

## Functional Monomers and Polymers CXXIII.<sup>†</sup> Interaction of Polymethacrylates Containing Nucleic Acid Bases: Effect of Methylation of Nucleic Acid Bases

Shi-Bi FANG,\* Yoshiaki INAKI, and Kiichi TAKEMOTO

*Faculty of Engineering, Osaka University,  
Suita, Osaka 565, Japan*

(Received May 10, 1984)

**ABSTRACT:** A study was made of the interaction of the polymethacrylate derivatives of nucleic acid bases, particularly the effect of methylation of nucleic acid bases in the polymers. One of the complementary polymers is a copolymer containing uracil and 3-methyluracil units, and the other, a copolymer containing adenine and *N*<sup>6</sup>-methyladenine units. For the interaction of these copolymers, an optimum content of the methylated nucleic acid base unit in the copolymer was found. This was explained by opposite factors: an inhibition of hydrogen bonding and increase in compatibility or mutual solubility of two kinds of the copolymers.

**KEY WORDS** Interaction / Polymer Complex / Polymethacrylates / Nucleic Acid Bases / Uracil / Adenine /

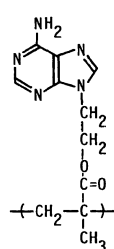
The most essential function of nucleic acids is based on the formation of specific base-base pairing through hydrogen bonding between purine and pyrimidine bases. It has recently been found that the generation of carcinogenesis is connected with the loss of such specific base-base interaction of nucleic acid bases.<sup>1-4</sup> It is therefore of interest to study the effects of the alkylation of nucleic acid bases on the specific interaction.

A number of nucleic acid analogs have been prepared, and the specific interactions between these analogs have been studied.<sup>5</sup> It has been found that these synthetic polymers containing nucleic acid bases cause the specific interaction even in organic solvent by hydrogen bonding. In a series of our preceding papers,<sup>6,7</sup> the methylation reactions of polymethacrylates containing nucleic acid bases were studied, as well as the specific interactions of these polymers.

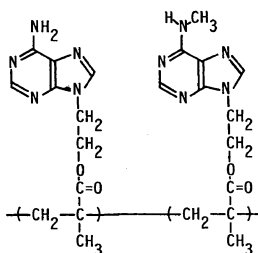
Poly[9-(2-methacryloyloxyethyl)adenine] (**1**; polyMAOA) was methylated by methyl iodide, and was found to give the methylated polyMAOA (Me-polyMAOA) containing both 1-methyladenine and 1,*N*<sup>6</sup>-methyladenine units. From the study of the interaction of the methylated polymer, Me-polyMAOA, with complementary poly[1-(2-methacryloyloxyethyl)uracil] (**3**; polyMAOU), it was found that the interaction ability of Me-polyMAOA decreased with increasing methylated adenine units in the polymer.<sup>6</sup> The methylation of polyMAOU gave the methylated polyMAOU (Me-polyMAOU) containing 3-methyluracil units, and the interaction of Me-polyMAOU was studied with polyMAOA. In contrast to the case of Me-polyMAOA, it was found that the interaction ability of Me-polyMAOU increased with increasing methylated uracil units in the polymer, and an optimum content of methylated uracil units in

<sup>†</sup> For Part CXXII, see S. Fang, Y. Inaki, and K. Takemoto, *Polym. J.*, **17**, 433 (1985).

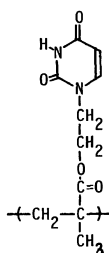
\* Present Address: *Institute of Chemistry, Academia Sinica, Beijing, China.*



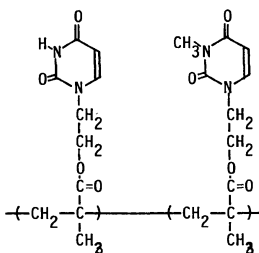
1  
Poly(MAOA)



2  
Poly(MAOA-co-Me-MAOA)



3  
Poly(MAOU)



4  
Poly(MAOU-co-Me-MAOU)

the polymer was observed.<sup>7</sup>

In the preceding paper,<sup>8</sup> a copolymer containing adenine and *N*<sup>6</sup>-methyladenine units was prepared by the free radical copolymerization of the corresponding monomers, which is poly[9-(2-methacryloyloxyethyl)adenine-*co*-*N*<sup>6</sup>-methyl-9-(2-methacryloyloxyethyl)adenine] (2; poly(MAOA-*co*-Me-MAOA)). The uracil containing copolymer was also prepared: poly[1-(2-methacryloyloxyethyl)uracil-*co*-1-(2-methacryloyloxyethyl)-3-methyluracil] (4; poly(MAOU-*co*-Me-MAOU)). The present paper concerns the interaction behavior of these copolymers studied by UV spectroscopy. The object of this study is to clarify the effects of the methylation of nucleic acid bases on their specific interaction, and the differences in polymer structure between the methylated polymer ob-

tained by a polymer reaction such as Me-polyMAOU and a copolymer such as poly(MAOU-*co*-Me-MAOU).

