Synthesis, Characterization and Catalytic Activity of
Resin-based Diiron(III) Catalysts:
Model for Methane Monooxygenase

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Abstract：Six novel resin-based μ-oxo diiron (III) catalysts have been successfully prepared to model the functional properties of Fe_2O enzyme methane monooxygenase (MMO). The catalysts were characterized and exhibited the monooxygenase-like ability to oxidize cyclohexane or ethylbenzene to responding alcohols and ketones in acetonitrile at room temperature by 30 % hydroperoxide (H_2O_2) or tert-butyl hydroperoxide (TBHP) as the oxidant. The catalyst prepared through macroporous aminomethyl resin showed the most efficient in the catalytic oxidation of ethylbenzene with moderate yields (total yield up to 69.9 %, turnover number 55.3). The effects of the particle size of resins and the anion donor were also studied, showing that the perchlorate anion is better than chloride and nitrate. The most efficient catalytic activity was observed by the catalyst synthesized through particle size 0.675—0.280 nm of resin and ClO_4^-. Furthermore, the catalysts show a potential for recover and reuse.

Key words：methane monooxygenase, model, resin, catalytic activity

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Methane monooxygenase (MMO) is one of the most important enzymes during the metabolism of methantrophs, wherein a μ-oxo binuclear diiron cluster found in the hydroxylase can catalyze a remarkable reaction, the oxidation of methane to methanol by molecular oxygen. MMO can also oxidize a broad range of hydrocarbons, including aromatic compounds[1~5] which boosts the wide potential application in chemical and pharmaceutical industries, environmental management, and so on. In view of its implication, synthesis of small molecule models containing the μ-oxo diiron unit to mimic the catalytic activity of the MMO to the hydroxylation of alkanes or epoxidation of alkenes by various oxidants would be of significant interest to synthetic chemistry, industry process and depollution process. Numerous model compounds, based on the self-assembly method, have been prepared using poly-

dentate nitrogen/oxygen-based ligands (e. g. phenol[6,7], bipyridine[8,9], naphthyridine[10,11], phthalazine[12,13], TPA[14,15]) to form μ-oxo bridging diiron centers and showed some catalytic activity. It provides very useful tools for studying the reactivity of these fascinating unique enzymes. However, most of these model compounds as homogeneous catalysts are difficult to recover and reuse, especially to separate products from the reagents and to apply to industry. Therefore, the replacement of current homogenous catalysts for the environmentally acceptable heterogenous catalysts is one of the major tasks in green chemistry. In order to accomplish this goal, solid-based interface catalysts were prepared by immobilizing the synthetically active centers onto a solid carrier[16,17]. During the past several years, resins have been a powerful tool in solid-phase organic synthesis for the rapid generation of

large numbers of organic compounds [18~21]. Macroporous aminomethyl resin, N-methylaminomethyl resin, benzhydrylamine hydrochloride salt resin (BHA) and 4-methylbenzhydrylamine hydrochloride salt resin (MBHA) were chosen because of their chemical stability to the conditions used in this synthesis and catalytic systems. In this paper, we describe the preparation, characterization and catalytic performance in the oxidation reactions of cyclohexane and ethylbenzene under novel resin-supported diiron complexes as recoverable heterogeneous catalysts and the models of MMO.

1 Experimental

1.1 Materials and measurements

All chemicals were of regent grade unless other-wise noted. Solvents and substrates used for the reaction were purified by distillation. The resins were purchased from Tianjin Nankai Hecheng S&T Company. The atomic absorption spectra were recorded on a Shimadzu Z-5000 spectrophotometer. Infrared spectra (4 000 ~ 500 cm^{-1}) were recorded from KBr pellets on a Bruker EQUNOX 55 FTIR. Gas chromatography was performed on a Agilent 6 820 with FID detector using a DB-Wax capillary column, and GC-MS were performed on a Shimadzu QP2010.

1.2 Synthesis of catalysts

We found a simple synthetic method which was similar to our previously work[22] to generate resin-based diiron (Ⅲ) interface catalysts, and the catalysts were prepared (Schemes 1 and 2) and characterized.

