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Mitsuru Akashi[a]; Koichi Beppu[a]; Ikuo Kikuchi[a]; Noriyuki Miyauchi[a]
[a] Department of Applied Chemistry Faculty of Engineering, Kagoshima University, Kagoshima, Japan

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Synthesis and Properties of Hydrophilic Copolymers Containing 5-Fluorouracil, Thymine, or Adenine

MITSURU AKASHI,* KOICHI BEPPU, IKUO KIKUCHI, and NORIYUKI MIYAUCHI

Department of Applied Chemistry
Faculty of Engineering
Kagoshima University
1-21-40 Korimoto, Kagoshima 890, Japan

ABSTRACT

Hydrophilic copolymers containing 5-fluorouracil (5-FU), thymine, or adenine were prepared by the free-radical copolymerization of methacryloyl-type monomers containing them with water-soluble vinyl monomers such as acrylic acid, methacrylic acid, vinylpyrrolidone, acrylamide, and 4(5)-vinylimidazole with AIBN as initiator. Complex formation between the copolymers and RNA and between the copolymers having complementary nucleic acid bases in aqueous solution and a DMSO-ethylene glycol was studied by means of UV spectroscopy. These copolymers were found to release the N-hydroxyethyl derivatives of 5-FU, thymine, or adenine by hydrolysis of the ester of the polymer side chain under mild conditions. The effects of the kind of water-soluble comonomer, temperature, pH, and the imidazole group as catalyst on the hydrolysis of the ester are discussed.

*To whom correspondence should be addressed.

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INTRODUCTION

Polymers containing nucleic acid bases such as adenine, uracil, thymine, and their derivatives have been of interest in the field of biological and biomedical applications of synthetic polymers [1-4]. Poly(vinyladenine), for instance, forms complexes with natural RNA, polyU, and poly(vinyluracil) similar to those of polyA with polyU [3, 5-7]. Methacryloyl-type polymers containing nucleic acid bases also have been found to form complexes between complementary base polymers through the hydrogen bond interaction as in biological systems [8].

Since the general idea of polymeric drugs was proposed by Ringsdorf [9], research on the design and preparation of them has become very active. Some of the synthetic analogs of nucleic acids [3] have already been found to have pharmaceutical activity [3, 10]. Further, since some of the nucleic acid base derivatives, such as 5-FU and mercaptopurine, are highly pharmaceutically active [11], polymers having such nucleic acid base derivatives have also become important as polymeric drugs [12].

In the present paper we describe the synthesis and properties of hydrophilic copolymers containing 5-FU, thymine, or adenine as a first part of our developing study of polymeric drugs. Since most of the drug molecules have reactive functional groups, free-radical polymerization would be more suitable for the preparation of polymeric drugs than ionic or condensation polymerization. When a monomeric drugs, such as 5-FU, is introduced into a macromolecule, the pharmaceutically active group must be released from the macromolecule to realize its high activity. Methacryloyl-type monomers of 5-FU, thymine, or adenine were, therefore, selected for the design of a polymeric drug. They can easily copolymerize with various vinyl monomers which can control the solubility, and their ester group can be expected to be hydrolyzed by suitable catalysts or enzymes.

EXPERIMENTAL

Materials

N-β-Methacryloyloxyethyl derivatives of thymine (MAOT) and adenine (MAOA), and N-β-acryloyloxyethylthymine (AOT) were prepared according to methods reported earlier [13, 14]. Vinylimidazole (VIm) was prepared by the method described in the literature [15]. All the reagents and solvents were purified in the usual manner. The polymerization was carried out in sealed tubes in vacuo, after repeated degassing, in the presence of AIBN in 10 mL of solvent.

N-β-Methacryloyloxyethyl-5-fluorouracil (MAOFU)

To a solution of 5.5 g (20 mmol) of 5-fluoro-2,4,-bis(trimethylsiloxy)pyrimidine (1) in 100 mL of dry acetonitrile, 5.8 g (30 mmol) of 2-bromoethyl methacrylate was added at reflux for 10 days. After acetonitrile was distilled off, 100 mL of methanol was added to the reaction mixture to decompose the silylated compounds. After filtration, 0.98 g (20%) of MAOFU was crystallized from water (colorless needles, mp 143-144°C).

Analysis: Calculated for $C_{10}H_{11}N_2O_4F$ (242.2): C, 49.59; H, 4.58; N, 11.57%. Found: C, 49.68; H, 4.69; N, 11.55%, IR (KBr): 1 170 cm^{-1} (ν–C–O of ester), 1 640 cm^{-1} (ν–C=C) of vinyl). ^{1}H-NMR (100 MHz, DMSO-d_6) δ: 1.85 (s, CH_3 on vinyl), 3.96 (t, CCH_2O), 4.32 (t,NCH_2C), 5.68 (s, $C=C\overset{H_a}{\diagup}$), 6.04 (s, $C=C\underset{H_b}{\diagdown}$), 8.10 (d, 6H of pyrimidine ring), 11.78 (s, 3-NH of pyrimidine ring), UV (ethanol) λ_{max}: 272.5 nm (ϵ = 7 400).

