamine-hydrochloride The filtrate was concentrated under reduced pressure and distilled 1 65g (90%) of 2-chloroethylmethacrylate was obtained IR 1720(C=0 stretching) 1620 (C=C stretching) 653 (C—Cl stretching) NMR δ 6 2 and 5 7(=CH, d), 3 7(0—CH, t) 2 1(—CH, s) 1 4(CH-Cl) Fig 1(IR spectrum), Fig 2(NMR Spectrum)

4. Synthesis of 1-(Methacryloylethyl)-5-fluorouracil(MAOEFU)

To a three-necked round bottom flask equipped with a reflux condenser was added 5-fluorouracil(2g, 0 02mol) and DMF of 70ml and the mixture was dissolved Sodium hydride of 0 02mol was added to the reaction mixture and the solution was refluxed at 60°C for 3h when a clear solution resulted When the reaction was complete, the reaction mixture was allowed to stand at room temperature It was then treated dropwise with 2-chloroethyl methacrylate of 0 02mole over 1 5h(ice bath cooling) Sulfur as polymerization inhibitor was added to the reaction mixture After heating at 50°C for 60h the mixture was evaporated under vaccum to provide a crystalline product The product was washed throughly with water and recrystallized from ethanol Yield 3 40g(70%) mp175-177°C IR 3040(=C-H stretching) 2810(C-H stretching) 1720(C=0 stretching), 1630(C=C stretching), 815(C-F stretching)NMR δ 7 9(=CH d), 6 1 and 5 7(=CH, d), 4 3(CH-0 t), 3 7(NCH, t), 1 7(-CH, s) Fig 3(IR Spectrum) Fig 4(NMR Spectrum)

5. Homopolymerization of MAOEFU

MAOEFU(0 01M/1), AIBN(4 9x10^{4}M/1) and cyclohexanone were charged into a polymerization and degassed The tube was sealed and heated at 60°C for 24hr The solution was poured into excess n-hexane with stirring The precipitated polymer was dried in vacuum at 25°C for 48hr

6. Copolymerization of MAOEFU and MA

MAOEFU (0 01M/1), MA(0 01M/1) AIBN(4 9x10^{4}M/1), and ethanol were charged into a polymerization tube The solution was degassed with three freeze-thaw cycles The thbe was then sealed and heated at 60°C for 48 hr The copolymerizations were stopped after·a few percent conversion to avoid composition drift and to obtain good reactivity ratios The solution was poured into excess n-haxane with vigorous stirring The precipitate was filtered and dried in vacuum at 25°C for 48hr

D. Analysis of copolymers

The specific absorptivity of the copolymer of MAOEFU and MA was measured by means of UV spectrophotometer at 268 5nm in THF The calibration curve was obtained by changing concentration ratios of both homopolymers (Fig 5)

E. Hydrolysis

1. Hydrolysis of MAOEFU and homo (MAOEFU)

The rate of formation of 2-hydroxyethyl-5-fluorouracil were studied under pseudo-first order conditions, using ethanol-water(1 1) mixtures The solution was kept in water bath at 37°C

2. Hydrolysis of copolymer

Hydrolysis of copolymer was conducted in a 20ml test tube containing 8mg of copolymer in 10ml of 0 1M phosphate buffer solution with shaking for a given time at 37°C. Copolymer and its hydrolyzates were separated by TLC(Kieselgel 60F254, Merck) after collecting 0.5ml of the mixture, The hydrolyzate was dissolved in 8ml of methanol, and its concentration was determined by optical density reading in a UV spectrophotometer at 271nm for the 2-hydroxyethyl-5-fluorouracil