## EXPERIMENTAL

### Materials

PolyMAOA (1), poly(MAOA-*co*-Me-MAOA) (2), polyMAOU (3), and poly(MAOU-*co*-Me-MAOU) (4) were prepared as reported in the preceding paper.<sup>8</sup>

### Interaction between the Polymers

Interaction of the polymers was estimated by hypochromicity values in the UV spectra as reported previously.<sup>6,7</sup> The UV spectra were measured with a UNION SM-401 spectrophotometer equipped with a temperature controller at 25°C. The adenine containing polymer was dissolved in ethylene glycol (EG), and the uracil containing polymer in dimethyl sulfoxide (DMSO) as a stock solution. These solutions were mixed to give a polymer mixture of 10<sup>-4</sup> mol l<sup>-1</sup> total concentration of the nucleic acid base units in DMSO-EG (3:2, v/v). The solution thus prepared was kept for 2 days at 50°C, and for 7 days at 25°C in order to measure the UV spectra.

## RESULTS AND DISCUSSION

There has been extensive discussion on the intermolecular and intramolecular interactions in a series of natural and synthetic polynucleotides, and their synthetic analogs.<sup>5</sup> The intramolecular interaction of nucleic acid bases in the polymer influences the formation of the intermolecular polymer complex. A strong intramolecular interaction inhibits the intermolecular polymer complex formation. On the other hand, an intramolecular interaction such as a stacking interaction makes the intermolecular polymer complex stable, following formation of the polymer complex. For example, polyuridylic acid (polyU) does not exist in an ordered structure at room tem-

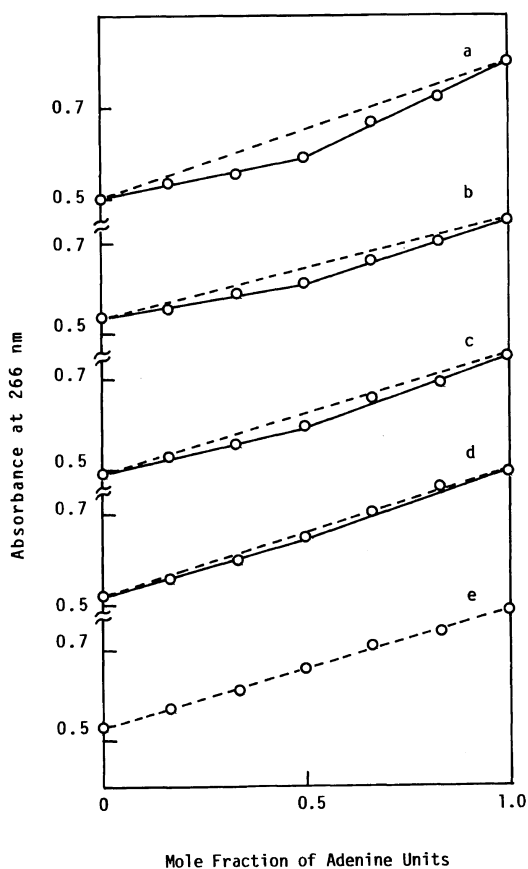
perature, and consequently forms a stable polymer complex with a synthetic polymer having pendant adenine bases.<sup>9</sup> In contrast, polyadenylic acid (poly A) is known to form a double or single stranded helix at room temperature by the stacking interaction, and has low ability to form a polymer complex with a synthetic polymer having pendant thymine bases.<sup>9</sup>

The same was also observed for the interaction of the polymethacrylates containing adenine and uracil (or, thymine) bases.<sup>10</sup> The intermolecular interaction of the polymers, that is the polymer complex formation, could be affected by stereoregularity of the polymers, temperature of the system, and solvent used. These facts are closely related to the intramolecular interaction of the nucleic acid bases in the polymer chain.<sup>10</sup> From the study of the interaction of Me-polyMAOU obtained by a polymer reaction, the interaction between polyMAOU and polyMAOA was found to be greatly enhanced by methylation of the uracil units in polyMAOU as mentioned above.<sup>7</sup> This is also related to the intramolecular interaction of uracil bases in polyMAOU; a partial methylation of polyMAOU releases uracil bases from the self-association, and allows the polymer to form a stable intermolecular polymer complex with polyMAOA.<sup>7</sup>

From these considerations, the interaction of the copolymers containing methylated nucleic acid bases are fully discussed in this paper, with attention directed to the intermolecular and the intramolecular interactions of the nucleic acid bases.