N-β-Hydroxyethyl-5-fluorouracil

To 2.7 g (10 mmol) of (1) was added 3.4 g (20 mmol) of 2-bromoethyl acetate, and the solution was stirred at 60°C for 10 days. Then, 100 mL of methanol and 10 mL of 6 N HCl were added to the reaction mixture. After additional refluxing for 1 h, the methanol was distilled off under vacuum to provide a crystalline product. Recrystallization from ethanol gave 0.54 g (31%) of the product (colorless cubes, mp 158-159°C) (mp 159-160°C [16])). The UV and NMR data agreed with those in the literature [16].

Spectroscopy

IR and NMR spectra were measured on a JASCO A-3 spectrophotometer and JNM-4H-100(JEOL) instrument, respectively.

UV spectra in ethanol, a DMSO-ethylene glycol mixture (3:2, v/v) and an aqueous solution were measured by use of a Hitachi 200-20 spectrometer with a temperature controller. Absorbance values given were obtained in a 10-mm quartz cell at 0.1 mmol/L total concentration (monomer units). Hypochromicity was calculated by following equation [17]:

$$\text{Hypochromicity } (\%) = 100[1 - I^{a+b}/(mI^a + nI^b)],$$

where a and b are the interactants and m and n are their volume fractions, while I^a, I^b, and I^{a+b} are the absorbances for solutions of a, b, and their combination, respectively.

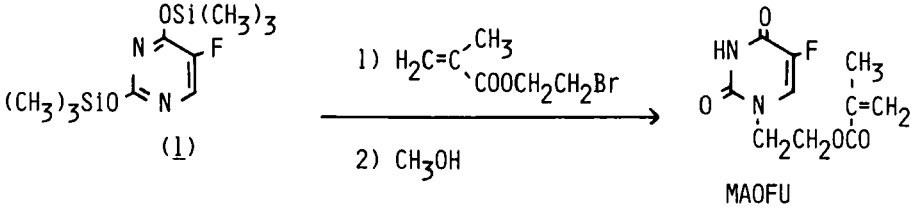
Hydrolysis of Copolymers

Hydrolysis of copolymers was conducted in a 20-mL test tube containing ~8 mg of copolymer in 10 mL of appropriate buffer solution with shaking for a given time at 25, 37, and 60°C. Copolymers and their hydrolyzates were separated by thin layer-chromatography (TLC) (Kieselgel $60F_{254}$, Merck) after collecting 0.5 mL of the mixture. The isolated hydrolyzate was dissolved in 8 mL of methanol, and its concentration was determined by optical density reading in a UV spectrometer at 271 nm for the N-β-hydroxyethyl derivatives of 5-FU and thymine and at 262 nm for the N-β-hydroxyethyl derivatives of adenine.

RESULTS AND DISCUSSION

Synthesis and Polymerization of Vinyl Monomers Containing 5-FU

For the preparation of MAOFU, which is a polymerizable monomer containing 5-FU, the silylated 5-FU (1) was allowed to react with 2-bromoethyl methacrylate in acetonitrile at refluxing temperature, followed by decomposition of the silylated compound in a manner similar to that described previously [13].



When no solvent was used, the yield was extremely low, as for the reaction of silylated uracil with 2-bromoethyl methacrylate [13].

MAOFU was polymerized in the presence of AIBN, and the results are shown in Table 1. The polymerizability of MAOFU was almost comparable to that of the other methacryloyl-type monomers containing thymine and adenine [18]. The resulting polymer, polyMAOFU, was colorless, amorphous, and soluble in DMSO, DMF, and pyrimidine.

Synthesis of Hydrophilic Copolymers

Most of the polymers containing nucleic acid bases are insoluble in water due to the hydrophobic purine and pyrimidine moiety [3]. In order

TABLE 1. Polymerization of MAOFU, MAOT, and MAOA

Monomer	Solvent	Conversion, %[a]
MAOFU	Ethanol	73
	Dioxane	56
	DMSO	72
	DMF	42
MAOT	Ethanol	76
MAOA	Ethanol	76
	Dioxane	54

[a][Monomer] = 40 mmol/L, [AIBN] = 2 mmol/L, 10 mL of solvent, 60°C, 6 h.

to examine the biological and biomedical characters of the polymers and their complexing abilities with natural polymers such as DNA and RNA, the hydrophilic nature of the polymers containing 5-FU, thymine, or adenine could be significant. When hydrophilic polymers containing methacryloyl-type monomers are to be prepared, copolymerization with water-soluble vinyl monomers is one possible approach. In the present case, MAOFU, MAOT, AOT, and MAOA were copolymerized with acrylamide (AAm), acrylic acid (AA), methacrylic acid (MA), and vinylpyrrolidone (VP) in the presence of AIBN at 60°C. The results are summarized in Table 2. The copolymers obtained, except for the copolymer containing VP units, had water solubility sufficient for the present experiments. Electronegative monomers, such as AA and MA, were more effective as solubilizers than electroneutral ones, such as AAm and VP.