RESULT AND DISCUSSION

A. 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[17] Each kind of radical may react with either kind of monomer molecule

$$M_1\cdot+M_1 \overset{k_{11}}{\cdots\cdots\cdots\cdots} M_1 \quad (1)$$

$$M_1\cdot+M_2 \overset{k_{12}}{\cdots\cdots\cdots\cdots} M_2 \quad (2)$$

$$M_2\cdot+M_2 \overset{k_{22}}{\cdots\cdots\cdots\cdots} M_2 \quad (3)$$

$$M_2\cdot+M_1 \overset{k_{21}}{\cdots\cdots\cdots\cdots} M_1 \quad (4)$$

In the copolymerization of two monomers. M_1 and M_2 according to equations (1) - (4), the rates of disappearance of M_1 alone and of M_2 alone are given by the expressions

$$\frac{d[M_1]}{dt}=k_{11}[M_1\cdot][M_1]+k_{21}[M_2\cdot][M_1] \quad (5)$$

$$\frac{d[M_2]}{dt}=k_{12}[M_1\cdot][M_2]+k_{22}[M_2\cdot][M_2] \quad (6)$$

where the monomer consumed in chain initiation and termination is neglected

Meehan[18] found that the adsorption curves were of the same form for the seven polystyrene samples which he studied, regardless of the molecular weight of the sample, and that the molar absorption coefficient at the absorption maximum(260nm) was directly proportional to molecular weight Since the molar absorption coefficient of polystyrene at 260nm is directly to the molecular weight of the polymer, the specific extinction cofficient, referred to weight concentration of polystyrene, is constant (independent of molecular weight) UV spectrophotometric measurements do not permit the determination of the molecular weight of a polystyrene sample, but they do permit the determination of the weight concentration of polystyrene in an unknown sample

The problem of determining the fraction of a (MAOEFU-MA) copolymer which is MAOEFU. is formally analogous to the problem of determining the weight concentration of polystyrene in an unknown solution. Therefore, by UV spectrophotometric means a determination of absorption intensity allows the calculation of the chemical composition of the copolymer When the copolymer is made up of MAOEFU and MA, which act independently with regard to the absorption of light, and which follow Beer's law, it is clear that E*=xEs*+(1-x)Eb* in which E*=specific absorptivity of the copolymer at a given wave length Es* and Eb*=specific absorptivity of pure poly(MAOEFU) and pure poly(MA) respectively, and x= weight fraction of the copolymer which is MAOEFU From the extinction coefficient (E*) of the copolymer samples at 268.5nm, the weight fraction of poly(MAOEFU) (x) could then be calculated from the following equation E*=0.14+0.8x $d[M_1]/d[M_2]$ in equation(10) correspond to x in the above equation Dividing (5) by (6) gives

$$\frac{d[M_1]}{d[M_2]}=\frac{k_{11}[M_1\cdot][M_1]+k_{21}[M_2\cdot][M_1]}{k_{11}[M_1\cdot][M_2]+k_{22}[M_2\cdot][M_2]} \quad (7)$$

It is now assumed that a steady state is soon reached and maintained where the rate at which M_1 type radicals are converted to M_2 type radicals is equal to the rate at which M_2 type radicals are converted to M_1 type radicals, so that $k_{12}[M_1\cdot][M_2] = k_{21}[M_2\cdot][M_1]$ (8)

Substituting $[M_1\cdot]$ in (7)by its equivalent from (8). and multiplying numerator and denominator of the right-hand member of (7) by $[M_2]/k_{21}[M_2\cdot]$. we obtain

$$\frac{d[M_1]}{d[M_2]} = \frac{[M_1]}{[M_2]}\,\frac{(k_{11}/k_{12})[M_1]+[M_2]}{[M_1]+(k_{22}/k_{21})[M_2]}$$

$$= \frac{[M_1]}{[M_2]}\,\frac{r_1[M_1]+[M_2]}{[M_1]+r_2[M_2]} \qquad (9)$$

where the reactivity ratios are defined as:

$r_1 = k_{11}/k_{12}$ $r_2 = k_{22}/k_{21}$

and $[M_1]$ and $[M_2]$ are the mole composition of the monomer feed. and $d[M_1]$ and $d[M_2]$ are the mole compostition of the copolymer formed instantaneously