#### *Interaction of PolyMAOA with Poly(MAOU-co-Me-MAOU)*

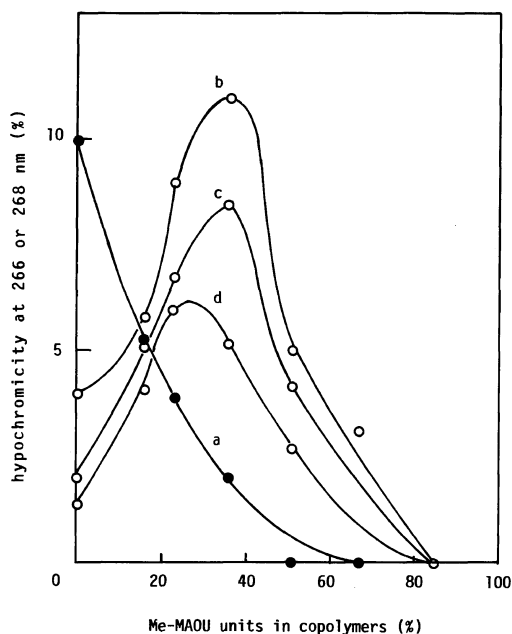
The mixing curves obtained for the system of polyMAOA with poly(MAOU-co-Me-MAOU) are shown in Figure 1. The stoichiometry of the polymer complex estimated from the highest hypochromicity was 1:1 (adenine : uracil), and independent of the Me-



**Figure 1.** Mixing curves between polyMAOA and poly(MAOU-co-Me-MAOU) in DMSO-EG (3:2, v/v) at 25°C. Content of Me-MAOU units in poly(MAOU-co-Me-MAOU) (%): a) 0; b) 16; c) 23; d) 36; e) 51.

MAOU unit content in poly(MAOU-co-Me-MAOU). In the case of the polyMAOA-Me-polyMAOU,<sup>7</sup> the stoichiometry of the polymer complex was always adenine > uracil. Thus, the distribution of Me-MAOU units in Me-polyMAOU may differ from that in poly(MAOU-co-Me-MAOU).

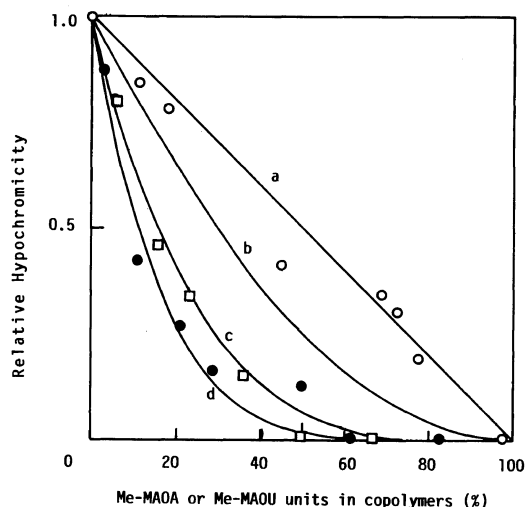
The highest hypochromicity of the mixing curve in Figure 1 is plotted against the content of Me-MAOU units in the copolymer (a in Figure 2). The values shown in Figure 2 (a) tend to decrease with increasing Me-MAOU units in the copolymer. This is quite different from that of the system of Me-polyMAOU with polyMAOA,<sup>7</sup> where the hypochromicity



**Figure 2.** Hypochromicity for the polyMAOA-poly(MAOU-*co*-Me-MAOU) system (a), and the poly(MAOA-*co*-Me-MAOA)-poly(MAOU-*co*-Me-MAOU) systems (b-d). Content of Me-MAOA units in poly(MAOA-*co*-Me-MAOA) (%): a) 0; b) 10; c) 29; d) 50.

value increased largely with an increase in the methylated uracil units in Me-polyMAOU up to 20% methylation. This can be explained by the considerable decrease in intramolecular interaction of the uracil bases in polyMAOU at this range of methylation followed by conformational transition of the polymer observed from the viscosity measurement for the methylated polymer.<sup>7</sup> For poly(MAOU-*co*-Me-MAOU), however, it was difficult to show conformational transition of the copolymers by increasing the Me-MAOU units.<sup>8</sup>