Polymer-Polymer Interaction

The functionality of 5-FU, thymine, and adenine of the hydrophilic copolymers was studied by UV spectroscopy. Mixing curves between copolymers and between copolymers and RNA are shown in Figs. 1-3. The interaction between copoly(MAOFU-AAm) (No. 1), copoly(MAOT-AAm) (No. 9), or copoly(MAOA-AAm) (No. 16) with RNA was observed, as shown in Fig. 1. The overall stoichiometry of the complexes was about 1:1 and the hypochromicity was ~2% for the copolymer-RNA systems under the conditions used. The observed interaction was not as strong as for the poly(vinyladenine)-RNA system [5] and polyMAOA-polyMAOT system [8], since the solubilizer, AAm, in the copolymers

TABLE 2. Copolymerization of MAOFU, MAOT, AOT, and MAOA with Water-Soluble Vinyl Monomers

Sample no.	M Monomer, mmol/L			Solvent	Conversion, %[a]	Content, %[b]		
	M_1	M_2	M_3			M_1	M_2	M_3
1	MAOFU (1)	AAm (19)	-	DMF	77	6	94	-
2	MAOFU (2)	AAm (18)	-	DMF	73	12	88	-
3	MAOFU (1)	AA (19)	-	MeOH	27	14	86	-
4	MAOFU (1)	MA (19)	-	MeOH	41	7	93	-
5	MAOFU (2)	MA (18)	-	MeOH	45	15	85	-
6	MAOFU (3)	MA (17)	-	MeOH	49	21	79	-
7	MAOFU (2)	AA (14)	VIm (4)	MeOH	27	12	57	31
8	MAOT (1)	AA (19)	-	MeOH	43	12	88	-
9	MAOT (1)	AAm (19)	-	DMF	68	7	93	-

10	MAOT (6)	AA (14)	-	MeOH	66	43	57	-
11	MAOT (2)	AA (9)	VIm (9)	MeOH	25	8	52	40
12	AOT (10)	AA (10)	-	MeOH	48	35	65	-
13	AOT (2)	AA (9)	VIm (9)	MeOH	19	4	56	40
14	AOT (4)	AA (12)	VIm (4)	MeOH	27	11	56	33
15	MAOA (2)	AA (18)	-	MeOH	43	23	77	-
16	MAOA (1)	AAm (19)	-	DMF	56	8	92	-
17	MAOA (2)	AA (9)	VIm (9)	MeOH	26	5	52	43
18	MAOA (4)	AA (12)	VIm (4)	MeOH	31	18	58	24
19	MAOA (1)	VP (99)	-	DMF	12	16	84	-

[a] [AIBN] = 1 mmol/L, 60°C, 6 h.
[b] Determined by nitrogen analysis and UV.

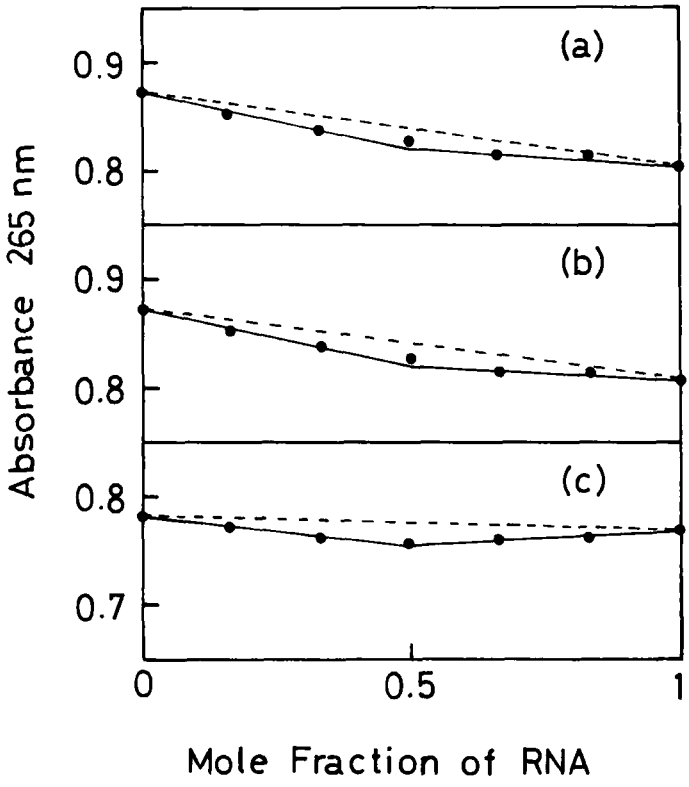


FIG. 1. Mixing curve between copolymers and RNA in water. Absorbance at 265 nm obtained in a 10-mm cell at 20°C. (a) Copoly-(MAOFU-AAm) (No. 1)-RNA system, (b) copoly(MAOT-AAm) (No. 9)-RNA system, (c) copoly(MAOA-AAm) (No. 16)-RNA system.

interrupts the base-base stacking in the polymer side chain and weakens complex formation.

