

SYNTHESIS OF ACTIVE ACRYLIC ESTERS OF 8-HYDROXYQUINOLINE AND IMMOBILIZATION OF PROTEINS WITH THEIR POLYMERS

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ABSTRACT

Active esters, 8-acryloyloxyquinoline (AQ) and 8-methacryloyloxyquinoline (MAQ) were synthesized and characterized. It was found that polymers of these two monomers can react with nucleophilic reagents such as amines or alcohols under mild condition. The ammonolysis yield of P(AQ) for aliphatic amines were above 60% and the specific activity of the immobilized glucoamylase on P(AQ) and P(MAQ) was 5328 u/g and 4320 u/g, respectively.

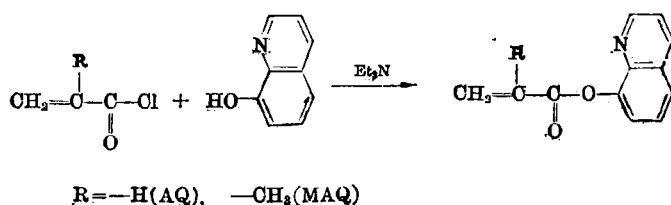
Recently, there are many reports concerning with the investigation of active esters and active amides of acrylic acids. Most of these active esters and active amides are heterocyclic compounds with nitrogen or oxygen hetero-atoms, such as derivatives of N-hydroxysuccinimide and benzotriazole. In our previous papers^[1-3], the preparation of macroreticular water-insoluble polyvinyl alcohol carriers bearing active ester groups or active amide groups and the application of these active carriers for affinity chromatography have been presented. 8-Hydroxyquinoline (oxine), due to the neighbouring group effect of the nitrogen atom, may be used to prepare active esters. Ho^[4] have studied the selective N-acylating reaction of various acyloxyquinoline compounds. Manecke^[5] and Okawara et al.^[6] have prepared polymeric acylating reagents for solid phase synthesis of polypeptide by introducing 8-hydroxyquinoline onto polystyrene supports. As regards 8-acryloyloxyquinoline (AQ) and methacryloyloxyquinoline (MAQ) and their polymers as active carriers, there have been few reports. Huber-Emden, Pittmann Jr. et al.^[7-11] have investigated the fungicidal activity of these two polymers and their application in mildew defacement of organic coatings. Sakaguchi et al.^[12] have reported the preparation of tactic poly(acrylic acid) via the hydrolysis of AQ polymer.

In this article, the comparison of the ammonolysis and alcoholysis reactions of AQ polymer and MAQ polymer for the purpose of immobilization of proteins are presented. The primary results obtained show that 8-acryloyloxyquinoline polymers can be used as macromolecular reactive carriers to immobilize biosubstances under mild condition.

Synthesis and Polymerization

8-Acryloyloxyquinoline (AQ) and 8-methacryloyloxyquinoline (MAQ) were synthesized.

by the reaction of 8-hydroxyquinoline with corresponding acryl chloride in the presence of triethylamine^[6, 10]:



The melting points, elementary analysis of the obtained products are summarized in Table 1.

Table 1 Melting points and elementary analysis of AQ and MAQ

Monomer	Yield (%)	m.p. (°C)	M.W.		Elementary analysis**		
			Cal.	Found*	C%	H%	N%
AQ	73	48—50	119	119	72.02 (72.35)	4.29 (4.55)	6.95 (7.03)
MAQ	69	61—63	213	213	72.82 (73.22)	5.15 (5.99)	6.46 (6.57)

* Determined by MS, EI.

** The value in parenthesis are calculated according to the expected structures.

The monomers are easy to dissolve in such solvents as ethyl ether, alcohol, dimethylformamide (DMF), etc.

Initiated with radical initiator such as azoisobutyronitrile (AIBN), AQ and MAQ can be polymerized easily, given light yellow or white polymers, respectively. These polymers are insoluble in ethyl ether, alcohol, etc., but are soluble in DMF. Table 2 shows the elementary analysis and specific viscosity of these polymers.

