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SYNTHESIS OF ALTERNATING COPOLYMERS CONTAINING 5-FLUOROURACIL AND THEIR HYDROLYTIC VELOCITY

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= Abstract =

The copolymerizations of 1-(2-carbomethoxyacrylolyl) -5-fluorouracil (CMAFU) with methymethacrylate (MMA) and styrene (St) were carried out with AIBN in cyclohexanone at 60°C. The specific absorptivity on the copolymer of CMAFU and MMA in 2-methoxyethanol at 267.5nm was measured by UV spectrophotometer. The specific absorptivity on the copolymer of CMAFU and St in N, N-dimethylformamide at 280nm was measured by UV spectrophotometer. The monomer reactivity ratios, r_1 and r_2 were determined by Kelen-Tüdös method. r_1(CMAFU)0.62, r_2(MMA)4.20, r_1(CMAFU)0.15, r_2(St)0.95. The hydrolytic velocity of CMAFU and copolymer was determined by NMR and UV spectrophotometer.

INTRODUCTION

Synthetic pharmacologically active polymers have become of interest from the standpoint of drug development in recent years.[1,6,10] A polymeric drug can be expected to have reduced toxicity and increased durability of drug activity. Perhaps one of the most widely studied polymer system is the divinylether-maleic anhydride copolymer(DVEMA), commonly known as "Pyran copolymer". This copolymer has been shown to possess interesting biological activity.[4]

It has been reported that 5-fluorouracil(5-FU) has not only the remarkable antitumor activity but also strong side effects. In order to counter the latter effects, 1-(tetrahydro-2-furanoyl)-5-fluorouracil (Ftorafur) and 5-fluorouridine(FUR)[11] were synthesized.

It was of interest to utilize this pharmacologically active polymer as a carrier of 5-fluorouracil(5-FU). Butler[3] reported on the modificatory incorporation of 5-FU into the 1 : 2 reguarly alternating cyclocopolymer(DIVEMA) of divinyl ether and maleic anhydride. On the other hand, Ohashi and Hirano et al. synthesized DIVEMA fixing FUR and tested its antitumor activity.

In order to provide the polymeric drug, increasing durability of drug activity and reducing toxicity, the present paper deals with the synthesis of 1-(2-Carbomethoxyacrylolyl)-5 Fluorouracil(CMAFU) and the copolymerization of it with methylmethacrylate(MMA) and styrene(St). Furthermore, the CMAFU and its copolymers were investigated from the standpoint of the slow release of 5-FU residue.

EXPERIMENTAL

A. Materials

Styrene and methylmethacrylate were purified by usual methods.[2)] Azobisisobutyronitrile(AIBN) was recrystallized from methanol. 5-Fluorouracil, maleic anhydride, thionyl chloride, and triethylamine were of commercial grade and used without further purification. Solvents were purified before use by conventinal procedures.

B. Instruments

Infrared spectra were recorded on a Shimadzu 400 Infrared Spectrophotometer. H-NMR spectra were on a Varian EM-360A 60MHz spectrophotometer. UV spectra were recorded on a Hitachi spectrophotometer.

C. Preparation of Monomer and Copolymers

1. Synthesis of CMAFU

Methyl hydrogen fumarate was prepared according to the method of Eisener et al. Maleic anhydride was dissolved in warm methanol(50mℓ). This solution was treated with thionyl chloride(1mℓ) and refluxed for 10 min, during which time the mixture set to a solid. This was stirred with excess of sodium hydrogen carbomate, and the insoluble material(dimethyl fumarate) was removed by filtration. The aqueous filtrate was concentrated under reduced pressure thymol blue with concentrated hydrochloric acid. The precipitate was collected, washed with water. dried and extracted with boiling chloroform. On concentration and cooling, the extract afforded pure methyl hydrogen fumarate(yield 20%), mp 141℃. Methyl hydrogen fumarate(0.2mole) and excess of thionyl chloride(100mℓ) were heated on the oil bath under refluxing for 1hr, and the product fractionated to yield methyl fumaroyl chloride(yield 90%), bp 83℃ /28mmHg. A mixture of 1.3g 5-FU(0.01 mole), 1.01g(0.01 mole) triethylmaine, and 150mℓ DME was allowed to reflux for 3hr with precoution being taken to exclude moisture from the system The resultant clear solution was cooled to -5℃ in an ice-salt bath. To this cooled solution 1.49g(0.01 mole) methyl fumaroyl chloride in 30mℓ dry DME was slowly added with vigorous stirring over a period of 2hr. The triethylamine hydrochloride salt was filtered and the filtrate rapidly concentrated on a rotary evaporator. The concentrated solution was slowly added with vigorous stirring to 1000mℓ hexane. The pale brown precipitate was collected by filtration, dried in a vacuum desicator, and redissolved in 15mℓ dry acetone. After removing the insoluble material by filtration, the product was reprecipitated in 500mℓ hexane to yield 1.65g(70%) of CMAFU : m.p.145℃. NMR(acetone-d_6) : 8.22 (d.1.J=7Hz), 7.86(d.1.J=16Hz), 6.70(d.1.J=16Hz) 3.75(s.3). Fig.1, 2.