Figure 2 shows the interaction between copoly(MAOT-AAm) (No. 9) and copoly(MAOA-AAm) (No. 9) in water and DMSO-ethylene glycol, respectively. No hypochromicity was observed in water, while hyperchromicity [19] was observed in DMSO-ethylene glycol. These results can be explained by assuming that the nucleic acid bases are isolated along the copolymer chain and difficult to stack intramolecularly, hence it is easy to form complementary base pairs between copolymers through hydrogen bonding. Generally, hydrophobic interactions by base-base stacking are predominant in aqueous solution, and hydrogen bonding interactions are apparently observed in organic solvent [20]. This fact may support the present experimental results.

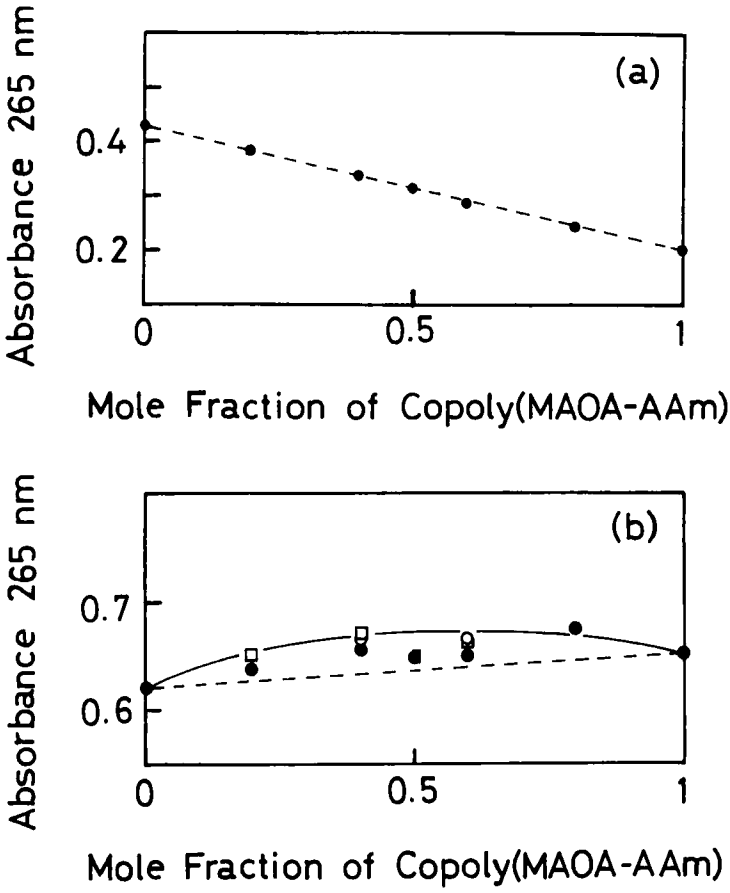


FIG. 2. Mixing curve between copoly(MAOT-AAm) (No. 9) and copoly(MAOA-AAm) (No. 15). Absorbance at 265 nm obtained in a 10-mm cell at 20°C. (a) In water, (b) in DMSO-ethylene glycol mixture (3:2 v/v): (●) after 5 h, (○) after 15 h, (□) after 30 h.

Figure 3 shows the mixing curve between copolymers containing AA and RNA in water. In aqueous solution, purine and pyrimidine bases in copolymers can interact by base-base stacking, as shown in Fig. 1. However, even for copolymers with high contents of nucleic acid bases such as copoly(MAOFU-AA) (No. 3), copoly(MAOT-AA) (No. 8), and copoly(MAOA-AA) (No. 15), no interaction was observed. This is explained by the repulsion between electronegative charges of carboxyl groups in the copolymers and those of phosphate groups in RNA, thus preventing a mutual approach of nucleic acid bases.

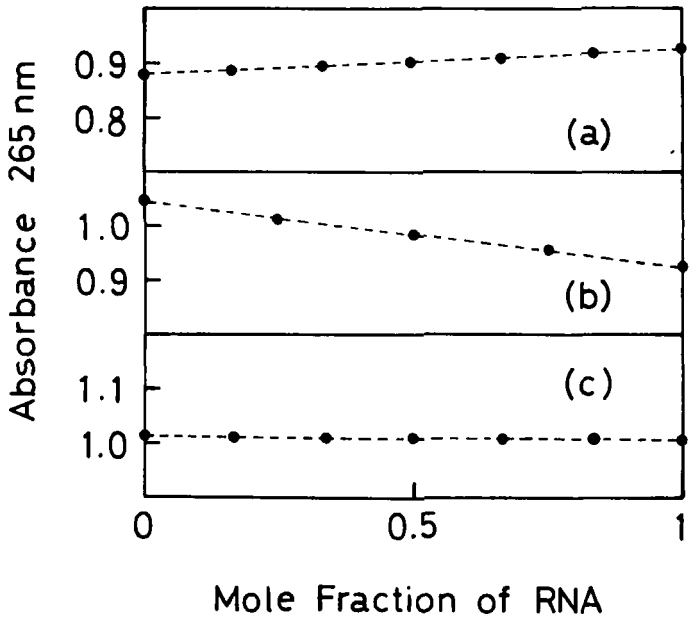


FIG. 3. Mixing curve between copolymers and RNA in a 0.1 M phosphate buffer (pH 7.8). Absorbance at 265 nm obtained in a 10-mm cell at 20°C. (a) Copoly(MAOFU-AA) (No. 3)-RNA system, (b) copoly(MAOT-AA) (No. 8)-RNA system, (c) copoly(MAOA-AA) (No. 15)-RNA system.