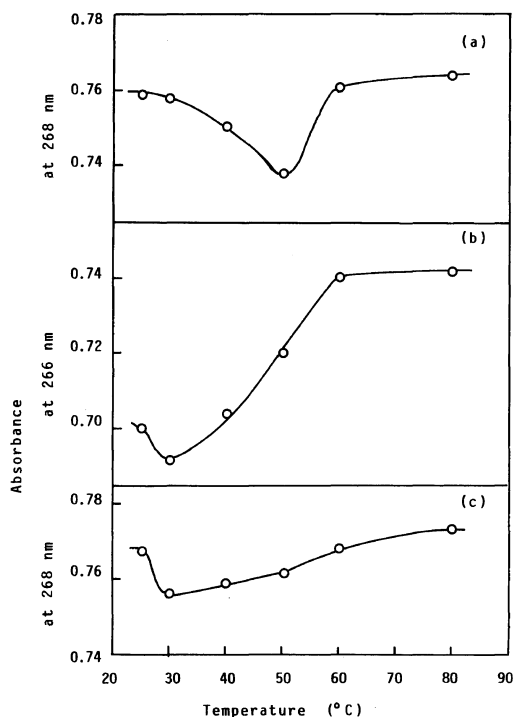
In Figure 3, the relative hypochromicity values for the polyMAOA-poly(MAOU-*co*-Me-MAOU) system and other systems are plotted against Me-MAOU unit content in the copolymer. The curves in this figure are the calculated values<sup>11</sup> of the probability of uracil unit sequences in the polymers. Hypochromicity values for the polyMAOA-poly-



**Figure 3.** Relative hypochromicity of the polymer complexes. (□), polyMAOA-poly(MAOU-*co*-Me-MAOU); (●), polyMAOU-poly(MAOA-*co*-Me-MAOA); (○), polyMAOU-Me-polyMAOA.<sup>6</sup> Normalized probability of the uracil unit sequences: a) diad; b) triad; c) pentad; d) heptad.<sup>11</sup>

(MAOU-*co*-Me-MAOU) system were on the curve for the pentad uracil sequence (curve c in Figure 3). Consequently, a certain length of uracil unit sequences in the copolymer chain may be necessary to form the polymer complex with polyMAOA.

Next, the effect of temperature on the polymer interactions was studied. The UV absorbance of the 1:1 mixture of polyMAOA and poly(MAOU-*co*-Me-MAOU) was plotted against the temperature of the system (Figure 4b). The absorbance decreased slightly from 25 to 30°C, and increased gradually by raising the temperature from 30 to 60°C. From this figure, the melting temperature,  $T_m$ , of the polymer complex was evaluated to about 45°C. The melting profile for the polyMAOA-polyMAOU system<sup>10</sup> is analogous to that for polyMAOU-poly(MAOA-*co*-Me-MAOA) system (Figure 4a). In these systems, the hypochromicity values at 25°C are rather small, and increase by heating up to 50°C and decrease again on further heating to 80°C. This behavior can be explained as follows. At room



**Figure 4.** Temperature dependence of UV absorbance. (a), polyMAOU-poly(MAOA-co-Me-MAOA, 10%); (b), polyMAOA-poly(MAOU-co-Me-MAOU, 16%); (c), poly(MAOU-co-Me-MAOU, 36%)-poly(MAOA-co-Me-MAOA, 10%).

temperature, the formation of the polymer complex cannot take place because of the stable intramolecular interaction of the uracil units in polyMAOU. By heating the system to 50°C, the self-association of the uracil bases in polyMAOU melt away, and the intermolecular polymer complex is formed between the complementary polymers. In contrast, for the polyMAOA-Me-polyMAOU system,<sup>7</sup> the intramolecular interaction of the uracil units in Me-polyMAOU containing 20% methylated uracil units is negligibly small at 25°C, and the polymer forms the intermolecular polymer complex with polyMAOA even at 25°C. The melting profile for the polyMAOA-poly(MAOU-co-Me-MAOU) system in Figure 4b resembles that for the polyMAOA-Me-polyMAOU system,<sup>7</sup> except the range from 25 to