2. Poly(CMAFU)

Monomer CMAFU was dissolved in 5mℓ of a cyclohexanone and AIBN(0.082g) was added. The solution was placed in a polymerization tube, degassed, and the tube was sealed under vacuum. The polymerization was conducted at 60℃ for 24hrs. The solution pour into 100mℓ hexane with stirring, to precipitate the formed polymer. The product was dried in vacuo at room temperature 48hrs to yellow polymer. The homopolymer was identified from the IR absorption bands of the functional groups of monomer.

3. Poly(CMAFU-MMA)

A solution of 0.242g CMAFU, 1.00g MMA, and 0.082g AIBN in 7.5mℓ dry cyclohexanone was introduced into a dry polymerization tube. The procedure followed was identical to that used for poly(CMAFU). The copolymer was identified from the IR absorption bands of the functional groups of both monomers.

4. Poly(CMAFU-St)

The procedure followed was identical to that used for poly(CMAFU-MMA) copolymer. The amount of reagents used were 1.04g styrene, 0.242g CMAFU, 0.082g AIBN, and 7.5mℓ cyclohexanone. The copolymer was identified from the IR absorption bands of the functional group of both monomers.

D. Analysis of Copolymers

1. Poly(CMAFU-MMA) Copolymer

Poly(CMAFU), poly(MMA), and poly(CMAFU-co-MMA) were dissolved in 2-methoxyethanol. The copolymer composition was determined from UV spectra at 267.5nm.[8,9]

2. Poly(CMAFU-St) Copolymer

Poly(CMAFU), poly(St), and poly(CMAFU-co-St) were dissoloved in N,N-Dimethylformamide. The copolymer composition was determined from UV spectra at 280nm.

E. Measurements of Hydrolytic Velocity of Monomer and Polymers

1. Use of UV Spectrophotometer

Hydrolysis of CMAFU in H_2O-Dioxane at 25℃ was measured by following the decrease of absorbance at 300nm. As the UV spectra of the copolymers were very close to that of 5-FU and also the solubility in water was very slow, their hydrolysis was observed in heterogeneous system. The copolymers were dispersed in 0.5M NaCl solution: Samples were removed periodically. After removing polymer by filtration, the filtrate was diluted to a predetermined amount and the amount of 5-FU released was determined by measuring its absorbance at 265.5nm. Fig.3, 4. Table 1.

2. Use of NMR Spectrophotometer

The rates of hydrolysis of CMAFU were measured in acetone-d_6-D_2O(5 : 1 V/V). Fig.6.

RESULT AND DISCUSSION

A. Determination of Monomer Reactivity Ratios

The copolymerization behavior of two monomers by addition copolymerization[8] is usually described by four propagation reaction

$$\sim\!\sim\!\sim A^* + A \xrightarrow{k_{AA}} \sim\!\sim\!\sim A - A^* \qquad (1)$$

$$\sim\!\sim\!\sim A^* + B \xrightarrow{k_{AB}} \sim\!\sim\!\sim A - B^* \qquad (2)$$

$$\sim\!\sim\!\sim B^* + A \xrightarrow{k_{BA}} \sim\!\sim\!\sim B - A^* \qquad (3)$$

$$\sim\!\sim\!\sim B^* + B \xrightarrow{k_{BB}} \sim\!\sim\!\sim B - B^* \qquad (4)$$