$[M_1]$ will be used to represent MAOEFU and $[M_2]$ will be MA

Reactivity ratios were calculated from the date in Table 1. according to the Kelen-Tudős equation[12]

$$\eta = (r_1 - r_2/\alpha)\xi - r_2/\alpha \qquad (10)$$

with $\eta = G/(\alpha+F)$, $\xi = F/(\alpha+F)$, $F = X2/Y$

$G = X(Y-1)/Y$,

$= \sqrt{F\min \times F\max}$, $X = M_1/M_2$,

and $Y = d[M_1]/d[M_2]$

By poltting η values calculated from the experimental data as a function of ξ a straight line is obtained, which on extrapolation to $\xi = 0$ and $\xi = 1$ gives $-r_2/\alpha$ and r_1, respectively both as intercepts Kelen-Tudős plots are shown in Fig 6 and Table 1 and the values of r_1 and r_2 calculated from these date are given by linear least-squares method To determine the relationship of these two monomers at different relative concentrations a monomer feed-copolymer composition study was carried out The feed ratios of monomer were varied from 0 47 to 2 13 for each monomer, keeping polymerization conversions to less than 10% The reactivity ratios for the copolymerization of MAOEFU(r_1) and MA (r_2) are 0 05 and 2 04 respectively

B. Hydrolysis of MAOEFU and poly (MAOEFU)

The reaction progress was followed by recording the increase in absorption at 265nm as a function of time Pseudo-first-order rate constants[11] were determined by linear least-squares method from the slopes of linear plots of $\log(A_\infty - A_t)$ or $\log(A_t - A_\infty)$ versus time. where A and At are the absorbance readings at infinity and at time, t respectively K for MAOEFU is 1 08 × 10^{4}/sec and K for poly(MAOEFU) is 3 3×10^{4}/sec

C. Hydrolysis of Copolymer

The hydrophilic copolymer was found to

Table 1. Determination of monomer reactivity ratios for the copolymerization of MAOEFU(M1) and MA(M2) AIBN=5 0x10^{4}M/1. =3.40

Exp No.	$X=\frac{M1}{M2}$	$Y=\frac{m1}{m2}$	X^2	$Y-1$	$F=\frac{X^2}{Y}$	$G=\frac{X(Y-1)}{Y}$	$\alpha+F$	$\eta=\frac{G}{\alpha+F}$	$\xi=\frac{F}{\alpha+F}$
1	0.47	0 19	0.22	−0.81	1.16	−2.0	4.56	−0.44	0 25
2	1 0	0 29	1.0	−0.71	3.45	−2.448	6.85	−0 36	0.50
3	1.5	0.38	2.25	−0.62	5.92	−2 44	9 32	−0.26	0 64
4	2 3	0.53	5.29	−0.47	9.98	−2.04	13 38	−0 15	0.75

be degraded in aqueous solution under mild conditions to give the derivatives of pyrimidines The derivatives released from the copolymer identified as 2-hydroxyethyl derivatives of 5-FU by UV spectroscopy after isolation by preparative TLC. This means that the ester groups of the polymer side chains were hydrolyzed in aqueous solution Fig 7 shows the degree of hydrolysis of copoly(MAOEFU-MA) at 37°C in a 0 1M phosphate buffer solution(pH=7.8) as a function of time

CONCLUSION

The new monomer MAOEFU was synthesized by the reaction of 5-fluorouracil with 2-chloroethylmethacrylate Poly(MAOEFU) and poly(MAOEFU-co-Methacrylic acid) were also obtained by radical polymerization with AIBN in ethanol at 60°C. The specific absorptivity of copolymer was measured by UV spectrophotometric means at 268 5nm in THF. The r_1 and r_2 values for the comonomer pair of MAOEFU (M_1) and MA (M_2) are 0.05 and 2 04 respectively First-order rate constant for hydrolysis of MAOEFU and HCl in ethanol-water mixture (1:1) at 265nm is 1.08×10^{-4}/sec The present hydrophilic copolymer containing 5-fluorouracil can be regarded as polymeric drug since the 5-fluorouracil derivatives in them can be released by hydrolysis under mild condition similar to those in biological systems.