Hydrolysis of Copolymers

Homopolymers of methacryloyl-type monomers containing 5-FU, thymine, or adenine were very stable toward acids and bases, while the hydrophilic copolymers of them were found to be degraded in aqueous solution under mild conditions to give the derivatives of purine and pyrimidines. The derivatives released from the copolymers were identified as N-hydroxyethyl derivatives of 5-FU, thymine, or adenine by UV spectroscopy after isolation by preparative TLC. This means that the ester groups of the polymer side chains were hydrolyzed in aqueous solution.

Figure 4 shows the degree of hydrolysis of copoly(MAOT-AA) (No. 10), copoly(AOT-AA) (No. 12), and copoly(MAOA-AA) (No. 15) at 60°C in a 0.1-M phosphate buffer solution (pH 7.8) as a function of time. Acryloyl-type copolymer (copoly(AOT-AA)) was hydrolyzed rather easily, but no significant difference between the kinds of leaving group was observed. The flexibility of the polymer chain is sup-

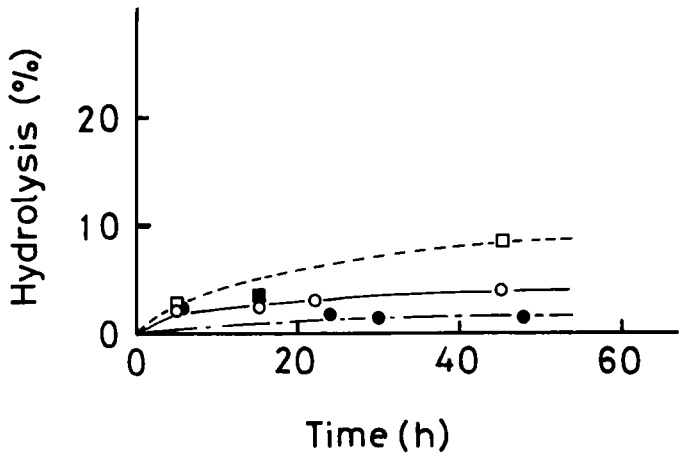


FIG. 4. Hydrolysis of copoly(MAOT-AA) (No. 10) (○), copoly-(AOT-AA) (No. 12) (□), and copoly(MAOA-AA) (No. 15) (●) at 60°C in a 0.1 M phosphate buffer (pH 7.8). Concentration: 0.8 mg/mL.

posed to be an important factor for the hydrolysis of the polymer side chain.

The hydrolysis of copolymers containing VIm as the third component, copoly(MAOT-AA-VIm) (No. 11), copoly(AOT-AA-VIm) (No. 13), and copoly(MAOA-AA-VIm) (No. 17), is shown in Fig. 5. These copolymers were easily degraded to give the hydroxyethyl derivatives, compared to the hydrophilic copolymers consisting of AA and methacryloyl-type monomers. It can be assumed that the imidazole group of the polymer side chain plays a role in the ester hydrolysis as seen in the enzyme model system containing polyVIm [21].

To elucidate the role of the imidazole group, the concentration dependence of the hydrolysis of copoly(AOT-AA-VIm) (No. 14) and copoly(MAOA-AA-VIm) (No. 18) was studied and the results are shown in Fig. 6. Evidently the hydrolysis does not depend on the copolymers concentration. Therefore, it can be concluded that the ester is hydrolyzed intramolecularly. The fact that the VIm unit of the polymer side chain does not catalyze the hydrolysis of the ester of another polymer side chain [22] also supports intramolecular catalysis by the neighboring imidazole group via six-membered ring formation as shown in Scheme 1.

Figure 7 shows the pH dependence of the hydrolysis of copoly-(MAOA-AA-VIm) (No. 18) at 37°C. As shown in this figure, hydrolysis proceeds more efficiently at higher pH. This is considered to be due to the increase of the nucleophilicity of the imidazole group as a hydrolysis catalyst at higher pH.

