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1-(2-카르보메톡시아크릴로일)-5-플루오르우라실과 초산비닐, 메틸메타크릴레이트 및 스티렌과의 공중합

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Copolymerization of 1-(2-Carbomethoxy acryloyl)-5-fluorouracil with Vinyl Acetate, Methyl Methacrylate, and Styrene

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요 약 : 1-(2-Carbomethoxyacryloyl)-5-fluorouracil(CMAFU)과 초산비닐(VAc), 메틸메타크릴레이트(MMA) 및 스티렌을 tetrahydrofuran을 용매로 사용하여 50℃에서 라디칼 공중합을 하였다. 공중합체내의 단량체조성은 공중합체의 IR 스펙트럼으로부터 정량분석하여 구하였다. Kelen-Tüdös 법에 의해 구한 각각의 단량체 반응성비의 값은 r_1(CMAFU)=0.04, r_2(VAc)=0.19 ; r_1(CMAFU)=0.01, r_2(MMA)=32.11 ; r_1(CMAFU)=0.01과 r_2(St)=7.65이었다. 얻어진 단량체 반응성비의 값들로부터 CMAFU와 VAc, MMA 및 St 공중합에서 CMAFU의 입체장애 효과가 큰 영향을 미치는 것을 알 수 있었다. 노랑초파리(*Drosophila melanogaster*)를 이용하여 5-플루오르우라실(5-FU), 합성한 CMAFU 단량체 및 공중합체들의 독성을 조사한 결과, 5-FU>공중합체>CMAFU순으로 독성이 감소하였다.

Abstract : Copolymerizations of 1-(2-carbomethoxyacryloyl)-5-fluorouracil(CMAFU) with vinyl acetate, methyl methacrylate, and styrene were carried out with 2,2′-azobisisobutyronitrile in tetrahydrofuran at 50℃. The copolymer compositions were determined by quantitative infrared(IR) analysis. The monomer reactivity ratios, r_1 and r_2 were determined by the Kelen-Tüdös method. The values of r_1 and r_2 for each monomer pair were as follows ; r_1(CMAFU)=0.04 and r_2(VAc)=0.19 ; r_1(CMAFU)=0.01 and r_2(MMA)=32.11 ; r_1(CMAFU)=0.01 and r_2(St)=7.65. These values imply that the copolymerizations were significantly affected by the steric hindrance of CMAFU. Toxicity of 5-fluorouracil(5-FU), CMAFU and its copolymers obtained for this work was tested in *Drosophila melanogaster*. It was observed that the toxicity decreased in the order of 5-FU> copolymers>CMAFU.

INTRODUCTION

The synthetic polycarboxylic acid polymers such as poly(acrylic acid), poly(methacrylic acid), poly (ethylene-co-maleic anhydride), and oxidized polysaccharides were found to have interferon-inducing ability and display antiviral and antitumor activity.[1~4] Especially, the copolymer of divinyl ether and maleic anhydride(DIVEMA) synthesized by Butler et al. has shown biological activities such as antibacterial and antifungal activity; Moreover, it has significant antitumor and antiviral activity.[1,5~12] The copolymers containing pyran or furan derivatives together with carboxyl groups along their backbone, studied by Han et al.[13~15] are another typical examples of those biologically active polymers. They reported the syntheses and biological activities of poly[(tetrahydropyran-2,3-diyl)(1,2-dicarboxyethylene)], poly[(tetrahydrofuran-2,3-diyl)(1,2-dicarboxyethylene)] and poly (tetrahydrofuran-2,3-diyl)(1,2-dihydroxyethylene)].

Recently lots of works on the new polymers containing 5-fluorouracil (5-FU) have been reported to reduce the toxicity and delivery problems of 5-FU for its use in cancer chemotherapy. For instance, Akashi et al.[16] synthesized 1-N-acryloyl-5-fluorouracil, 1-N-methacryloyl-5-fluorouracil, and their copolymers. Even though researches on the biological activities of those polymeric drugs are crucially important, the information on the reactivities of those monomers containing antitumor agents must be of importance as well. Furthermore no work on the toxicity of polymeric drugs against *Drosophila melanogaster* has been published. Thus, in this work, copolymerizations of 1-(2-carbomethoxymethacryloyl)-5-fluorouracil(CMAFU) with vinyl acetate(VAc), methyl methacrylate(MMA), and styrene(St) were carried out by a free radical initiator. The copolymer compositions were analyzed quantitatively by infrared(IR) spectroscopy. The monomer reactivity ratios, r_1 and r_2, were determined by the Kelen-Tüdös method. Toxicity of 5-fluorouracil(5-FU), CMAFU and the copolymers against *Drosophila melanogaster* was also determined by the adult feeding method of Lewis and Bacher.[17]