Scheme 1 Preparation of Catalysts 1 and 2

1.3 Oxidation reaction

The catalytic oxidation activity of the catalysts have been evaluated in CH_3CN solvent at room temperature using 30% hydroperoxide (H_2O_2) or tert-butyl hydroperoxide (TBHP) as oxidant. In a typical oxidation reaction experiment of ethylbenzene, 20 mg of the solid catalyst were stirred with 5.02 mmol of the substrate, 10 mL of solvent, 70 μmol of an internal standard and 0.34 mmol oxidant at ambient temperature. The reaction was monitored by taking aliquots of the reaction mixture at different reaction times. The products were identified from the retention times of the pure compounds and analyzed quantitatively by GC-MS.

2 Results and Discussion

2.1 Characterization

Comparison of the IR spectrum of catalyst **1** with that of catalyst **2** shows that an additional characteristic stronger absorption band at 1668 cm^{-1} in catalyst **1**, assigned to the stretching vibration of C = N imine bond, was not detected in the catalyst **2**. The absorption bands at 1 603, 1 507, 1 453 and 1 376 cm^{-1} were assigned to the vibrations of pyridyl rings in catalyst **1** (at 1 603, 1 494, 1 448 and 1 362 cm^{-1} for catalyst **2**, at 1 598, 1 494, 1 439 and 1 371 cm^{-1} for catalyst **3**, at 1 639, 1 494, 1 409 and 1 366 cm^{-1} for catalyst **4**, at 1 603, 1 498 and 1 444 cm^{-1} for catalyst **5**, at 1 603,

1 498 and 1 444 cm^{-1} for catalyst **6**). The slightly broad band at 1100 cm^{-1} is attributed to the vibration of ClO_4^-. Iron contents of catalysts are shown in Table 1 (by atomic absorption spectra). These results in combination with our previous work [22] confirmed that the Fe-O-Fe units are covalently bound on the surface of resin supporter. The structure of catalysts is shown in Fig 1.
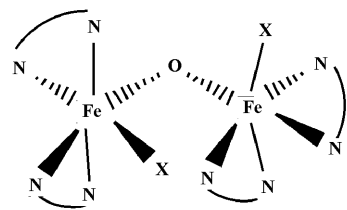

Fig 1 Molecular structure of catalysts

Table 1 Iron content of catalysts

| Catalysts	**1**	**2**	**3**	**4**	**5**	**6**
Loading of iron (mmol g^{-1})	0.16	0.24	0.27	0.89	0.05	0.10

2.2 Catalytic activity

A series of catalysts and substrates were applied in the catalytic oxidation reaction. The results for some different types of substrates are summarized in Table 2.

Table 2 Catalytic performance of resin-based diiron (Ⅲ) catalysts with oxidants in CH$_3$CN^a

Catalysts[a]	Oxidant	Substrate	Yield(μmol)[b]		Total yield (%)[c]	TON[d]
			Alcohol	Ketone		
1	TBHP	ethylbenzene	51.0	126.0	54.85	5.3
	H$_2$O$_2$		0.5	1.2	0.5	0.9
	TBHP	cyclohexane	1.5	n. p.[e]	0.4	0.9
	H$_2$O$_2$		trace	n. p.	trace	
2	TBHP	ethylbenzene	39.7	116.4	48.3	28.9
	H$_2$O$_2$		1.3	6.5	2.4	1.6
	TBHP	cyclohexane	3.6	n. p.	1.1	0.8
	H$_2$O$_2$		0.5	n. p.	0.2	0.1
3	TBHP	ethylbenzene	15.1	85.4	31.1	18.6
	H$_2$O$_2$		3.0	6.6	2.8	1.8
	TBHP	cyclohexane	10.9	n. p.	3.4	2.0
	H$_2$O$_2$		2.3	n. p.	0.7	0.4
4	TBHP	ethylbenzene	3.1	10.5	4.2	0.8
	H$_2$O$_2$		trace	trace	trace	
	TBHP	cyclohexane	trace	n. p.	trace	
	H$_2$O$_2$		trace	n. p.	trace	
5	TBHP	ethylbenzene	0.7	2.9	1.0	3.6
	H$_2$O$_2$		trace	trace	trace	
	TBHP	cyclohexane	1.2	n. p.	0.4	1.2
	H$_2$O$_2$		trace	n. p.	trace	
6	TBHP	ethylbenzene	0.7	2.4	1.1	3.1
	H$_2$O$_2$		trace	trace	trace	
	TBHP	cyclohexane	1.0	n. p.	0.3	0.5
	H$_2$O$_2$		trace	n. p.	trace	

