Studies on CoSalen Immobilized onto *N*-(4-Methylimidazole)-Chitosan

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Received 30 May 2003; accepted 4 January 2006
DOI 10.1002/app.24019
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A chitosan (CS) derivative, *N*-(4-methylimidazole)-chitosan (MIC), was synthesized, and a cobalt (II) complex of bis(salicylideneethylene diamine), (CoSalen), was immobilized on it. The structure of the polymer-immobilized CoSalen was characterized by elemental analysis, IR, XPS, fluorescence and ESR spectroscopy. It was demonstrated that the immobilization of CoSalen was realized through the coordination of a nitrogen atom of the pendant group, imidazole in MIC, to the Co(II) in CoSalen. The immobilized polymer complex is more efficient than the corresponding monomeric complex in catalyzing the oxidation of DOPA using oxygen. The results may be attributed to a site isolation effect offered by the supporting polymer chain. A mechanism similar to that for enzymatic catalysis was proposed. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 2431–2436, 2006

Key words: cobalt; chitosan; complex; immobilization

INTRODUCTION

The reversible oxygenation of cobalt (II) complex of bis(salicylideneethlene diamine) (CoSalen) was discovered by Tsumaki in 1938. Since then most of the work on the Schiff-base complexes of cobalt have concentrated on the synthesis, structure characterization, electronic, magnetic, and other properties.[1] The potential application of cobalt complexes as industrial catalysts has stimulated research in this area.[2] The basic consideration is that molecular oxygen could be activated by the formation of monomeric metal oxygen adducts (Co–O_2). To improve the catalytic activity, Bied-Charreton et al. investigated in some detail the synthesis and application of cobalt and other transition metal complexes attached to polymer surfaces or to polymer backbones as pendant group.[3] The main idea is to prevent the formation of μ-oxodimers and simple dimers of CoSalen by employing the site isolation effect of polymers. However, most of the polymers employed in these studies are synthetic polymers, which lack biodegradability, biocompatibility, and surface wetting ability, and thereby the system cannot be used in biological systems. In the future, it is likely that synthetic catalysts may find some use in biological systems and in food industries. Chitosan (CS), a polysaccharide, seems to be a good candidate for polymeric supporter of active metal complexes.

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Journal of Applied Polymer Science, Vol. 101, 2431–2436 (2006)

In this research, a CS derivative *N*-(4-methylimidazole)-chitosan (MIC) was prepared and used to immobilize CoSalen. The polymer-supported CoSalen was instrumentally characterized, and its catalytic activity was investigated using oxidation of DOPA by molecular oxygen as a model system.

EXPERIMENTAL

Instruments and chemicals

WFX-IF2 Model atomic absorption spectrometer, Mattson FTIR spectrometer, 756 MC Model UV–vis spectrometer, PerkinElmer TGS-2 thermal analyzer, PerkinElmer 2400 elemental analyzer, PerkinElmer PHI-5400 photoelectron spectrometer, Varian-E109 ESR spectrometer were used. All chemicals, except CS, 4-chloromethylimidazole, and *N*-(4-methylimidazole)-chitosan, were purchased commercially and of at least analytical grade.

Preparation and characteriation of CS

CS was prepared by deacetylation of chitin according to a literature method.[4] The deacetylation was confirmed by the disappearance or reduction of a peak at 1655 cm^{-1}, which may be found in the IR spectrum of chitin. Thermal decomposition behavior of the product was similar to that reported in the literature,[5] and ignition residue was zero. In addition, the results of elemental analysis are also in good agreement with those calculated theoretically. Found (%) (calcd. %) C: 39.89% (39.63), H: 7.74(7.38), N: 7.54 (7.90). The degree of deacetylation was determined by potentiometric

titration and was found to be about 100%.[6] The viscosity–average molecular weight ($\overline{W_v}$) of the sample is 7.86×10^5.

Synthesis of Co(II) complex of bis(salicylideneethylene diamine)

The Co(II) complex of bis(salicylideneethylene diamine) (CoSalen), reddish brown microcrystals, was prepared according a literature method.[7] Elemental analysis: found (%) C: 58.93; H: 4.32; N: 8.50; Calculated for $C_{16}H_{14}O_2N_2Co$ (%): C: 59.10; H: 4.352; N: 8.62. The characteristic IR band ($\nu_{C=N}$) for the complex is 1625 cm^{-1}.