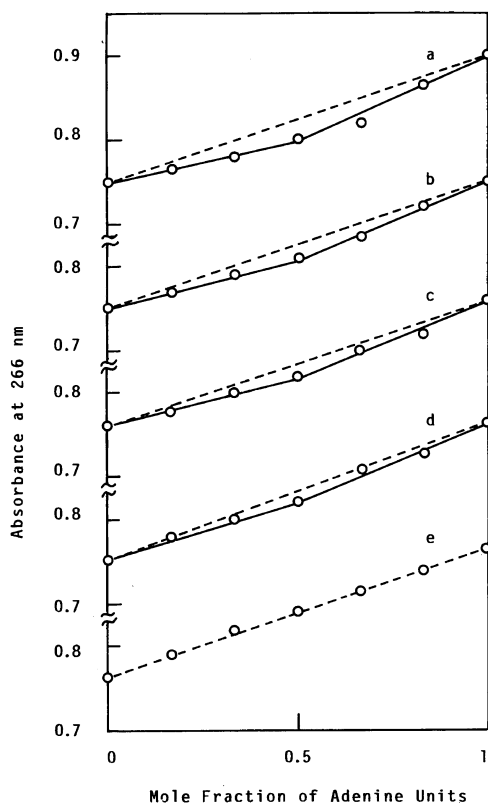
30°C. This suggests that the intramolecular interaction of the uracil units occurs in poly(MAOU-co-Me-MAOU) at 25°C, but to a small extent. Thus, the effect of temperature on the polymer interactions also suggests that the distribution of uracil units in poly(MAOU-co-Me-MAOU) is quite different from that of Me-polyMAOU.

The interaction of polyMAOA with poly(MAOU-co-Me-MAOU) can be summarized as follows. The interaction of polyMAOA with poly(MAOU-co-Me-MAOU) decreases with an increase in Me-MAOU units in the copolymer, suggesting the interaction of these polymers to be caused by hydrogen bonding interactions between adenine and uracil bases. The interaction depends on the distribution of uracil units and especially on the length of the uracil unit sequence in poly(MAOU-co-Me-MAOU), which is different from that in Me-polyMAOU obtained by the polymer reaction.

#### *Interaction of PolyMAOU with Poly(MAOA-co-Me-MAOA)*

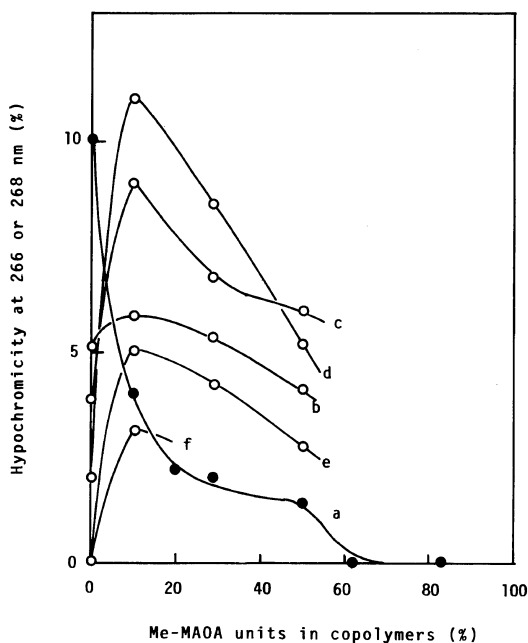
The mixing curves for the system of polyMAOU with poly(MAOA-co-Me-MAOA) in DMSO-EG (3:2, v/v) solution are shown in Figure 5. The stoichiometry of the polymer complex in this system was also 1:1, independent of the Me-MAOA unit content in poly(MAOA-co-Me-MAOA). When Me-polyMAOA obtained by the polymer reaction was used instead of poly(MAOA-co-Me-MAOA),<sup>6</sup> the stoichiometry of the polymer complex with polyMAOU was always uracil > adenine. This indicates that the distribution of the adenine units (MAOA) in the copolymer differs from that in Me-polyMAOA. The copolymer was obtained by free radical copolymerization and thus the adenine units and Me-MAOA units may be randomly distributed and Me-polyMAOA obtained by the polymer reaction<sup>6</sup> should be assumed to be a block type structure.

The highest hypochromicity value in the mixing curve (Figure 5) is plotted against the



**Figure 5.** Mixing curves between polyMAOU and poly(MAOA-co-Me-MAOA) in DMSO-EG (3:2, v/v) at 25°C. Content of the Me-MAOA units in poly(MAOA-co-Me-MAOA) (%): a) 10; b) 20; c) 29; d) 50; e) 62.

content of the Me-MAOA units in poly(MAOA-co-Me-MAOA) in Figure 6 (a). The methylation of adenine in MAOA at  $N^6$ -position was found to cause a decrease in hypochromicity for the polymer complex system with polyMAOU. The hypochromicity values depended on the MAOA unit content in the copolymer, but a linear relationship was not obtained. In Figure 3, the relative hypochromicity value for the polyMAOU-poly(MAOA-co-Me-MAOA) system was plotted with the results for the polyMAOU-Me-polyMAOA system.<sup>6</sup> The result for the polyMAOU-Me-polyMAOA system showed a linear relationship with the MAOA unit content in the polymer. The result for the