EXPERIMENTAL

Materials

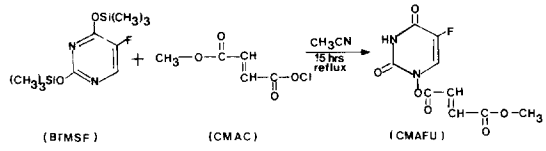
5-Fluorouracil(Aldrich Co.) was used as received. Vinyl acetate, methyl methacrylate, styrene and 2,2′-azobisisobutyronitrile(AIBN) were purified by the conventional methods. Acetonitrile, acetone, *n*-hexane, and cyclohexanone were used after purification.

Instruments

IR spectra were taken on a Perkin-Elmer 1330 spectrophotometer using KBr pellet or liquid cell for quantitave analysis. Elemental analyses were performed by Elemental Analyzer (Perkin-Elmer 240C). UV spectra were taken on a Shimadzu 200 A spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian A-60 spectrophotometer.

Synthesis of Monomer

1-(2-Carbomethoxyacryloyl)-5-fluorouracil (CMAFU) : CMAFU was prepared by the reaction of 2,4-bis(trimethylsilyloxy)-5-fluoropyrimidine (BTMSF)[18] with trans-β-carbomethoxyacryloyl chloride(CMAC) in acetonitrile, according to the Butler's method.[19](as shown in Scheme 1)



Scheme 1

A solution of 5.46 g(20 mmole) BTMSF and 2.97 g(20 mmole) CMAC in 100 ml dry acetonitrile was refluxed for 15 hrs under dry condition. Cooling to room temperature, the acetonitrile was distilled off under reduced pressure to dryness. The residue was dissolved in 30 ml dry acetone. After filtration the acetone solution was slowly added with vigorous stirring to 600 ml hexane and the precipitate

was collected by filtration to yield 3.24g(67%) of CMAFU(m.p. : 149~150℃, lit.[19] m.p. : 150~153 ℃).

Analysis : Cal. for $C_9H_7N_2O_5F$(242.2) : C, 44.63 ; H. 2.91 ; N, 11.57%. Found : C, 44.47 ; H, 2.71 ; N. 11.60%. IR(KBr, cm^{-1}) : 3450(−NH), 3080(=CH), 2840 (aliphatic C−H), 1725 & 1705(−C=O), 1250(−C−O) and 815(−NH). ^{1}H-NMR(acetone-d_6) : δ 8.2(d, 6H of pyrimidine ring), 7.8 & 6.7(d, 2H of ethylenic hydrogen) and 3.7ppm(s, 3 H, $-CH_3$).

Syntheses of Polymers

Poly(vinyl acetate)(PVAc) : A solution of 1.72g (0.02 mol) vinyl acetate and 0.086g AIBN in 25ml dry tetrahydrofuran(THF) was introduced into a dry polymerization tube and degassed twice by purging with purified N_2 gas. The tube was sealed and placed in a regulated thermostat at 50 ± 0.05 ℃ for 24 hrs. The polymer solution was precipitated in excess *n*-hexane. The precipitate was collected by filtration and dried until a constant weight under vacuum.

Poly(methyl methacrylate)(PMMA) : Poly(methylmethacrylate) was prepared by the polymerization of MMA. The procedure was the same as that of poly(vinyl acetate).

Polystyrene(PS) : Polystyrene was prepared from styrene by the same procedure as described for poly(vinyl acetate).