Preparation of 4-(chloromethyl)-imidazole (CMI)

4-(Hydroxymethyl)-imidazole hydrochloride was prepared according to Totter and Darby.[8] A white crystal was obtained [m.p. 106.8–108.5°C (literature 107.5–108.5°C)]. 4-(Chloromethyl)-imidazole was prepared using 4-(hydroxymethyl)-imidazole in accordance with the directions of Jones.[9] Found (%) (calcd. %): C: 31.60 (31.41); H: 4.72 (4.94); N: 15.82 (15.73).

Preparation of *N*-(4-methylimidazole)-chitosan (MIC)

MIC was prepared according to a modified literature method.[10] Chitosan (2.0 g, 0.0124 mol glucosamine residue) and CMI (1.9 g, 0.0124 mol CMI) were added to a flask, followed by 100 mL of DMSO and 6 mL triethylamine. The mixture was heated and stirred at 80°C for 10 h. The solvent was evaporated *in vacuo*, a brown precipitate was obtained after adding 150 mL of acetone, and the precipitate was filtered, and washed with water and acetone consequently. The precipitate dried *in vacuo* at 80°C was ground with a mortar and sieved with 200 mesh sieve.

Cosalen immobilized onto MIC (MIC-CoSalen)

A given amount of MIC was mixed with the saturated aqueous solution of CoSalen, and the mixture was shaken for 24 h. Then, the precipitate was filtered, and washed with ethanol in a Soxhlet extractor till the eluate became colorless. A light brown powder of MIC-CoSalen was obtained after vacuum drying at 80°C for 4 h.

DOPA oxidation catalyzed by MIC-CoSalen

It is reported that the catalytic oxygenation reaction of DOPA may be monitored by measuring the product's concentration as a function of time using absorbance data at 475 nm for dopaquinone.[11] In the present study, aqueous solution of DOPA (2×10^{-3} mol^{-1} 4 mL) was added to jacketed glass reactor containing MIC-CoSalen (0.05 g), then oxygen was immediately passed through the mixture at a constant rate and timing. To compare the catalyzing activity of the immobilized complex with CoSalen, the catalytic activities of CaSalen and auto-oxidation of DOPA were also determined. In the course of the reaction, the reactor was maintained at 25 ± 0.05(°C) by a continuous supply of thermostated water through the jacketed cell.

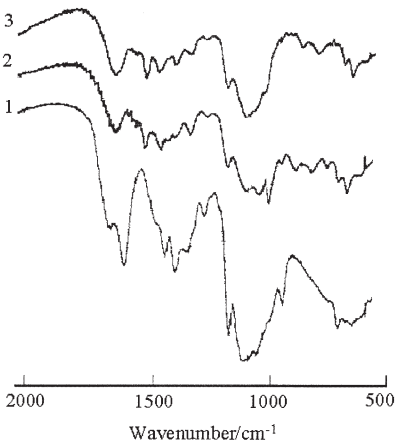


Figure 1 IR spectra of CS (1) MIC (2), and MIC-CoSalen (3).

RESULTS AND DISCUSSION

IR spectra of MIC and MIC-CoSalen

The IR spectra of the CS, MIC, and MIC-CoSalen are shown in Figure 1. A profile of MIC differs from that of the CS. In the spectrum of MIC, the peaks that appeared in the range from 1500 to 1440 cm^{-1} may originate from the C=N of imidazole group. The peaks in the range from 900 to 840 cm^{-1} are assigned to ring bending of imidazole. The peak at 770 cm^{-1} is attributed to $\nu_{(C—Cl)}$ in 4-(chloromethyl)-imidazole and disappears in the IR spectrum of MIC. This result indicates that the imidazole group is linked to CS through eliminating HCl from 4-(chloromethyl)-imidazole and CS.

There are no obvious differences between the spectra of MIC and MIC-CoSalen due to the small amount of CoSalen immobilized onto MIC. As a result, it is difficult to characterize CoSalen using IR spectroscopy.