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

$$\frac{a}{b} = \frac{[A]r_1\,([A]/[B]) + 1}{[B]r_2 + ([A]/[B])} \qquad (5)$$

where the reactivity ratios are defined as :

$$r_1 = \frac{k_{AA}}{k_{AB}} \qquad r_2 = \frac{k_{BB}}{k_{BA}} \qquad (6)$$

and [A] and [B] are the mole composition of the monomer feed, and a and b are the mole composition of the copolymer formed instantaneously. A will be used to represent CMAFU and B will be MMA or St. Various equaion determining the reactivity ratios from (5) are known, Kelen-Tüdős equation[7] was employed to calculate the reactivity ratios in these cases. Kelen-Tüdős equation

$$\eta = \left(r_1 + \frac{r_2}{\alpha}\right)\xi - \frac{r_2}{\alpha} \quad \text{with } \eta = \frac{G}{\alpha+F},\ \xi = \frac{F}{\alpha+F},$$

$$F = \frac{X^2}{Y},\ G = \frac{X(Y-1)}{Y},\ \alpha = \sqrt{F\text{min} \times F\text{max}},$$

$$X = \frac{M_1}{M_2},\ \text{and } Y = \frac{m_1}{m_2}$$

(where M_1 and M_2 are the moles of monomer 1 and monomer 2 in the feed and m_1 and m_2 are moles of the respective monomers in the copolymer) was used with a least-square procedure to fit the data. Plotting η against ξ, a straight line is obtained which on extrapolation to $\xi = 0$ and $\xi = 1$ gives $-r_2/\alpha$ and r_1(as intercepts), respectively 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 the degree of polymeriaztion to less than 10%. The parameters of Kelen-Tüdös equation are shown in Table 2.3 and Fig. 9, 10. The reactivity ratio for the copolymerization of CMAFU(r_1) and MMA(r_2) is r_1=0.62, r_2=4.20. The reactivity ratio for the copolymerization of CMAFU(r_1) and St(r_2) is r_1=0.15, r_2=0.95.

Table 1. First-order rate constants for hydrolysis of CMAFU in H_2O-Dioxane at ℃

H_2O: Dioxane	k_1 (liter/mole-sec)
1 : 5	3.46×10^{-5}
1 : 3	1.03×10^{-4}
1 : 2	2.14×10^{-4}
1 : 1	6.54×10^{-4}
2 : 1	1.71×10^{-3}
5 : 1	3.22×10^{-3}
10 : 1	4.12×10^{-3}

B. Hydrolysis of monomer and polymer

1. Rate of Hydrolysis by Use of NMR

Figure 6 shows typical examples of NMR spectra before and after hydrolysis. Figure 7 shows the time-conversion curves. As shown in Figure 7, CMAFU was hydrolyzed relatively fast, while the copolymers released 5-FU gradually. In the case of the copolymers almost half of the 5-FU units remained on the polymer chain even after two days. In the case of CMAFU, a first-order plot gave a straight line, and k_1 calculated was 3.67×10^{-5} liter/molesec.

2. Hydrolytic rate by Use of UV

Figure 8 shows the UV spectra of 5-FU and CMAFU λ_{max} and ϵ in the region of 250~300 nm are shown in Table 4.

In the case of CMAFU, the absorption at 300nm was sufficiently strong that the rate of hydrolysis could be measured by following the decrease of absorbance. Figures 3 and 4 show the time-conversion curves and their first-order plots in the hydrolysis of CMAFU in water-dioxane. Table 1

Table 2. Detemimation of monomer reactivity ratios for the copolymerization of CMAFU(M_1) and MMA(M_2). [M]=0.02M/ℓ, α=3.18

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	0.47	0.13	0.22	−0.87	1.69	−3.15	4.87	−0.65	0.35
2	1.00	0.30	1.00	−0.70	3.33	−2.33	6.51	−0.36	0.51
3	1.50	0.52	2.25	−0.40	4.33	−1.15	7.51	−0.15	0.58
4	2.13	0.76	2.54	−0.24	5.97	−0.67	9.15	−0.07	0.65

Table 3. Determination of monomer reactivity ratios for the copolymerization of CMAFU(M_1) and St(M_2). [M]=0.02M/ℓ, $\alpha=1.73$