Syntheses of Copolymers

Poly[1-(2-carbomethoxyacryloyl)-5-fluorouracil-co-vinyl acetate][Poly(CMAFU-co-VAc)] : Copolymerization of CMAFU with vinyl acetate(VAc) was carried out with AIBN in THF at 50℃. A series of copolymerizations, in which the feed ratios of CMAFU(M_1) to VAc(M_2) were varied in the range of 0.50 to 2.50, yielded copolymer over a wide range of compositions. The copolymerizations were stopped before 10% conversion was reached. As a typical copolymerization of $M_1/M_2=1$, for example, a solution of 0.81g(3.3 mmol) CMAFU, 0.28g(3.3 mmol) VAc and 0.055g AIBN in 25 ml dry THF was introduced into a dry polymerization tube. After degassing twice by purging with purified N_2 gas, the tube was sealed and placed in a regulated thermostat at 50 ± 0.05℃ for fixed periods of time. The polymer solution obtained was precipitated in excess *n*-hexane several times. The precipitate was collected by filtration and dried until a constant weight under vacuum.

Poly[1-(2-carbomethoxyacryloyl)-5-fluorouracil-co-methyl methacrylate] [Poly(CMAFU-co-MMA)] : Poly(CMAFU-co-MMA) was prepared by the same procedure as that of poly(CAMFU-co-VAc).

Poly[1-(2-carbomethoxyacryloyl)-5-fluorouracil-co-styrene] [Poly(CMAFU-co-St)] : Poly (CMAFU-co-St) was prepared by the same procedure as that of poly(CMAFU-co-VAc).

Analyses of Copolymer Compositions : The copolymer compositions were determined quantitatively by infrared(IR) spectroscopy according to the literature.[20,21] For the analysis of poly(CMAFU-co-VAc) and poly(CMAFU-co-St), a definite amount of copolymer was dissolved in N,N-dimethylformamide(DMF) of spectrophotometric grade. The solution was placed in a liquid cell and quantitative analysis was performed on IR spectrum. The composition of CMAFU was determined by a characteristic peak of N-H out-of-plane bending at 815 cm^{-1}. For poly(CMAFU-co-MMA), a liquid cell filled with the copolymer solution of 2-methoxyethanol(spectrophotometric grade) was used for analysis. A quantitative analysis of copolymer composition was done using a characteristic peak at 790 cm^{-1}. The composition of the copolymer samples was also determined by elemental analysis and correlated with respective IR spectra to give a calibration curve.

Measurement of Intrinsic Viscosity : The intrinsic viscosity(η) of polymers was measured in N,N-dimethylformamide(DMF) at 30 ± 0.01℃ with Cannon-Fenske viscometer.

Toxicity Test : Toxicity of 5-FU, CMAFU, poly (CMAFU-co-VAc), poly(CMAFU-co-MMA) and poly(CMAFU-co-St) against *D. melanogaster* was tested by the Lewis and Bacher's adult feeding method. The compositions of CMAFU in the copo-

lymers for tests were 47, 49 and 50 mole%, for poly(CMAFU-co-VAc), poly(CMAFU-co-MMA) and poly(CMAFU-co-St), respectively. The intrinsic viscosities of the polymers were 0.09, 0.08 and 0.07 for poly(CMAFU-co-VAc), poly(CMAFU-co-MMA) and poly(CMAFU-co-St), respectively.

D. melanogaster was cultured in the standard medium. Except when needed for counting or transferring, the cultures were kept in a constant-temperature cabinet at 25℃ over all experimental runs. The treatment medium for control group consisted of 5% sucrose in ethanol-water(1 : 1, v/v) mixture. For other experimental groups, 300 ppm of 5-FU, CMAFU or its copolymers was added to the treatment solution. Every 12 hr the number of dead flies is scored for each experimental group.

RESULTS AND DISCUSSION

Monomer Reactivity

The copolymer compositions were determined by quantitative IR analyses. The IR spectra of CMAFU and its copolymers are shown in Figures 1 through 4, where 815 cm^{-1} is selected as the characteristic wavenumbers for analyses of poly(CMAFU-co-VAc) and poly(CMAFU-co-St), whereas 790cm^{-1} for poly(CMAFU-co-MMA), because PVAc, PS, and PMMA show no absorbance at these wavenumbers.

The datails of quantitative IR analysis are found in the literatures[20,21] but a brief explanation can be described as follows : The IR spectra of copolymers of CMAFU and VAc, MMA or St with several feed ratios were used to find the absorbances of the N-H out-of-plane bending of CMAFU. A base line was constructed in the region of the characteristic wavenumbers. A straight-line calibration plot was obtained for the absorbance values by using the Beer-Lambert Law versus the mole ratio of the two monomer units in the polymer mixtures. The calibration curve was correlated with the elemental analysis data. Using the calibration plot, the mole ratio of the two monomers in each of the copolymers was obtained. The characteristic wavenumbers were represented by arrows in Figures 2 through 4. The copolymer compositions in poly(CMAFU-co-VAc) were listed in Table 1.