Fluorescence spectra of MIC-CoSalen

The excitation and emission spectra of MIC and MIC-CoSalen are shown in Figure 2. We know that CS has hot fluorescence, so the fluorescence of MIC is originated from imidazole group. The excitation and emission appears at 391 and 460 nm, respec-

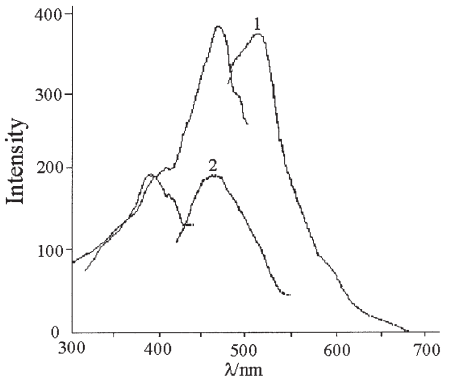


Figure 2 Excitation and emission spectra of MIC (2) (λ_{ex} = 391 nm, λ_{em} = 460 nm) and MIC-CoSalen (1) (λ_{ex} = 466 nm, λ_{em} 511 nm).

tively. This indicates that there is an imidazole group in MIC. The fluorescence spectrum of MIC-CoSalen is different from that of MIC. For MIC-CoSalen, the excitation and emission appear at 466 and 511 nm, respectively. Compared with that of MIC, the excitation and emission of MIC-CoSalen is red-shifted due to the imidazole group participating in the immobilizing reaction.

It can be seen from the spectrum of MIC-CoSalen that there is a fine structure. The spectra as shown in Figure 3 can be obtained when MIC-CoSalen is excited using light of 398 nm. The excitation and emission appear at 398 and 594 nm, respectively. This indicates that there are two fluorophores in MIC-CoSalen. Compared with that of CoSalen, a profile of the spectra of MIC-Cosalen, as shown in Figure 3, (peak 1) resembles those of CoSalen and (peak 2) is strong evidence for the existence of a CoSalen moiety in the polymer.

ESR spectra of MIC-CoSalen

Electron spin resonance spectroscopy provides a very sensitive probe for the existence of low-spin

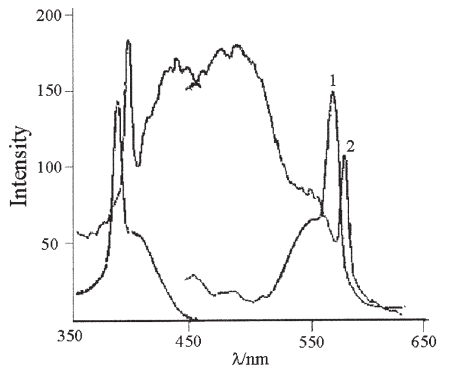


Figure 3 Excitation and emission spectra of CoSalen (1) (λ_{ex} = 385 nm, λ_{em} = 584 nm) and MIC-CoSalen (2) (λ_{ex} = 390 nm, λ_{em} = 605).

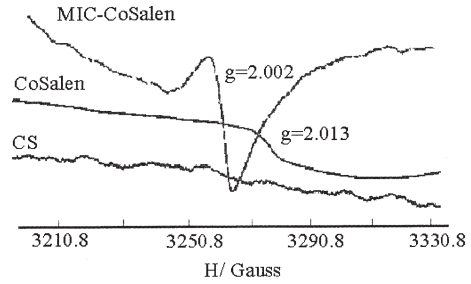


Figure 4 ESR spectra of CS, CoSalen, and MIC-CoSalen.

(Co(II)) and of superoxo-cobalt(II) centers. It is possible to distinguish the symmetry of a ligand-field and the electronic configuration of the central ion in CoSalen-like chelates from the nature of the ESR signal. The dioxygen adduct gives rise to a characteristic ESR signal at about g = 2.000. Any peroxo-bridge species of type $(CoSalen)_2O_2$ will, of course, be ESR silent. The typical ESR spectrum of Cosalen, CS, and MIC-CoSalen are shown in Figure 4. CoSalen gives a very broad poorly resolved signal. A clear signal at g = 2.002 appears in the ESR spectrum of MIC-CoSalen. This indicates that there is superoxo moiety (Co (III)O_2^-) in the MIC-CoSalen or an axial donor coordinating to cobalt of CoSalen, or both.[12] This result indicates that an oxygen molecule binds to the MIC-CoSalen through the vacant sixth coordination site.