^{a}The mesh of resins used in catalysts **1**, **2**, **3** is 200~400. The anion of all catalysts is ClO_4^-. Catalysts **1**, **2**, **3** are synthesized by macroporous aminomethyl resin, and catalysts synthesized **4**, **5**, **6** are synthesized by N-methylaminomethyl resin, BHA resin and MBHA Resin ^{b} After complete reaction. ^{c} Total yield based on oxidant ^{d} turnover number based on iron content of catalyst. ^{e} n. p. no species of the oxidation products was determined.
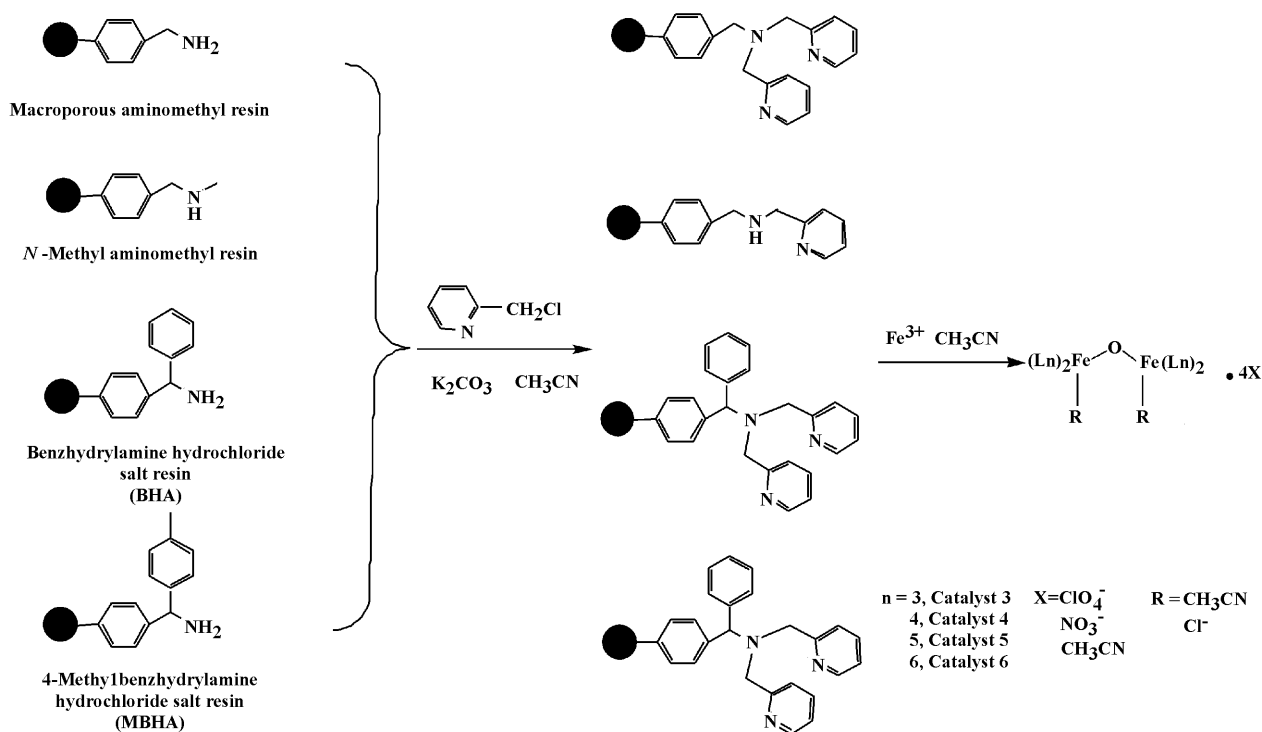
Scheme 2 Preparation of catalysts 3, 4, 5, and 6