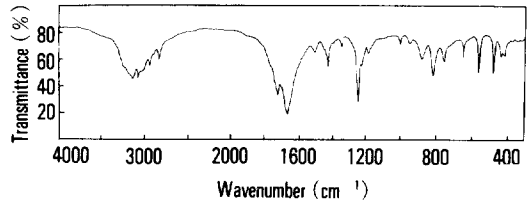


Fig. 1. IR spectrum of CMAFU.

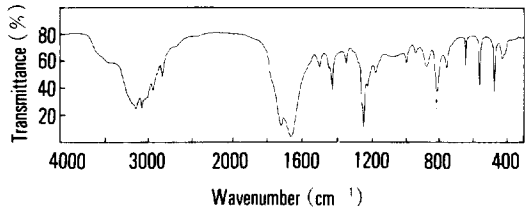


Fig. 2. IR spectrum of poly(CMAFU-co-VAc).

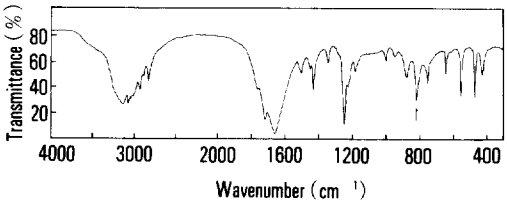


Fig. 3. IR spectrum of poly(CMAFU-co-MMA).

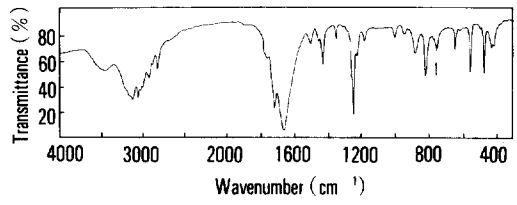


Fig. 4. IR spectrum of poly(CMAFU-co-St).

The reactivity ratio of each monomer was estimated by the Kelen-Tüdös method.[22] Figure 5 shows a typical Kelen-Tüdös plot to determine monomer reactivity ratios, in which the ordinate η and the abscissa ξ are explained in Table 1 along with other several parameters.

The Kelen-Tüdös plot gives r_1 value of 0.04 (CMAFU) and r_2 value of 0.19(VAc). Since r_1 (k_{11}

Table 1. Kelen-Tüdös Parameters for Determination of Monomer Reactivity Ratios for the Copolymerization of CMAFU(M_1) and VAc(M_2) $\alpha = 1.41$

EXP. NO.	$X=\frac{M_1}{M_2}$	$Y=\frac{m_1}{m_2}$	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	0.50	0.75	0.25	−0.25	0.33	−0.17	1.74	−0.10	0.19
2	1.00	0.87	1.00	−0.13	1.14	−0.15	2.55	−0.06	0.44
3	1.50	0.93	2.25	−0.07	2.41	−0.12	3.82	−0.03	0.63
4	2.00	1.00	4.00	−0.00	4.00	−0.00	5.40	0.00	0.73
5	2.50	1.05	6.25	−0.04	5.95	−0.11	7.36	0.01	0.80

Table 2. Kelen-Tüdös Parameters for Determination of Monomer Reactivity Ratios for the Copolymerization of CMAFU(M_1) and MMA(M_2) $\alpha = 34.52$, r_1(CMAFU) = 0.01, r_2(MMA) = 32.11

EXP. NO.	$X=\frac{M_1}{M_2}$	$Y=\frac{m_1}{m_2}$	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	0.50	0.016	0.25	−0.98	15.63	−30.75	50.15	−0.61	0.31
2	1.00	0.029	1.00	−0.97	34.48	−33.48	68.99	−0.49	0.45
3	1.50	0.043	2.25	−0.96	52.33	−33.38	86.85	−0.39	0.60
4	2.00	0.060	4.00	−0.94	66.67	−31.33	101.19	−0.31	0.66
5	2.50	0.082	6.25	−0.92	76.22	−27.99	110.74	−0.25	0.69