**Figure 6.** Hypochromicity for the polyMAOU-poly(MAOA-co-Me-MAOA) system (a), and the poly(MAOU-co-Me-MAOU)-poly(MAOA-co-Me-MAOA) systems (b-f). Content of the Me-MAOA units in poly(MAOU-co-Me-MAOU) (%): a) 0; b) 16; c) 23; d) 36; e) 51; f) 67.

polyMAOU-poly(MAOA-co-Me-MAOA) system, however, fitted well with the calculated curve<sup>11</sup> of the probability for the heptad sequences of the MAOA units in the copolymer (curve **d** in Figure 3). This suggests that the intermolecular interaction between polyMAOU and poly(MAOA-co-Me-MAOA) depends on the sequence length of the MAOA units in poly(MAOA-co-Me-MAOA). This difference in behavior of the interaction for the copolymer and Me-polyMAOA may arise from differences in the distribution of the MAOA units in each polymer. The conformational transition by the methylated adenine units was not observed for the copolymer nor Me-polyMAOA from the viscosity measurements in DMSO-EG mixed solvent system, but was so for the Me-polyMAOU polymer. This may be explained by the fact that both DMSO and EG are good solvents for polyMAOA.

The UV absorbance of the 1:1 mixture of polyMAOU and poly(MAOA-*co*-Me-MAOA, 10%) which contains 10% Me-MAOA units, depended on the temperature of the system as shown in Figure 4a. The melting profile in this figure is analogous to that of the polyMAOA-polyMAOU system,<sup>10</sup> as mentioned above. These abnormal melting profiles may be caused by an intramolecular interaction of the uracil units in polyMAOU, and be independent of the adenine containing polymers. The melting temperature ( $T_m$ ) of the polymer complex between polyMAOU and poly(MAOA-*co*-Me-MAOA) is around 60°C, which is higher than that for the system of polyMAOA with poly(MAOU-*co*-Me-MAOU) (Figure 5b).

The interaction of polyMAOU with poly(MAOA-*co*-Me-MAOA) can thus be summarized as follows. The interaction of polyMAOU with poly(MAOA-*co*-Me-MAOA) decreases by increasing the Me-MAOA units in the copolymer, suggesting that the amino group at the 6-position of adenine base is necessary for such an interaction. The interaction depends on the length of the MAOA sequences in the copolymer, which is different from that in Me-polyMAOA obtained by the polymer reaction.

*Interaction of Poly(MAOU-*co*-Me-MAOU) with Poly(MAOA-*co*-Me-MAOA)*

It was shown that blocking of either the amino group of adenine or the amido groups of uracil bases by methylation resulted in an inhibition of the specific interaction between polyMAOU and polyMAOA. Thus, the interaction between poly(MAOU-*co*-Me-MAOU) and poly(MAOA-*co*-MAOA) was considered negligibly weak. This interaction could not be observed for the system of Me-polyMAOU with Me-polyMAOA obtained by the polymer reaction.<sup>6</sup> However, the poly(MAOU-*co*-Me-MAOU)-poly(MAOA-*co*-Me-MAOA) system showed a significant interaction in the present study.