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	0.47	0,36	0.22	−0.64	0.61	−0.84	2.34	−0.36	0.26
2	1.00	0,58	1.00	−0.42	1.72	−0.72	3.45	−0.21	0.50
3	1.50	0,76	2.25	−2.25	2.96	−0.47	4.69	−0.10	0.63
4	2.13	0,92	4.54	−0.08	4.93	−0.19	6.66	−0.03	0.74

Table 4. UV Spectra($_{max}$ and) of 5-FU and CMAFU

	Solvent	λ_{max}(nm)	$\varepsilon\times10^{-3}$
5-FU	H_2O	265.6	7.19
CMAFU	Dioxane	275.5	8.00

shows their first-order rate constants When the water content was lower, k_1 was in the range of 10^{-4}–10^{-5}, which agreed with the results obtained by using NMR spectrophotometer. When the water content became higher, k_1 increased to reach almost the order of 10^{-2} in the absence of water. As the UV spectra of the copolymer were very close to that of 5-FU and also the solubility in water was very low, their hydrolysis in dioxane-water could not be investigated.

Alternatively, hydrolysis in heterogeneous system was observed. The copolymers were dispersed in 0.5M NaCl solution in order to decrease their solubility in water. Samples were removed periodically. After removing polymer by filtration, the filtrate was diluted to a predetermined amount and the amount of 5-FU released was determined by measuring its absorbance at 265.5nm. The results are shown in Fig. 5. Under these conditions, CMAFU was hydrolyzed almost instantaneously. On the other hand, St/CMAFU and MMA/CMAFU were resistant to hydrolysis and released 5-FU gradually. Especially St/CMAFU gave good results. These results suggested that the hydrophobic character of the polymer as well as the strength of the bond with 5-FU is very important in decreasing the rate of hydrolysis. From the data, it was shown that these copolymers were hydrolyzed much more slowly than CMAFU and released 5-FU very slowly.

CONCLUSION

1. The specific absorptivity of copolymer, k=3+(1−x) 0.008 where x is the weight fraction of poly(CMAFU).
2. The r_1 and r_2 values for the comonomer pair of CMAFU(M_1) and MMA(M_2) are 0.62 and 4.20.

   The r_1 and r_2 values for the comonomer pair of CMAFU(M_1) and St(M_2) are 0.15 and 0.95
3. First-order rate constant for hydrolysis of CMAFU in acetoned$_6$-D_2O(5 : 1) is 3.67×10^{-5} liter/mole·sec.
4. Poly(CMAFU-MMA) and poly(CMAFU-St) were hydrolyzed much more slowly than CMAFU and released 5-FU very slowly.

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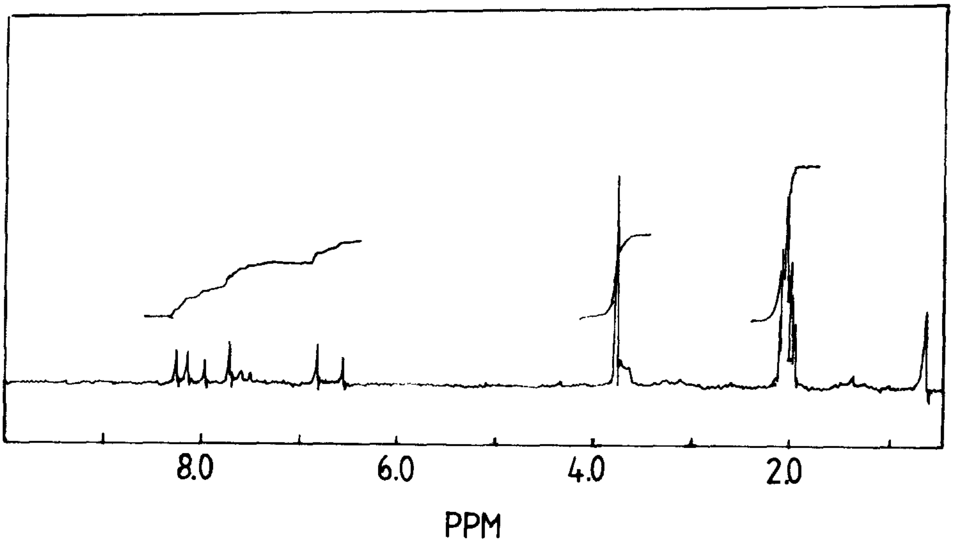

Fig. 1. NMR spectrum of CMAFU(acetone-d_6).