As shown in Table 2, the catalytic oxidation reactions were effectively performed with the catalysts and TBHP. The yields have been obtained with catalysts synthesized by macroporous aminomethyl resin (up to 54.8%), followed by N-methylaminomethyl resin (up to 4.2%), then BHA (up to 1.0%) and MBHA resin (up to 1.1%). The macroporous aminomethyl resin afforded a high yield due to numerous permanence holes on it and the weak stereospecific blockade with fewer side chains, which facilitates the substrate to get close to the active center of catalyst. It is also not difficult to find that higher activity in the oxidation reaction was found with TBHP than those with H_2O_2, which may be caused by the dismutation of H_2O_2 to O_2 and H_2O catalyzed by the catalyst [23]. For the ethylbenzene oxidation with TBHP, alkyl arene is oxidized selectively at the benzylic position to afford the corresponding only two benzylic oxygenation products: 1-phenylethanol and acetophenone; however, when the oxidant was replaced by H_2O_2, three additional products, ortho-, mata- and para-ethylphenol, were found in the oxidation process. Interestingly, three additional products disappeared when adding acetate in reaction systems. It is supposed that acetate group plays a cru-cial role to mimick the function of MMO in high selectivity by forming the carboxylate-bridged diiron center, which is close to the geometric feature of the active site of natural MMO. The result also suggests that the cyclohexane substrate has much lower yield and TON but higher selectivity (only the alcohol product was observed, see Table 2) than ethylbenzene substrate.

2.3 Effect of particle size (mesh) of solid support on the catalytic activity

Catalysts **1** and **2** were synthesized by different particle sizes of macroporous aminomethyl resins and their catalytic activities were evaluated with ethylbenzene and TBHP oxidant. The results are shown in Fig. 2. Both catalysts **1** and **2** efficiently catalyzed the oxidation of ethylbenzene in a good yield (32.5% - 69.9%) under mild conditions. Apparently, the best yield was given by catalysts **1** and **2** synthesized by the larger particle size of solid support (0.675 - 0.280 mm), followed by 0.079 - 0.034 mm and 0.154 - 0.079 mm. Accordingly, the activity of catalysts does not have the direct relationship with the particle size of solid supports. It is possible that the aperture of resins with particle size 0.675 - 0.280 mm is suitable for loading model compounds to form the more active site.
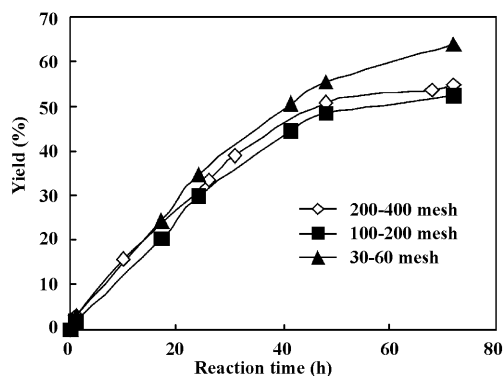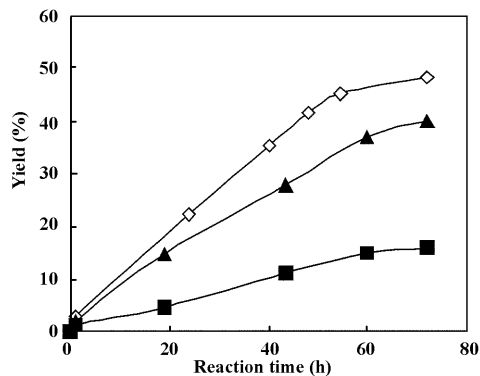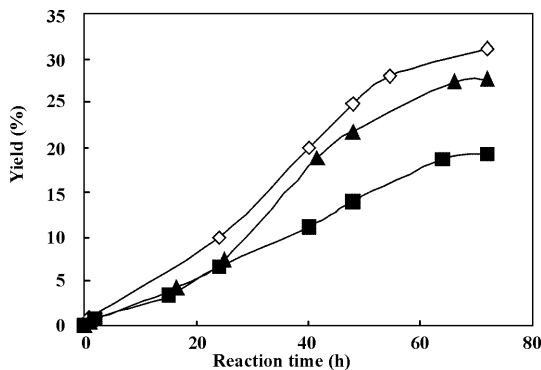
(a) Catalyst **1**
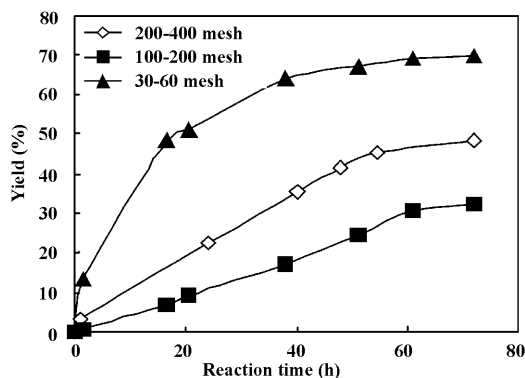
(b) Catalyst **2**