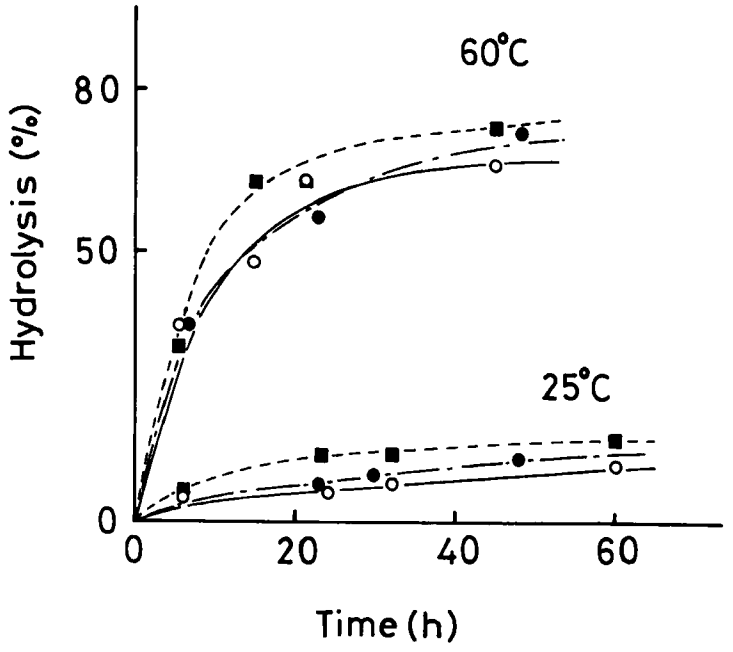
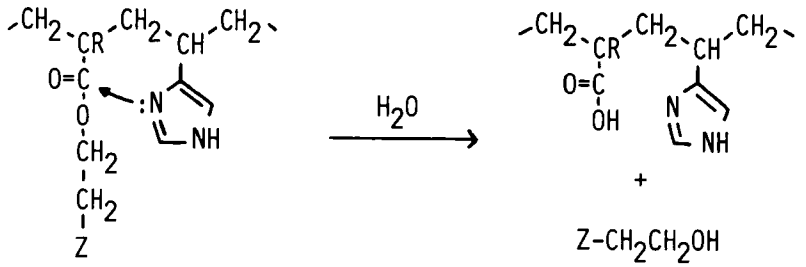


FIG. 5. Hydrolysis of copoly(MAOT-AA-VIm) (No. 11) (●), copoly-(AOT-AA-VIm) (No. 13) (■), and copoly(MAOA-AA-VIm) (No. 17) (○) at 60 and 25°C in a 0.1 M phosphate buffer (pH 7.8). Concentration: 0.8 mg/mL.



R; H or CH_3

Z-H; 5-FU, Thymine, Adenine

SCHEME 1.

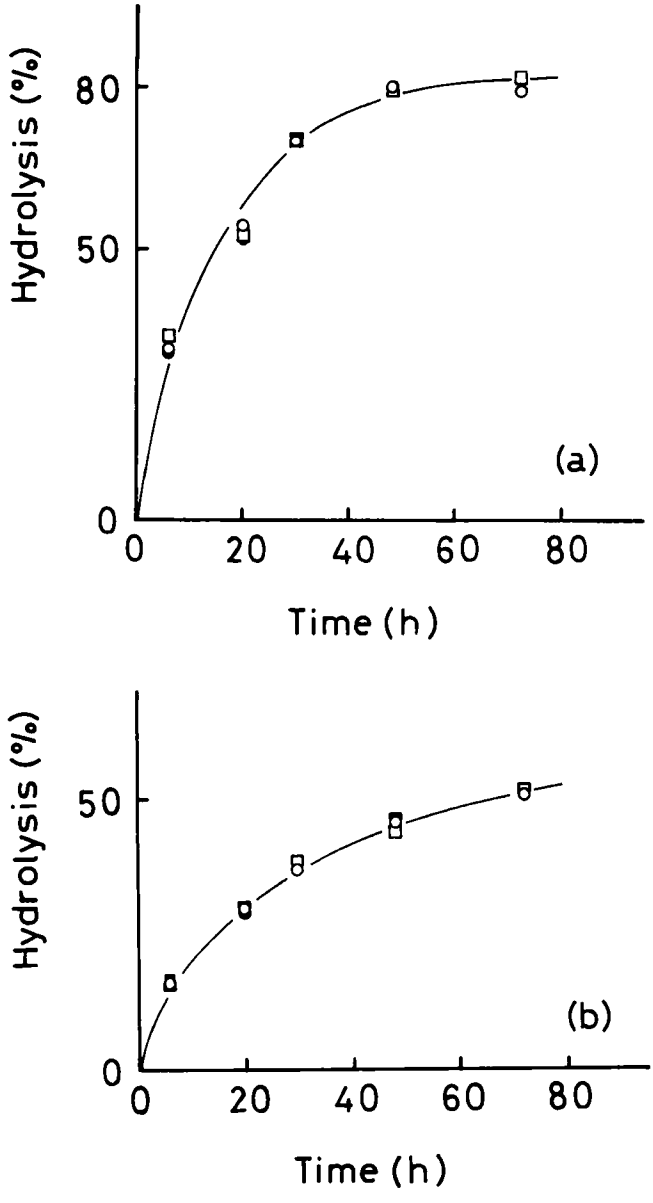


FIG. 6. Concentration dependence of the hydrolysis of copoly(AOT-AA-VIm) (No. 14) and copoly(MAOA-AA-VIm) (No. 18) at 60°C in a 0.1 M phosphate buffer (pH 7.8). (a) copoly(AOT-AA-VIm) (No. 14), (b) copoly(MAOA-AA-VIm) (No. 18). Concentration: (○) 0.8 mg/mL, (□) 0.4 mg/mL, (●) 0.2 mg/mL, (■) 0.1 mg/mL.