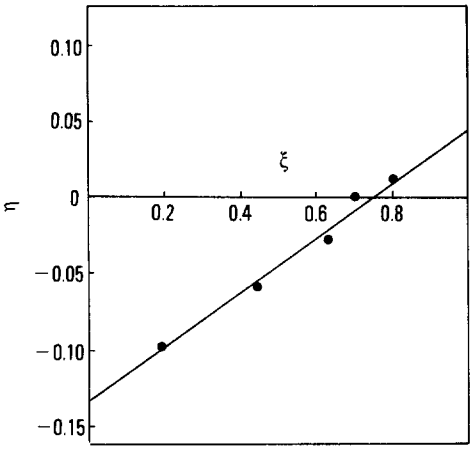


Fig. 5. Kelen-Tüdös plot for the copolymerization of CMAFU and VAc r_1(CMAFU) = 0.04, r_2(VAc) = 0.19.

$/k_{12}$) is less than unity whereas $r_2(k_{22}/k_{11})$ is much larger than unity for the copolymerization of CMAFU and VAc, CMAFU radical addition to VAc monomer occurs more readily than addition of CMAFU radical to CMAFU monomer. This is probably attributed to the steric hindrance of CMAFU.

The monomer reactivity ratios in the copolymerizations of CMAFU with MMA and St were determined by the same method as described for the copolymerization of CMAFU and VAc. The copolymer compositions were also determined by quantitative IR analyses. Each reactivity ratio shows clearly the same steric effect of CMAFU in both copolymerizations, as observed in the copolymerization system of CMAFU and VAc. The copolymer compositions were summarized in Tables 2 and 3. Several other parameters related to Kelen-Tüdös plot were also shown in the same tables.

Toxicity of Materials

The effects of 5-FU, CMAFU, and its copolymers at the concentration of 300ppm on the mortality of *D. melanogaster* are shown in Figure 6. There is no significant change in mortality up to 516 hrs for a control group. However, the mortality of *D. melanogaster* treated with all the other experimental groups increased remarkably after about 250 hrs. The lethal times for 100% at the same concentration were found to be 384, 480, 465, 456 and 441 hrs for 5-FU, CMAFU, poly(CMAFU-co-

Table 3. Kelen-Tüdös Parameters for Determination of Monomer Reactivity Ratios for the Copolymerization of CMAFU(M_1) and St(M_2) $\alpha = 10.21$, r_1(CMAFU) = 0.01, r_2(St) = 7.65

EXP. NO.	$X=\frac{M_1}{M_2}$	$Y=\frac{m_1}{m_2}$	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	0.50	0.06	0.25	−0.94	4.17	−7.83	14.38	−0.54	0.29
2	1.00	0.13	1.00	−0.89	7.69	−6.85	17.90	−0.38	0.43
3	1.50	0.18	2.25	−0.82	12.50	−6.83	22.71	−0.30	0.55
4	2.00	0.21	4.00	−0.79	19.04	−7.52	29.26	−0.26	0.65
5	2.50	0.25	6.25	−0.75	25.00	−7.50	35.21	−0.21	0.71

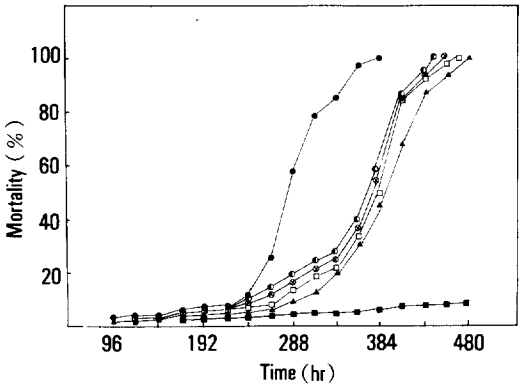


Fig. 6. Exposure-mortality relationship for OR males treated with various chemicals after adult feeding(300 ppm) : (■) control group, (●) 5-FU, (▲) CMAFU, (□) poly(CMAFU-co-VAc), (⊗) poly(CMAFU-co-MMA), (◐) poly(CMAFU-co-St).