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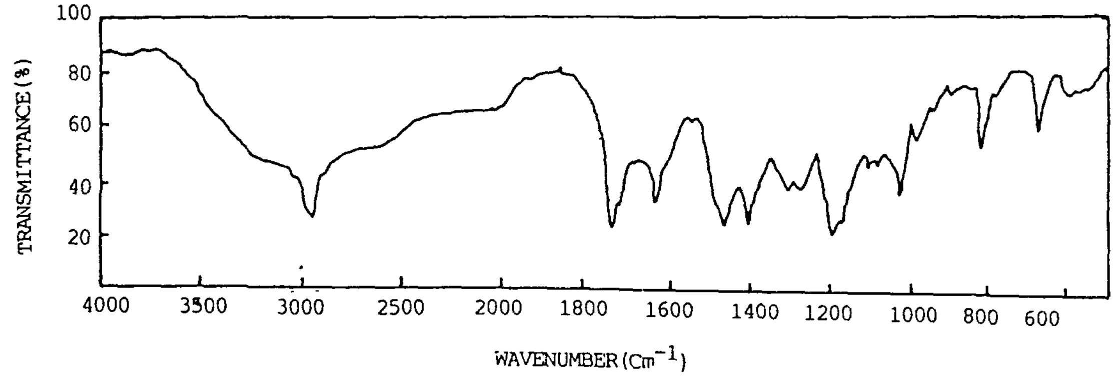

Fig. 1. IR spectrum of CEMA, (Neat phase)

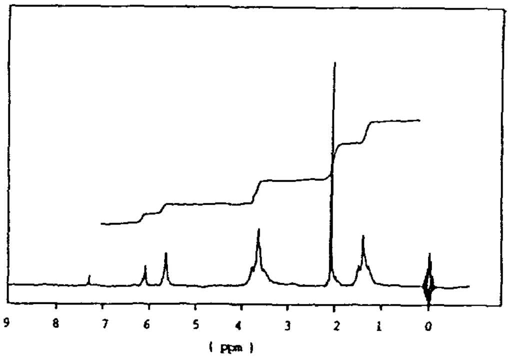

Fig. 2. NMR spectrum of CEMA (Chloroform-dl)

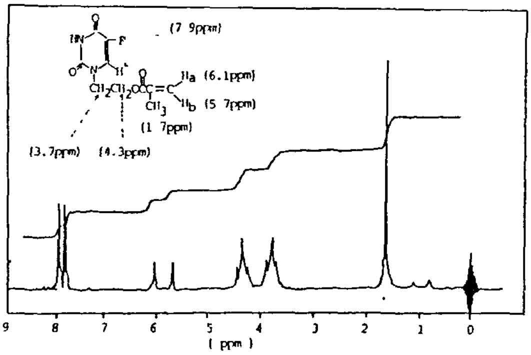

Fig. 4. NMR spectrum of MAOEFU (DMSO-d6)

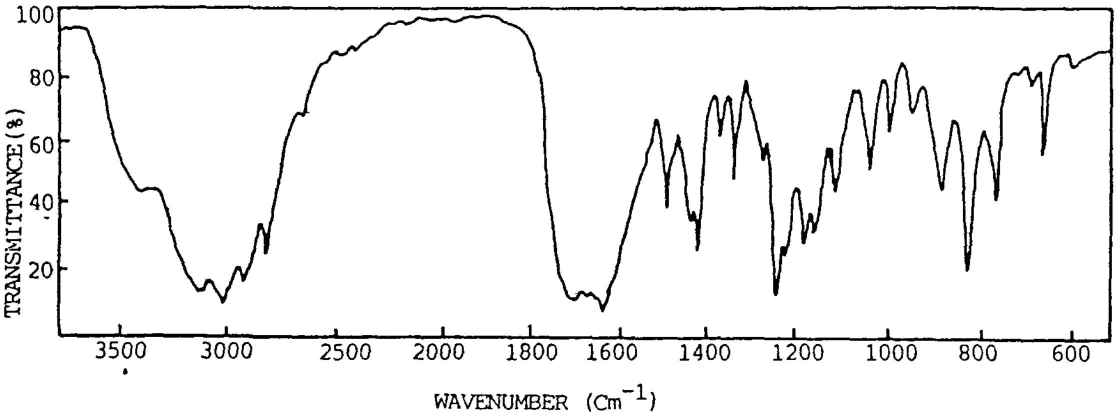

Fig. 3. IR spectrum of MAOEFU (Solid phase, KBr)

