# Oxidation of Catecholamines on Chitosan-Immobilized Co(II) Salen Complexes

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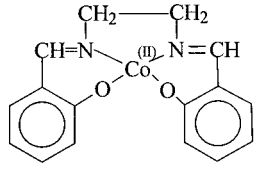
**Summary:** L-Adrenaline and 3,4-dihydroxyphenylalanine (dopa) oxidation by molecular oxygen in the presence of the Co(II) complex of bis(salicylideneethylene diamine) (CoSalen) immobilized on unmodified chitosan and chitosan modified by 4-pyridinecarboxaldehyde is studied in aqueous solutions under mild conditions. The catalysts prepared are selective with respect to oxidation of the chosen substrates. Preparation of binary composite egg-shell systems, with a thin film of low-loaded CoSalen-chitosan supported on macroporous $SiO_2$ makes it possible to increase sufficiently the specific surface area and the efficiency of the catalyst.

**Keywords:** catecholamines;catalysis; chitosan; CoSalen complexes; metal-polymer complexes

## Introduction

The capability of some cobalt complexes of reversibly binding molecular oxygen due to the formation of monomer adducts makes them especially attractive for oxidation catalysis. Furthermore, these complexes can be used as convenient model moieties in studying the mechanism of action of metal-containing oxygen transferring enzymes.[1]

One of the most widely used Co(II) complexes with a handful of valuable properties is a tetra-coordinated Co(II) complex of bis(salicylideneethylene diamine) (CoSalen):




CoSalen

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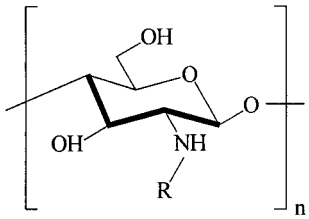
It is well known that the presence of monodentate Lewis basis intensifies the ability of such complexes to retain molecular oxygen. However, the catalytic activity of these complexes tends to decrease because of easy formation of dimers and peroxy-bonded adducts. These compounds are inactive toward reversible oxygen binding. To avoid the formation of the dimers, diverse polymeric supports including biopolymers, which exhibit an isolating effect, are used as carriers.[2,3]

Chitosan, i.e. N-deacetylated chitin (poly(1-4)N-acetyl-$\beta$-D-glucosamine), is a biodegradable polysaccharide containing different functional groups.




Chitosan

This polymer demonstrates unique adsorption ability toward many metal cations and atoms of Periodic Table.[4,5]

CoSalen complex can be immobilized on initial chitosan via the amino group (CoSalen-Chit complex preparation, where Chit stands for chitosan)[6] as well as on polymer whose amino group is modified with 4-pyridinecarboxaldehyde (modification results in the formation of N-(4-pyridine methylidene) chitosan (PM-Chit)). In this case, 4-pyridinecarboxaldehyde plays the role of a Lewis base and thereby it enhances the complex ability to bind molecular oxygen.[7]

The goal of this investigation is to develop effective catalysts based on chitosan-immobilized Co(II) Salen complexes for the reaction of catecholamine oxidation.

## Experimental

### Materials

CoSalen (Aldrich Chem. Co.) and L-adrenaline (Fluka AG) were purchased as analytical grade reagents, L-3,4-dihydroxyphenylalanine (dopa), adrenochrom (3-hydroxy-1-methyl-5,6-indolinedione) were also purchased as analytical grade reagents from Sigma AG, Germany. 4-Pyridinecarboxaldehyde (chemically pure grade) was used as a modifying agent.

***Initial chitosan.*** Two types of chitosan powder (made in Korea from crab shells, molecular weight 100,000-150,000) were used without further purification. Data of elemental analysis and main characteristics for these chitosans are presented in Tables 1 and 2.

Table 1. Elemental Analysis data.

| Element | Obtained | | Calculated* | Literature data |
|---|---|---|---|---|
| | Sample No.1 | Sample No.2 | | [8] |
| C | 41.41 | 41.34 | 44.72 | 41.13 |
| H | 6.34 | 6.32 | 6.88 | 7.31 |
| N | 7.24 | 7.36 | 8.69 | 7.35 |

*for chitosan with deacetylation degree 100%.