VAc), poly(CMAFU-co-MMA) and poly(CMAFU-co-St), respectively. The compounds have shown significant differences in toxicity as listed in decreasing order of mortality as follow ; 5-FU>poly (CMAFU-co-St)>poly(CMAFU-co-MMA)>poly (CMAFU-co-VAc)>CMAFU>control

CONCLUSIONS

The monomer, 1-(2-carbomethoxyacryloyl)-5-fluorouracil(CMAFU) was synthesized from 2,4-bis(trimethylsilyloxy)-5-fluoropyrimidine(BTMSF). Copolymerizations of 1-(2-carbomethoxy acryloyl)-5-fluorouracil(CMAFU) with vinyl acetate(VAc), methyl methacrylate(MMA) and styrene(St) were carried out with AIBN in THF at 50℃. The copolymer compositions were quantitatively analyzed by IR spectoscopy. The monomer reactivity ratios, r_1 and r_2 were determined by the Kelen-Tüdös method ; r_1(CMAFU) = 0.04 and r_2 (VAc) = 0.19 ; r_1(CMAFU) = 0.01 and r_2(MMA) = 32.11 ; r_1(CMAFU) = 0.01 and r_2(St) = 7.65. These values imply that the copolymerizations were significantly affected by the steric hindrance of CMAFU. The toxicity of CMAFU and its polymers against *Drosophila melanogaster* was investigated by the adult feeding method of Lewis and Bacher. The toxicity decreased in the order of 5-FU > copolymers > CMAFU > control.

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REFERENCES

1. L. G. Donaruma, "Anionic Polymeric Drugs", L. G. Donaruma, R. M. Ottenbrite and O. Vogl, Eds., John Wiley & Sons Inc., New York, Vol. 1, p. 50, 1980.
2. T. C. Merigan and W. Regelson, *N. Engl. J. Med.*, **277**, 1283 (1967).
3. P. S. Morahan, W. Regelson and A. E. Munson, "Antimicrobiological Agents and Chemotheraphy",

p. 16, 1972.
4. R. M. Ottenbrite, "The Antitumor and Antiviral Effects of Polycarboxylic acid Polymers" in Biological Activities of Polymers, ACS Symposium Series 186, Amer. Chem. Soc., Washington D. C., 1982.
5. R. M. Ottenbrite, E. Goodell and A. Munson, *Polymer*, **18**, 461 (1977).
6. G. B. Butler, *J. Macromol. Sci. Rev., Macromol. Chem. Phys.*, **C22**, 89 (1982).
7. D. S. Breslow, *Pure & Appl. Chem.*, **46**, 103 (1979).
8. G. B. Butler, *J. Macromol. Sci.-Chem.*, **46**, 103 (1979).
9. E. DcClereq and T. C. Merigan, *Arch. Intern. Med.*, **126**, 94 (1970).
10. S. J. Mohr, M. A. Chirigos, F. S. Fuhrman and J. W. Pryor, *Cancer Res.*, **35**, 3750 (1975).
11. W. Regelson, A. Munson and W. Wooles, International Symposium on Standards of Interferon and Interferon Inducers, London, 1969, Symposium Series Immunobiological Standards, Vol. 14, Korger, Basel., p. 227, 1970.
12. P. S. Roberts, W. Regelson and B. Kingsbury, *J. Lab. Clin. Med.*, **2**, 822 (1973).
13. M. J. Han, D. H. Lee, W. Y. Lee and B. S. Hahn, *Bull. Korea Chem. Soc.*, **10**, 212 (1989).
14. M. J. Han, S. D. Kang and W. Y. Lee, *Bull. Korea Chem. Soc.*, **11**, 154 (1990).
15. M. J. Han, K. B. Choi, K. H. Kim, T. J. Cho, W. Y. Lee and W. N. Shim, *J. Bioactive and Compatible Polym.*, **5**, 420 (1990).
16. M. Akashi, Y. Tanaka, T. Miyazaki and N. Miyauchi, *J. Bioactive and Compatible Polym.*, **2**, 120 (1987).
17. E. B. Lewis and F. Bacher, *Dorosophila Inform. Ser.*, **43**, 193 (1968).
18. R. Duschinsky and T. F. Gabrel, U. S. Pat., No. 3, 354, 160 (1967).
19. P. P. Umrigar, S. Ohashi and G. B, Butler, *J. Polym. Sci., Polym. Chem.*, **17**, 351 (1979).
20. W. J. Cho, Ph. D. Dissertation, University of Paris VI, 1978.
21. S. Das and F. Rodriguez, *J. Appl. Polym. Sci.*, **39**, 1309 (1990).
22. T. Kelen and F. Tüdös, *J. Macromol. Sci. -Chem.* **A-9**, 1 (1975).