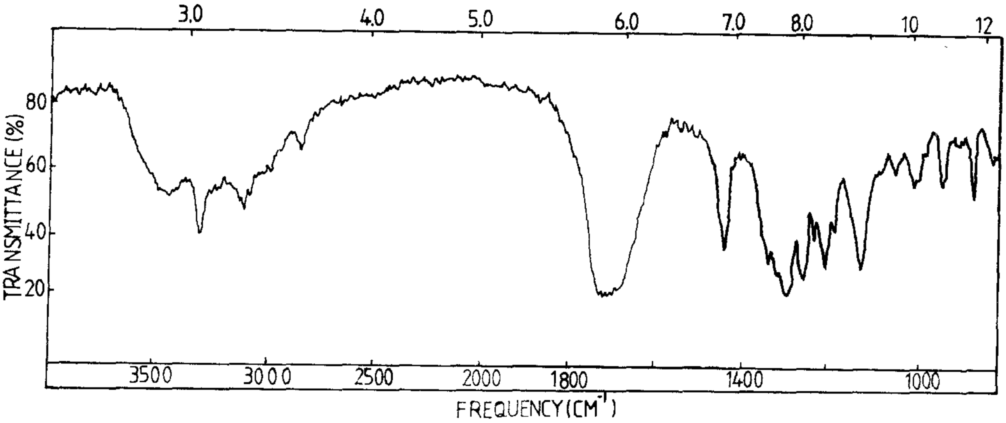

Fig. 2. IR spectrum of CMAFU(Solid phase, KBr).