XPS spectra of MIC-CoSalen

To look in detail at the interaction between MIC and CoSalen, CoSalen and MIC-CoSalen were characterized with X-ray photoelectron spectroscopy. The results are shown in Figure 5. The $Co_{2p3/2}$ binding energy for CoSalen is 780.5 eV, whereas for MIC-CoSalen is 781.2 eV. This seems a rather strange result because the coordination of nitrogen in imidazole to the cobalt in CoSalen would increase the electron density on cobalt. This donation of electron density would result

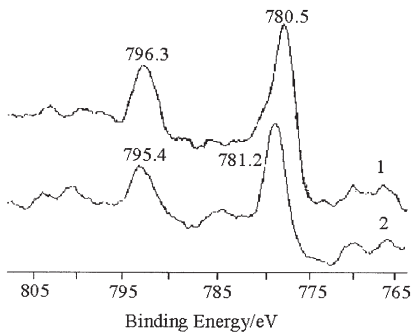


Figure 5 XPS spectra of CoSalen (1) and MIC-CoSalen (2) for $Co_{2p3/2}$.

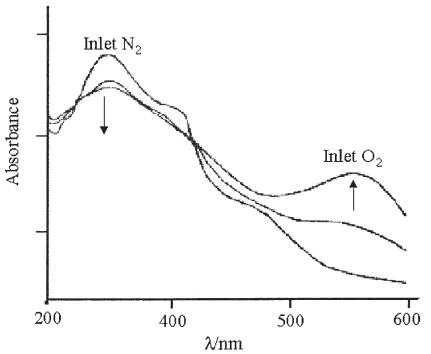


Figure 6 UV and visible spectral change after exposure of MIC-CoSalen to an atmosphere of oxygen or nitrogen.

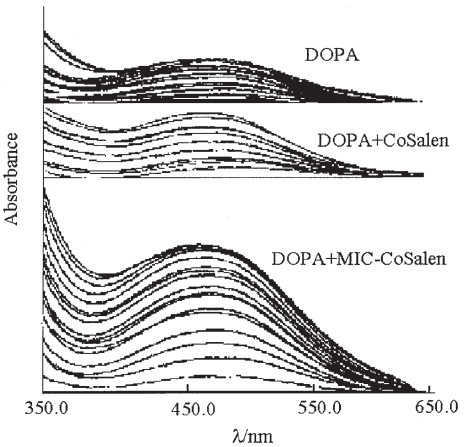


Figure 7 The UV–vis spectra of the products in the system self-oxidation of DOPA and DOPA oxidation catalyzed by MIC and MIC-CoSalen.

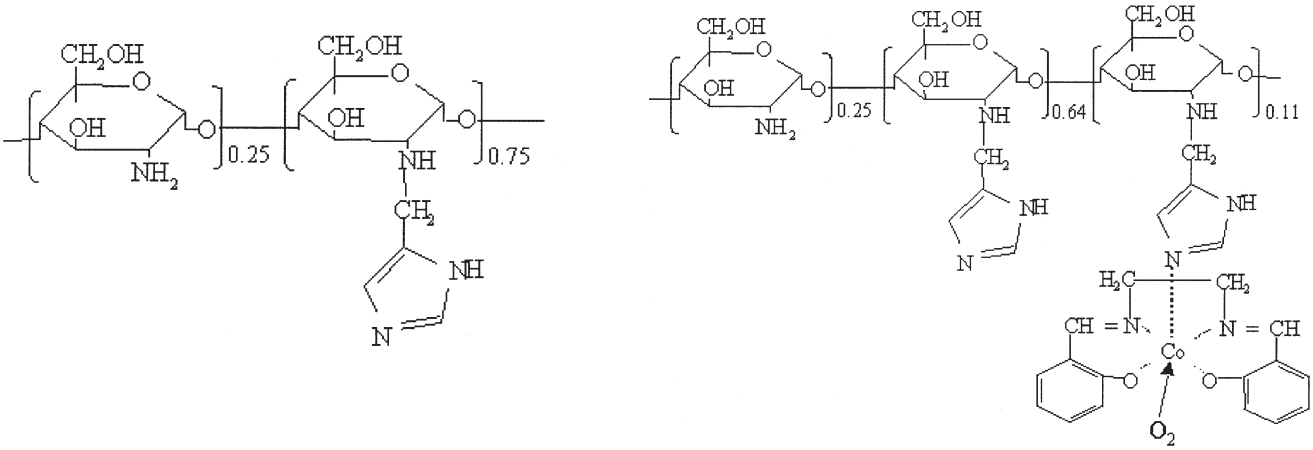
in a lower cobalt binding energy. The apparent contradiction may be explained by the fact that the cobalt ion in the five-coordinated MIC-CoSalen is in a high-spin state, whereas, CoSalen, the four-coordinated complex, is in a low-spin state. The electron–electron repulsion in the complex makes the nucleus of the metal more "shielded" from some of the electron density of the ligand in the high-spin form than in the low-spin form. Consequently, the positive charge on the metal ion would not be reduced as much as it would be if the complex were in a low-spin state. Therefore, the binding energy for the central ion in the five-coordinated complex is higher.[13]