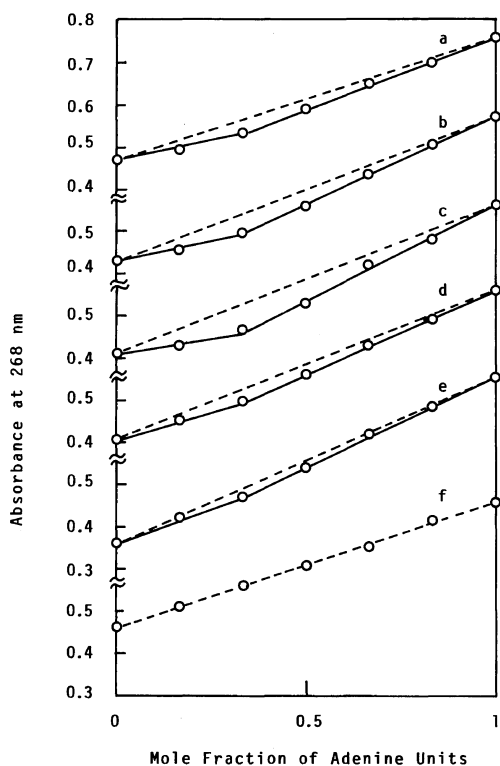
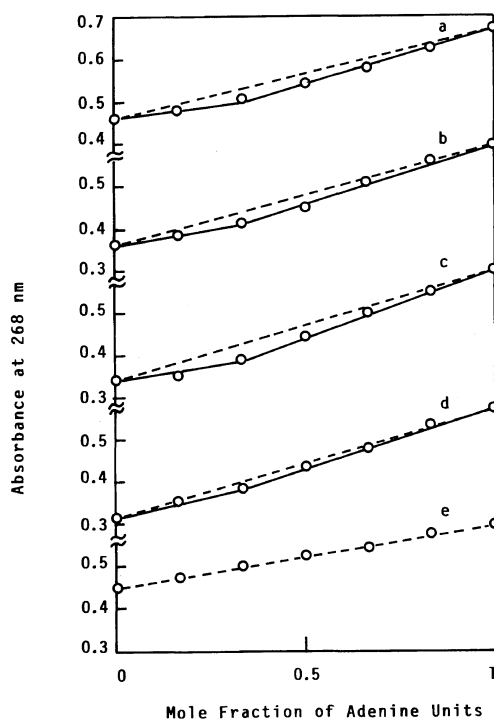


Figure 7. Mixing curves between poly(MAOA-*co*-Me-MAOA, 10%) and poly(MAOU-*co*-Me-MAOU) in MDSO-EG (3:2, v/v) at 25°C. Content of the Me-MAOU units in poly(MAOU-*co*-Me-MAOU) (%): a) 16; b) 23; c) 36; d) 51; e) 67; f) 85.

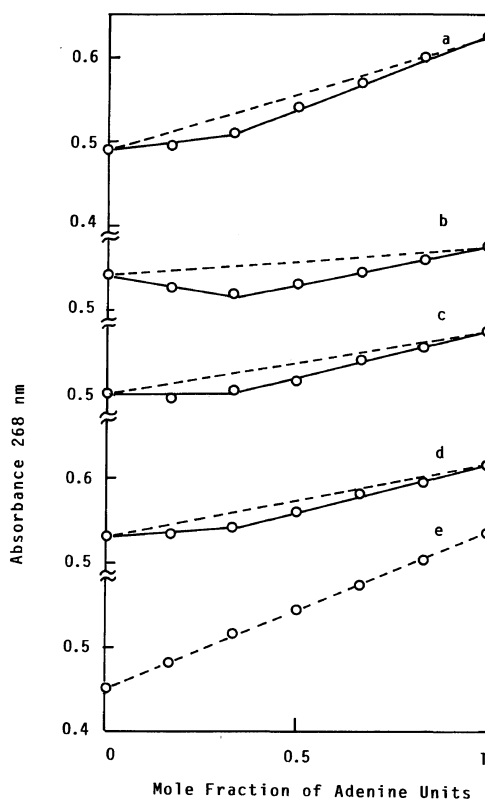
The mixing curves for the copolymer-copolymer systems are shown in Figures 7, 8, and 9. The stoichiometry of these polymer complexes was 2:1 (uracil:adenine) for all cases. The highest hypochromicity values of the mixing curves in these figures were plotted against the Me-MAOU unit content in poly(MAOU-*co*-Me-MAOU) (Figure 2, b-d), and against that of the Me-MAOA units in poly(MAOA-*co*-Me-MAOA) (Figure 6, b-f).

It has already been observed that the interaction in the polyMAOA-poly(MAOU-*co*-Me-MAOU) system decreased with increasing Me-MAOU units in poly(MAOU-*co*-Me-MAOU) (Figure 2, a). The copolymer-copolymer system (Figure 2, b-d), however, shows the existence of an optimum Me-



**Figure 8.** Mixing curves between poly(MAOA-co-MAOA, 29%) and poly(MAOU-co-Me-MAOU) in DMSO-EG (3:2, v/v) at 25°C. Content of the Me-MAOU units in poly(MAOU-co-Me-MAOU) (%): a) 16; b) 23; c) 36; d) 51; e) 67.