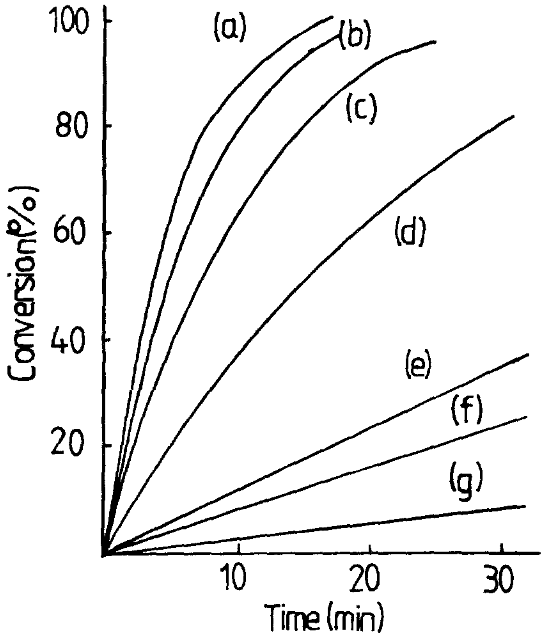

Fig. 3. Hydrolysis of CMAFU in dioxane-H_2O

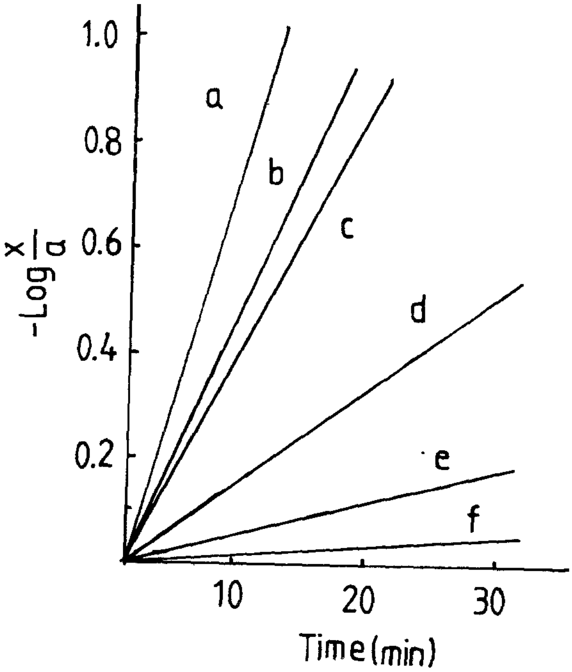

Fig. 4. Hydrolysis of CMAFU in dioxane-H_2O

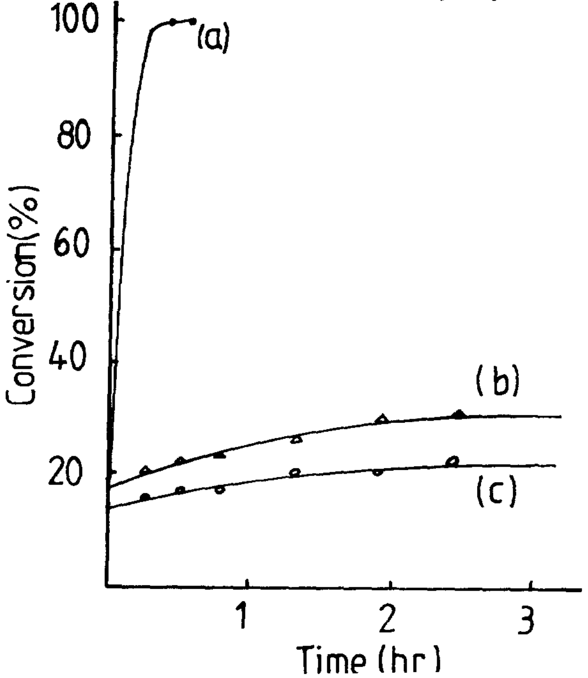

Fig. 5. Hydrolysis of 5-FU-containing compounds dispersed in 0.5 M NaCl (a) CMAFU, (b) MMA/CMAFU, (c) St/CMAFU.

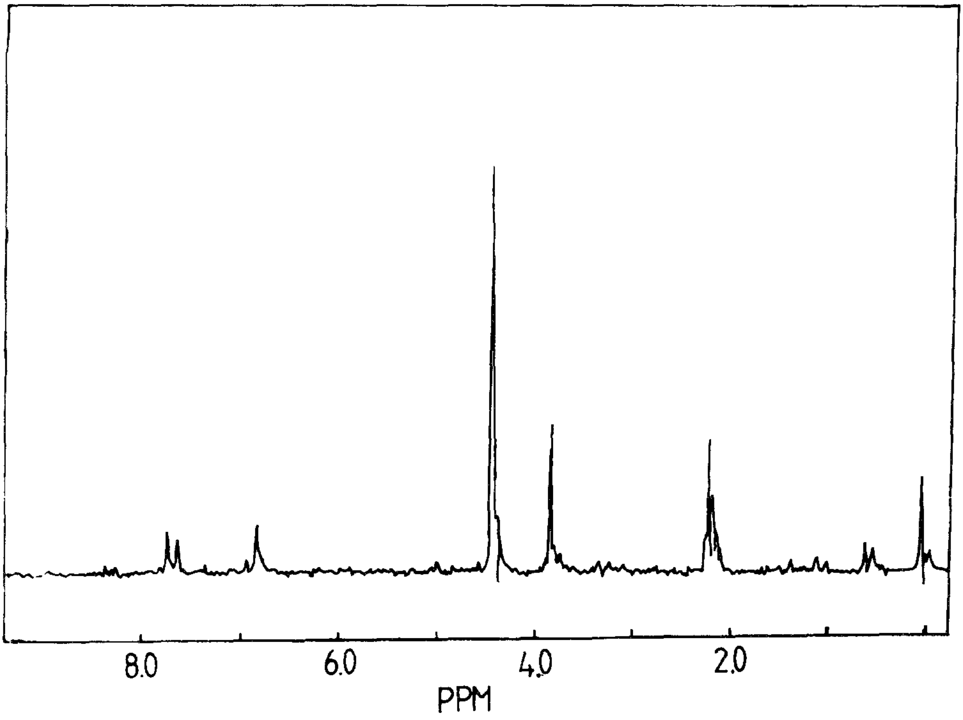

Fig. 6. NMR spectrum of CMAFU after hydrolysis in acetone-d_6-D_2O(5 : 1).