UV spectroscopy of MIC-CoSalen membrane

Reversible oxygen binding to MIC-CoSalen in the membrane was observed with a spectral change in the visible absorption. The spectra are shown in Figure 6. The color of the membrane changed reversibly from brown to deep violet on exposure to oxygen and nitrogen. UV and visible absorption maxima λ_{max} at 558 nm (oxy: Co:O_2 = 1:1 adduct), λ_{max} at 348 nm, 413 nm (deoxy: five-coordination), isosbestic point at 337 and 425 nm, which indicates that CoSalen is immobilized onto the polymer chain, and that CoSalen supported by polymer can reversibly bind oxygen.

Composition of MIC-CoSalen

MIC and MIC-CoSalen were further confirmed by elemental analysis. The amount of cobalt in MIC-CoSalen was determined by atomic absorption spectrometry. MIC, C (%): 48.87; H (%): 6.33; N (%): 15.84. MIC-CoSalen, C (%): 50.30; H (%): 6.05; N (%): 14.83; Co (%): 2.50. According to the results, immobilization of CoSalen onto MIC is realized through the binding of the imidazole group in MIC to the cobalt of CoSalen. Clearly, cobalt present in the MIC-CoSalen is in an unsaturated coordination state. The sixth coordination site of MIC-CoSalen may be occupied by molecular oxygen when it is exposed to air. The structure



Scheme 1 The structure and unit numbers of MIC and MIC-CoSalen.

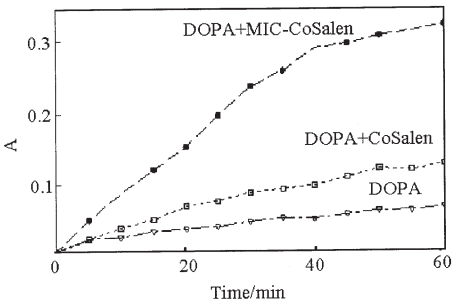


Figure 8 Catalytic effect of MIC-CoSalen and the related controls for the oxidation of DOPA.

and unit numbers of the polymer are shown in Scheme 1.

DOPA oxidation catalyzed by MIC-CoSalen

The oxidation of DOPA, catalyzed by the MIC-CoSalen, and the controls were quantified by measuring the absorbance of dopaquinone at 475 nm. The results are shown in Figures 7 and 8. It can be seen from Figure 7 that the UV–vis spectra of the products of self-oxidation catalyzed oxidation of DOPA were recorded. Examination of the spectra reveals that there are the same products in the system using MIC-CoSalen as for the catalysts and controls.

Figure 8 shows the absorbance at 475 nm, the characteristic absorbance of the catalysate as a function of time. It is obvious that the oxidation reaction occurring in the presence of catalyst is more efficient than in the blank system. Furthermore, the polymer-supported cobalt complex is much more efficient than CoSalen in catalyzing the oxidation of DOPA.

It is reported that CoSalen, the four-coordinated Co(II) complex, does not bind oxygen strongly, and forms inactive dimers easily. Although it can bind oxygen in the presence of a suitable monodentate Lewis base, the peroxo-bridged species of CoSalen, $(CoSalen)_2O_2$, which is inactive in catalyzing the oxidation, can be also formed.[3] Therefore, its catalyzing efficiency is limited. The site isolation effect presenting

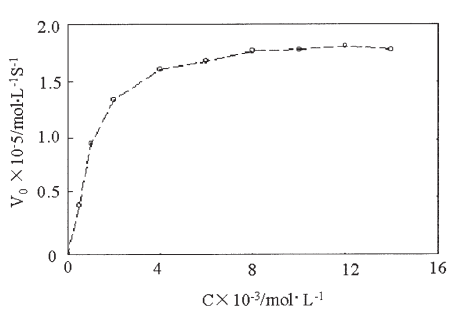


Figure 9 Initial oxidation rate of DOPA catalyzed by MIC-CoSalen as a function of initial DOPA concentration.