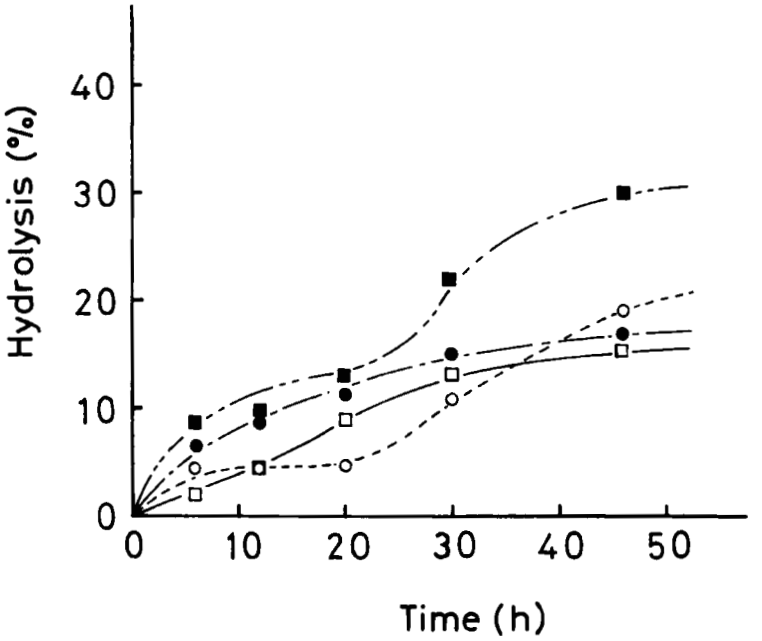


FIG. 7. pH dependence the hydrolysis of copoly(MAOA-AA-VIm) (No. 18) at 37°C. Concentration: 0.8 mg/mL. (○) pH 7.4 in a 0.1 M phosphate buffer, (□) pH 7.8 in a 0.1 M phosphate buffer, (●) 8.5 in a 0.05-M boric acid buffer, (■) pH 9.0 in a 0.05 M boric acid buffer.

Figure 8 shows the results of hydrolysis of hydrophilic copolymers having 5-FU, copoly(MAOFU-AAm) (No. 2), copoly(MAOFU-AA) (No. 3), copoly(MAOFU-MA) (No. 4), copoly(MAOFU-MA) (No. 5), copoly-(MAOFU-MA) (No. 6), and copoly(MAOFU-AA-VIm) (No. 7). As shown in the figures, the hydrolysis is dependent on the kind of solubilizer, its concentration, and the temperature. The hydrolysis of the copolymer containing AAm units took place very rapidly. This could be because hydroxyl anion can approach the ester group of the electroneutral copolymer rather easily. In contrast, the electronegative charges of the carboxyl groups in the AA and MA units cause intermolecular and intramolecular replusion, and the approach of the hydroxyl anion to the ester group could be prevented. Furthermore, increasing content of the solubilizer and/or rising temperature could facilitate the motions of the polymer chain to enhance the hydrolysis. When a VIm unit was used as the third component in the copolymers, the hydrolysis proceeded readily, similar to that or hydrophilic copolymers with adenine and thymine units.