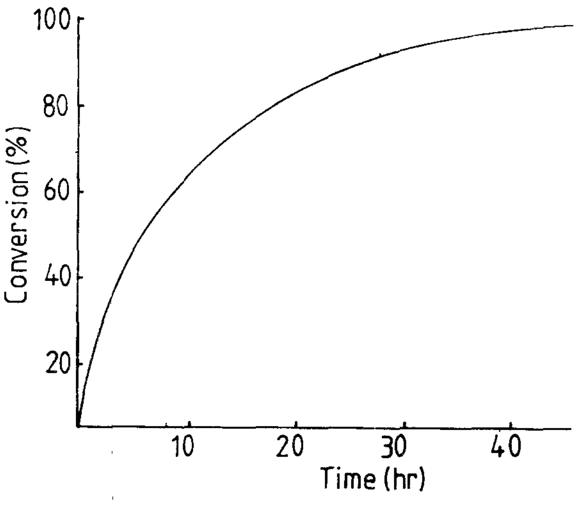

Fig. 7. Hydrolysis in acetone-d_6-D_2O(5 : 1).

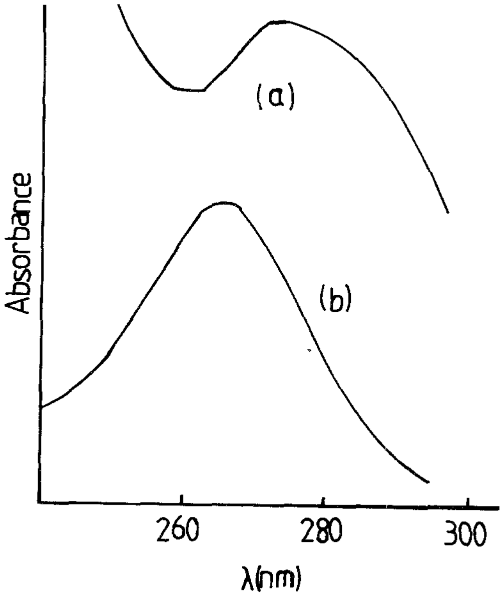

Fig. 8. UV spectra of 5-FU in H_2O (b); CMA-FU in dioxane (a)

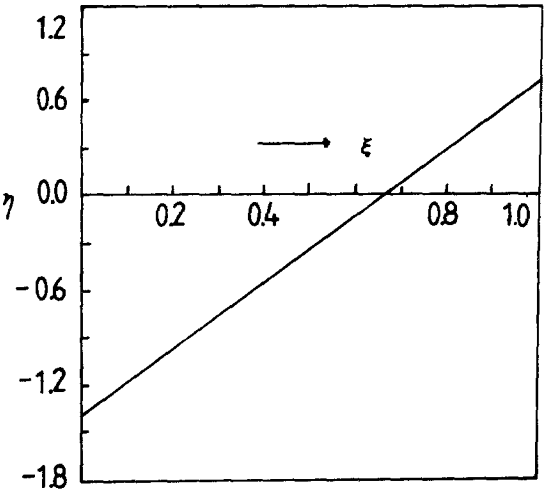

Fig. 9. Kelen-Tüdos plot for the copolymerization of CMAFU(r_1=0.61 and(r_2=4.20 at 60°C.

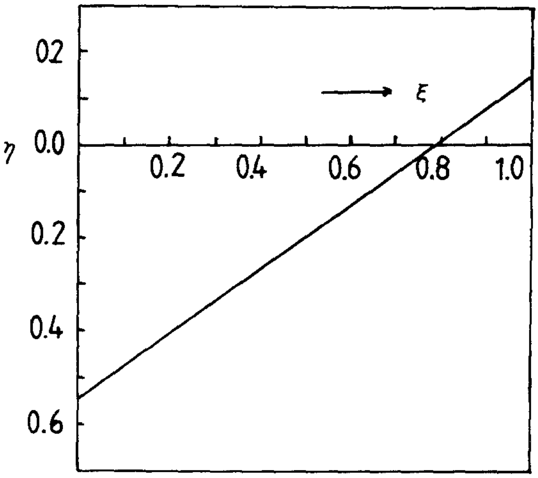

Fig. 10. Kelen-Tüdos plot for the copolymerization of CMAFU(r_1=0.15 and St(r_2=0.95) at 60° C.