The ^1H -NMR spectra of AQ and MAQ are showed in Fig. 1. There are two doublet

Table 2 Elementary analysis and specific viscosity of P(AQ) and P(MAQ)

Polymer	Elementary analysis*			Specific viscosity $[\eta]$ (DMF, 25°C)
	C%	H%	N%	
P(AQ)	71.45 (72.35)	4.69 (4.25)	6.85 (7.03)	0.10
P(MAQ)	72.47 (73.22)	5.08 (5.99)	5.95 (6.57)	0.13

* The value in parenthesis are calculated according to the expected structures.

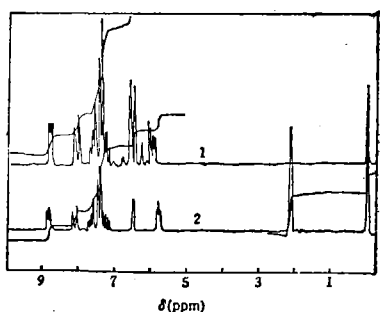


Fig. 1 ^1H -NMR spectra of AQ and MAQ
1. AQ; 2. MAQ.

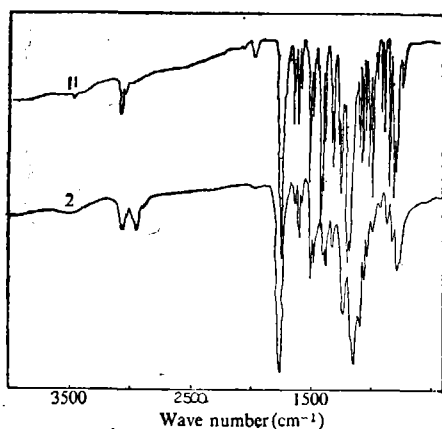


Fig. 2 IR spectra of AQ and its polymer
1. AQ; 2. P(AQ).

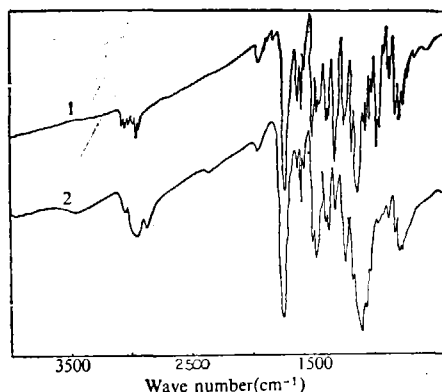


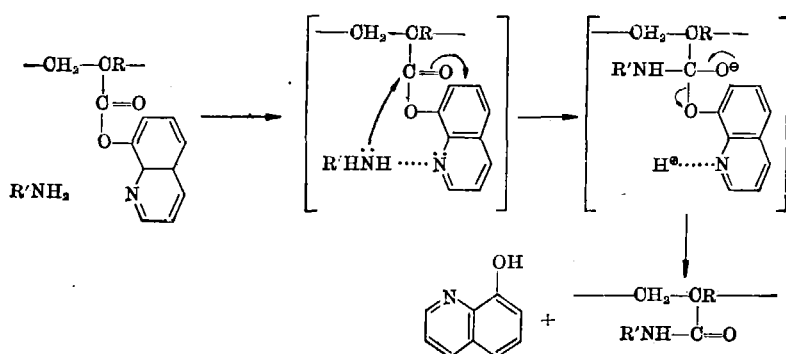
Fig. 3 IR spectra of MAQ and its polymer
1. MAQ; 2. P(MAQ).

peaks appear near δ 5.99—6.14 and δ 6.55—6.99 respectively, corresponding to the $\begin{array}{c} \text{H} \\ \diagup \\ \text{C}=\text{C} \\ \diagdown \\ \text{H} \end{array}$ protons of AQ and three multiplet appear near δ 8.86—8.94, δ 8.08—8.21 and δ 7.25—7.75, corresponding to the protons of quinolyl ring at 3, 5, 6, 7-site, 4-site and 2-site respectively. The singlet peak of methyl group protons of MAQ appear near δ 2.15 and the peaks for $\begin{array}{c} \text{H} \\ \diagup \\ \text{C}=\text{C} \\ \diagdown \\ \text{H} \end{array}$ protons of MAQ appear near δ 6.54 and δ 5.82—5.86, respectively.