Fig. 2 Effect of different particle sizes of resins on the catalytic activity of catalysts **1** and **2**

2.4 Effect of anion of iron donor on the catalytic activity

The effect on the catalytic activity by the anion of the catalysts was studied for the ethylbenzene oxidation with TBHP (Fig. 3). When $Fe(ClO_4)_3$ as iron donor for catalysts **1** and **3**, the yields were obtained up to 48.3% and 31.1%, respectively, and then followed by $Fe(NO_3)_3$ and $FeCl_3$ as iron donors, which is consist with the order of the coordinated capability $ClO_4^- < CH_3CN \sim NO_3^- < Cl^-$. The oxidant ($H_2O_2$ or TBHP) inserts easily into Fe-O-Fe centre by the removal of the solvent CH_3CN molecule and difficuttly to remove the Cl^- anion coordinated to Fe (III) ion, therefore the catalyst with ClO_4^- shows a high catalytic activity.
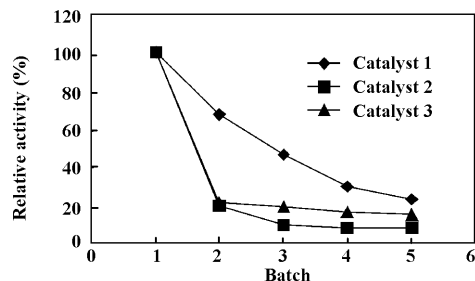

(a) Catalyst **2**

(b) Catalyst **3**

Fig. 3 Effect of different anion of iron donor on the catalytic activity of catalysts **2** and **3**

(the mesh resin is 200 – 400)

2.5 Recovery and reuse of catalysts

The reuse studies of catalysts **1**, **2** and **3** were carried out by recycling the catalyst with filtration and washing with CH_3CN. Recycling experiments were examined for the oxidation of ethylbenzene with TBHP and repeating the experiment. After being used for five times, the activity of the catalysts obviously declined (Fig. 4), the colour of the catalysts fades and reaction systems turned buff, which meant that the catalysts were deactivated. It is probable that the diiron core was cleaved and iron ions were dissolution in reaction solution. Effort to avoid cleavage of diiron corn is un-der investigation.

Fig. 4 Lifespan of catalysts **1**, **2** and **3**

3 Conclusions

We have successfully synthesized six novel resin-

based biomimetic models of MMO，which showed considerable catalytic activity under mild conditions with hydroperoxide or tert-butyl peroxide as an oxidant. The macroporous aminomethyl resin with fewer side chains is very suitable to act as a support to load the diiron（III）compounds as models of active centre of MMO. The effect of anion and carboxylate group on the catalytic activity is caused by the control of inserting the oxidant into the active center and by the coordination geometry of the active center.

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甲烷单加氧酶的模型化合物：以树脂为载体的双铁（III）催化剂的合成、表征及催化性能的研究

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摘　要：使用树脂为载体，合成了6种新的双铁（III）催化剂，成功的模拟了可溶性甲烷单加氧酶（MMO）的双核铁中心。这是首次使用树脂作为载体合成模拟 MMO 的非均相催化剂。在室温下以乙腈为溶剂，30% 的过氧化氢或特丁基过氧化氢做氧化剂，环己烷或乙苯作为底物的反应中，催化剂具有 MMO 相似的催化活性。其中，使用大孔氨基甲基树脂为载体合成的催化剂能有效地催化乙苯氧化得到较好的转化率（总收率达 69.9%，TON 值达 55.3）。同时研究了树脂的颗粒大小及阴离子供体对催化性能的影响。使用粒径 0.675～0.280 mm 的树脂和 ClO₄⁻ 的阴离子供体所合成的催化剂具有很好的催化活性。最后探讨了催化剂的回收和再利用。

关键词：甲烷单加氧酶；模型；树脂；催化活性