MAOU unit content for the interaction, being about 20–40% Me-MAOU units in poly(MAOU-co-Me-MAOU). This can be explained by compensation through opposite factors. A negative factor for the interaction is the inhibition of hydrogen bonding between adenine and uracil bases by methylation of nucleic acid bases. Positive factors for the interaction are a decrease in the intramolecular interaction of uracil bases in polyMAOU by methylation of the uracil units, and an increase in compatibility or mutual solubility of the polymers by methylation of nucleic acid bases. The methylated uracil units (Me-MAOU) in poly(MAOU-co-Me-MAOU) undoubtedly inhibit the hydrogen bonding interaction both with polyMAOA and poly(MAOA-co-Me-



**Figure 9.** Mixing curves between poly(MAOA-co-Me-MAOA, 50%) and poly(MAOU-co-Me-MAOU) in DMSO-EG (3:2, v/v) at 25°C. Content of the Me-MAOU units in poly(MAOU-co-Me-MAOU) (%): a) 16; b) 23; c) 36; d) 51; e) 67.

MAOA). The decrease in the intramolecular interaction of the uracil units in poly(MAOU-co-Me-MAOU) may equally influence both systems, polyMAOA-poly(MAOU-co-Me-MAOU) and poly(MAOA-co-Me-MAOA)-poly(MAOU-co-Me-MAOU). The increase in the hypochromicity of the copolymer-copolymer system around 30% Me-MAOU units in poly(MAOU-co-Me-MAOU) (Figure 2, b–d), may thus be caused by the predominantly increased compatibility or mutual solubility between the two copolymers by methylation of the adenine and uracil bases.

The optimum MAOA unit content in poly(MAOA-co-Me-MAOA) for the interaction was also observed in the copolymer-copolymer



system (Figure 6, b–f), while the hypochromicity decreased simply by an increase in the Me-MAOA units for the polyMAOU–poly(MAOA-*co*-Me-MAOA) system (Figure 6, a). This may also be explained by compensation through opposite factors as mentioned in Figure 2. Poly(MAOA-*co*-Me-MAOA) containing about 10% Me-MAOA units seemed to have high compatibility or mutual solubility with poly(MAOU-*co*-Me-MAOU), giving the highest hypochromicity value.

Figure 4c shows the melting profile for the polymer complex of poly(MAOU-*co*-Me-MAOU, 36%) with poly(MAOA-*co*-Me-MAOA, 10%). This melting profile resembles that for the polymer complex of polyMAOA with poly(MAOU-*co*-Me-MAOU, 16%) (Figure 4b), but differs from that for the polyMAOU–poly(MAOA-*co*-Me-MAOA, 10%) system (Figure 4a). This suggests that the intramolecular interaction of adenine bases in polyMAOA is negligibly small.

The interaction between poly(MAOU-*co*-Me-MAOU) and poly(MAOA-*co*-Me-MAOA) can be summarized as follows. For the interaction of the copolymers, the optimum content of methylated nucleic acid bases was observed. Poly(MAOU-*co*-Me-MAOU) containing about 30% Me-MAOU units gave the highest hypochromicity values for the interaction with poly(MAOA-*co*-Me-MAOA).

Similarly, poly(MAOA-*co*-Me-MAOA) containing about 10% Me-MAOU units gave the highest hypochromicity value for the interaction with poly(MAOU-*co*-Me-MAOU). This was explained on the basis of compensating factors: the negative factor is the inhibition of hydrogen bonding by methylation of the nucleic acid bases, and the positive factors, the decrease in the intramolecular interaction and increase in compatibility or mutual solubility of the polymers by methylation.

## REFERENCES

1. P. D. Lawley, *Nucl. Acid Res. Mol. Biol.*, **5**, 89 (1966).
2. B. Singer, *Nucl. Acid Res. Mol. Biol.*, **15**, 219 (1975).
3. W. Lijinsky, *Nucl. Acid Res. Mol. Biol.*, **17**, 247 (1977).
4. A. Razin and J. Friedman, *Nucl. Acid Res. Mol. Biol.*, **25**, 33 (1981).
5. K. Takemoto and Y. Inaki, *Adv. Polym. Sci.*, **41**, 1 (1981).
6. S. Fang, Y. Inaki, and K. Takemoto, *J. Polym. Sci., Polym. Chem. Ed.*, **22**, 2455 (1984).
7. S. Fang, Y. Inaki, and K. Takemoto, *J. Polym. Sci., Polym. Chem. Ed.*, in press.
8. S. Fang, Y. Inaki, and K. Takemoto, *Polym. J.*, **17**, 433 (1985).
9. C. G. Overberger, Y. Inaki, and Y. Nambu, *J. Polym. Sci., Polym. Chem. Ed.*, **17**, 1759 (1979).
10. M. Akashi, T. Okimoto, Y. Inaki, and K. Takemoto, *J. Polym. Sci., Polym. Chem. Ed.*, **17**, 905 (1979).
11. J. L. Koenig, "Chemical Microstructure of Polymer Chain," John Wiley & Sons, New York, N.Y., 1980.