The IR spectra of AQ and MAQ and their polymers are showed in Fig. 2 and Fig. 3. The $\nu_{\text{C}=\text{O}}$ absorption bands of the monomers near 1635 cm^{-1} disappear after polymerization.

Ammonolysis and Alcoholysis of P(AQ) and P(MAQ)

The ester bond of P(AQ) or P(MAQ), due to their neighbouring group effect of quinolyl group, may be attacked by some nucleophilic reagents such as amines or alcohols under mild condition. The reaction may be illustrated as follows:



The ammonolysis reaction is easy to take place even at room temperature whereas the alcoholysis reaction takes place at higher temperature (60°—70°C) and in the presence of triethylamine. Table 3 summarizes the ammonolysis and alcoholysis results of P(AQ) and P(MAQ).

Table 3 Yield of ammonolysis and alcoholysis of P(AQ) and P(MAQ)

Nucleophilic reagent	Yield (%) [*]	
	P(AQ)	P(MAQ)
Allylamine	66	<10
n-Butylamine	64	<10
Piperidine	60	<10
Aniline	<10	<10
Furfuryl alcohol	(60)	—
Cyclohexyl alcohol	20 (64)	<10
n-Butyl alcohol	18	<10
Phenol	<10	<10

* Reaction condition:

Ammonolysis, at room temperature, 4 hrs;

Alcoholysis, at 60°C for 4 hrs, in the presence of triethylamine (the value in parenthesis are the yield reacted for 48hrs).

From the result, it can be seen that P(AQ) is more reactive than P(MAQ). This may be due to the presence of methyl group which causes more steric hindrance and therefore higher potential energy in the tetrahedral configuration of ammonolysis or alcoholysis intermediate of P(MAQ). From the reaction of P(AQ), it can be concluded that the ammonolysis takes place more easily than the alcoholysis does. Aromatic amines, due to their weak basicity, are less reactive than aliphatic amines. Stirred with aliphatic amines at room temperature for 4hrs, the ammonolysis yield of P(AQ) was above 60%. This demonstrates that P(AQ) is a candidate polymer for coupling proteins.

Fig. 4 shows the spectra of P(AQ) and its ammonolysis products. After the exchange reaction, the $\nu_{C=O}$ absorption band of the ester bond in P(AQ) became weak whereas the amide I absorption band and amide III band appeared near 1630 cm^{-1} and 1280 cm^{-1} respectively.

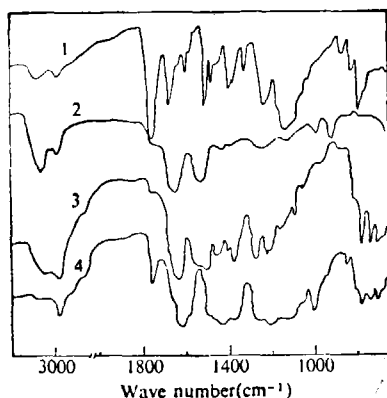


Fig. 4 IR spectra of P(AQ) and its ammonolysis products
Ammonolysis reagent: 1. —; 2. Allylamine;
3. n-Butylamine; 4. Piperidine.

P(AQ) and P(MAQ) were stirred with glucoamylase in borate buffer of pH7 at 0°C for 24hrs, given immobilized enzymes with specific activity of $5328\text{ }\mu\text{g}$ and $4320\text{ }\mu\text{g}$, respectively. This demonstrates that both P(AQ) and P(MAQ) can immobilizing proteins under mild condition without heavily deactivating the enzymes. The specific activity of P(AQ) immobilized glucoamylase was higher about 23% than that of P(MAQ) immobilized enzyme. This is probably due to the steric hindrance of methyl group in P(MAQ) structure.

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