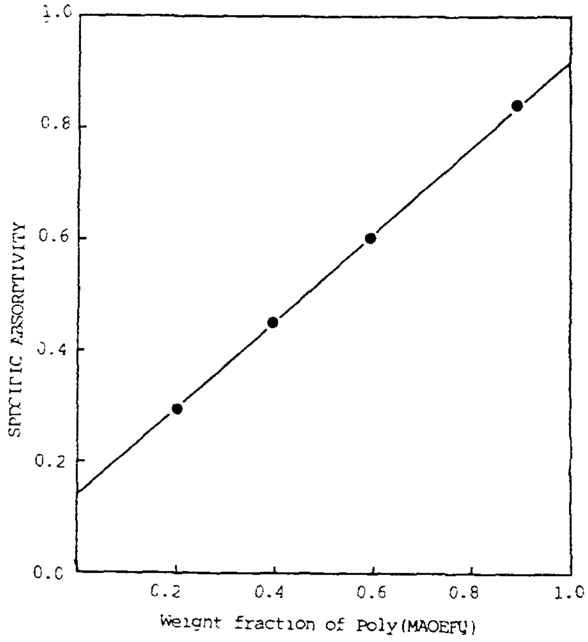

Fig. 5. Plot of specific absorptivity vs weight fraction of poly(MAOEFU) in THF at 268.5nm.

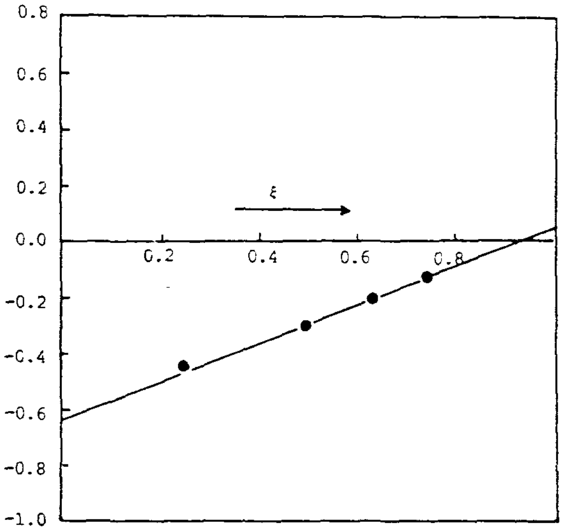

Fig. 6. Kelen-Tudos plot for the copolymerization of MAOEFU and MA at 60°C

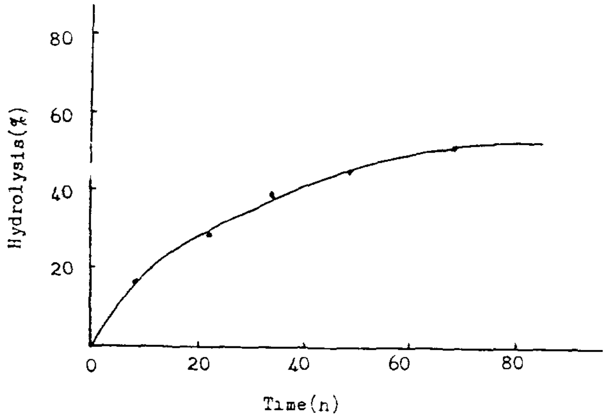

Fig. 7. Hydrolysis of copolymers having 5-Fu in a 0.1M phosphate buffer(pH=7.8) Concentration0.8mg/ml