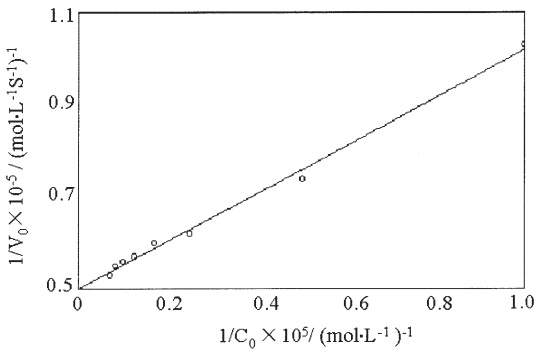
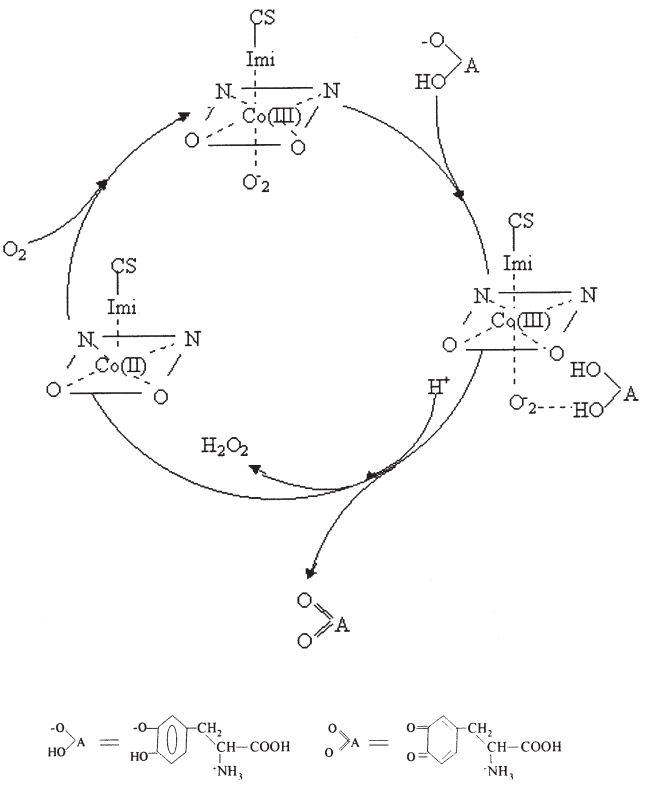


Figure 10 Double reciprocal plot for the oxidation of DOPA catalyzed by the polymer supported CoSalen.

in MIC-CoSalen inhibited the formation of dimers and peroxo-bridged dimers, and thereby promoted the formation of an active species of superoxo cobalt complex (Co(III) O_2^-).

Additionally, as shown in Figure 9, the rate of the oxidation is proportional to the DOPA concentration within a certain concentration range, and becomes independent of it in the high-concentration range. This is a typical behavior of Michaelis–Menten type kinetics, indicating the presence of a catalyst–substrate complex.[14]



Scheme 2 A proposed mechanism for the catalytic oxidation of DOPA.

If the reaction scheme is expressed as follows,

$$\text{Catalyst} + \text{Substrate} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{Catalyst} - \text{Substrate} \xrightarrow{k_3} \text{Product}$$

the following equation can be obtained:

$$\frac{1}{V_0} = \frac{1}{V_{\max}} + \frac{K_{\mathrm{m}}}{V_{\max}[\mathrm{S}]_0}$$

This, by definition, is the basis of Michaelis–Menten kinetics, where V_0, $V_{\max}$, and K_m represent the initial oxidation rate, the maximum oxidation rate, and Michaelis constant, respectively. Obviously, a plot of the reciprocal of the rate against the reciprocal of the substrate concentration should give a straight line. A typical example is shown in Figure 10. From the intercept of this line and its slope, $V_{\max}$ and K_m can be calculated. They were determined to be 2.0×10^{-5}mol L^{-1} S^{-1} and 9.6×10^{-6} mol L^{-1}, respectively.

On the basis of the earlier discussion and the results reported by others,[15] a mechanism for the catalytic oxidation of DOPA was proposed (Scheme 2).

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