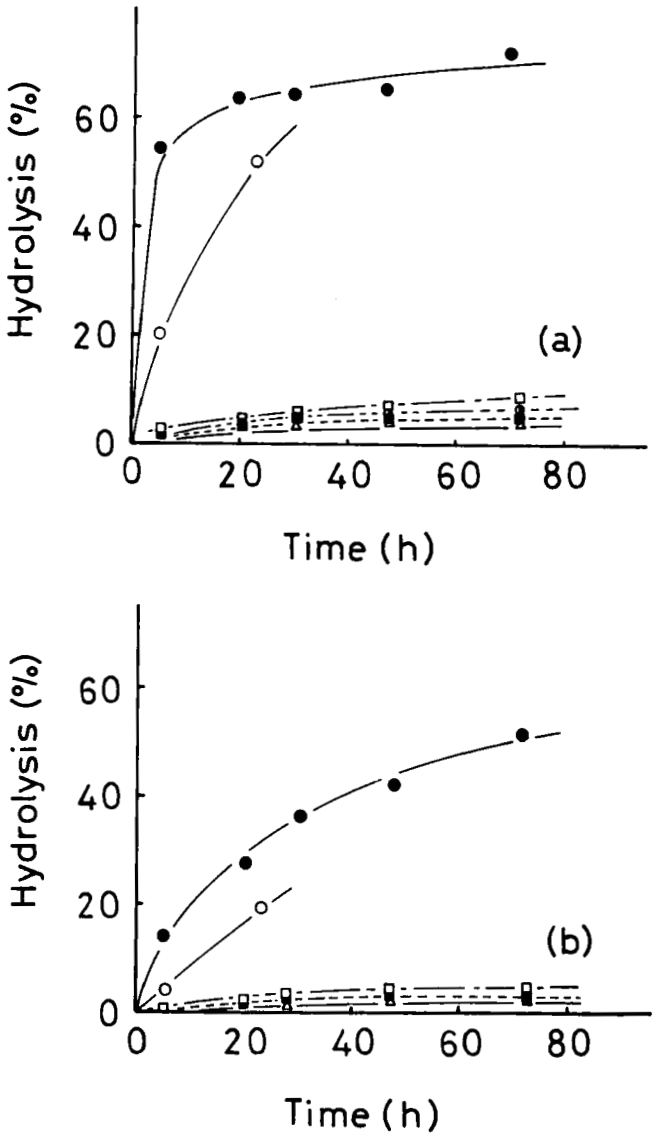


FIG. 8. Hydrolysis of copolymers having 5-FU in a 0.1 M phosphate buffer (pH 7.8). Concentration: 0.8 mg/mL. (a) At 60°C, (b) at 37°C. (●) Copoly(MAOFU-AAm) (No. 2), (△) copoly(MAOFU-AA) (No. 3), (□) copoly(MAOFU-MA) (No. 4), (◑) copoly(MAOFU-MA) (No. 5), (■) copoly(MAOFU-MA) (No. 6), (○) copoly(MAOFU-AA-VIm) (No. 7).

N-β-Hydroxyethyl-5-FU eliminated as a hydrolyzate is pharmaceutically active [23]. Consequently, the present hydrophilic copolymers containing 5-FU can be regarded as polymeric drugs since the 5-FU derivative in them can be released by hydrolysis under mild conditions similar to those in biological systems. Furthermore, this release can be controlled by changing the kind and the content of the solubilizer and the catalytic unit, such as imidazole.

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REFERENCES

[1] K. Takemoto, J. Polym. Sci., Polym. Symp., 55, 105 (1976).
[2] K. Takemoto, in Polymeric Drugs (L. G. Donaruma and O. Vogl, eds.), Academic, New York, 1978, p. 103.
[3] J. Pitha, M. Akashi, and M. Draminski, in Biomedical Polymer; Polymeric Materials and Pharmaceuticals for Biomedical Use (E. P. Goldberg and A. Nakajima, eds.), Academic, New York, 1980, p. 271.
[4] K. Takemoto and Y. Inaki, Adv. Polym. Sci., 41, 1 (1981).
[5] H. Kaye, J. Am. Chem. Soc., 92, 5777 (1970).
[6] J. Pitha, P. M. Pitha, and P. O. P. Ts'o, Biochem. Biophys. Acta, 204, 39 (1970).
[7] K. Kondo, H. Iwasaki, K. Nakatani, N. Ueda, K. Takemoto, and M. Imoto, Makromol. Chem., 125, 42 (1969).
[8] M. Akashi, T. Okimoto, Y. Inaki, and K. Takemoto, J. Polym. Sci., Polym. Chem. Ed., 17, 905 (1979).
[9] H. Ringsdorf, J. Polym. Sci., Polym. Symp., 51, 135 (1975).
[10] A. G. Ludwick and C. G. Overberger, J. Polym. Sci., Polym. Chem. Ed., 20, 2139 (1982).
[11] K. Sakaki, S. Furusawa, K. Monma, and G. Takayanagi, Chem. Pharm. Bull., 31, 2451 (1983).
[12] P. P. Umrigar, S. Ohashi, and G. B. Butler, J. Polym. Sci., Polym. Chem. Ed., 17, 351 (1979).
[13] M. Akashi, Y. Kita, Y. Inaki, and K. Takemoto, Makromol. Chem., 178, 1211 (1977).
[14] Y. Kita, Y. Inaki, and K. Takemoto, J. Polym. Sci., Polym. Chem. Ed., 18, 427 (1980).
[15] C. G. Overberger and N. Vorchheimer, J. Am. Chem. Soc., 85, 951 (1963).
[16] M. Gacek and K. Undheim, Acta Chem. Scand., B33, 515 (1979).
[17] I. Tinoko Jr., J. Am. Chem. Soc., 82, 4785 (1960).

[18] M. Akashi, Y. Inaki, and K. Takemoto, Makromol. Chem., 178, 535 (1977).
[19] G. J. Thomas Jr. and Y. Kyogoku, J. Am. Chem. Soc., 89, 4170 (1967).
[20] M. Akashi, Y. Kita, Y. Inaki, and K. Takemoto, J. Polym. Sci., Polym. Chem. Ed., 17, 301 (1979).
[21] C. G. Overberger, T. St. Pierre, C. Yaroslavsky, and S. Yaroslavsky, J. Am. Chem. Soc., 84, 1184 (1964).
[22] H. Morawetz and W. R. Song, Ibid., 88, 5714 (1966).
[23] K. Beppu, I. Kikuchi, M. Akashi, and N. Miyauchi, Polym. Prep. Jpn., 32, 1863 (1983).

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