Table 2. Key features of polymer carriers.

| Sample | Deacetylation degree: mol $NH_2$/(mol $NHCOOCH_3 + NH_2$) | Molecular weight | Moisture content, % |
|---|---|---|---|
| No. 1 | 0.68 | 150000÷300000 | 2.4 |
| No. 2 | 0.73 | 350000÷700000 | 1.8 |

***Chitosan modified with 4-pyridinecarboxaldehyde.*** Chitosan was modified with 4-pyridinecarboxaldehyde by the conventional methods (Shiff reaction) [7-8] A portion of chitosan (2.5 g, 0.0155 mol (based on monomer)) was dissolved in 300 ml of 1% acetic acid under heating and intensive stirring. 4-Pyridinecarboxaldehyde (0.87 g, 0.008 mol) dissolved in 10 ml of methanol was added dropwise to the obtained gel solution during 30 min. The reaction occurrence was controlled by color change (from colorless to yellow) of the reaction solution. The reaction mixture was stirred for 1.5 h, then pH of the solution was adjusted to 6.0, and the mixture was stirred again until the formation of a white-yellow gel product. The gel formed was repeatedly washed with water until neutral pH, and then the gel was washed several times with methanol. The obtained white powder polymer was air-dried.

**Catalyst Preparation**

***Adsorption method.*** A portion of chitosan (1 g) was placed in 20 ml of a saturated CoSalen aqueous solution. The mixture was shacked for 24 h. After this procedure, the catalyst particles were washed in a Soxhlet extractor with ethanol until the decoloration of the extract. The catalyst obtained was air dried for 24 h.[7]

***CoSalen complex with chitosan modified by 4-pyridinecarboxaldehyde.*** CoSalen complex with modified chitosan was obtained according to the procedure used for the unmodified catalyst. One

gramm of modified polymer was shacked with a saturated aqueous solution of CoSalen. Then the polymer was washed with ethanol in a Soxhlet extractor and air-dried for 24 h.[7]

***Homogeneous CoSalen-chitosan complex.*** A calculated amount of CoSalen was placed in a saturated aqueous solution of chitosan hydrochloride. The reaction mixture was stirred at room temperature until the dark-brown clear gel product was formed.

***Coprecipitation method.*** A dark-brown solution of the homogeneous CoSalen-chitosan complex was prepared as mentioned above. The complex obtained was added dropwise to a 0.5 M solution of NaOH. Spherical dark-brown globules formed were filtered off and washed repeatedly with distillated water until pH=7.0 and air-dried for 48 h.

***Immobilization of the CoSalen-chitosan complex on the surface of porous $SiO_2$.*** A portion of amorphous $SiO_2$ (*KSS-3,* 1 g, fraction 0.25-1 mm; $S_{BET}$=450 $m^2/g$; moisture capacity – 1.8 ml/g) was impregnated with 1.8 ml of a homogeneous CoSalen-chitosan complex solution. Silica gel impregnated was kept in 0.5 M NaOH for 15 min, and then the silica gel with the immobilized CoSalen-chitosan complex was washed repeatedly with distilled water until neutral pH. The catalyst obtained was air-dried for 24 h and then dried in a vacuum for 10 h.

**Characterization**

IR-Spectroscopy

Transmission FTIR spectra were recorded at 20°C using a Nicolet Protege 460 spectrometer in the range of 4000-400 $cm^{-1}$ at a resolution of 8 $cm^{-1}$, and also with a Matteson Galaxy Series FTIR 5000 spectrometer in the range of 4000-600 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. For the study of the homogeneous CoSalen-chitosan complex, some droplets of the solution were deposited on quartz plates and air-dried for 48 h. Dry globules of heterogeneous chitosan samples (adsorption and coprecipitation complexes) were ground in a mortar, the fine powder was mixed with KBr, pressed into a thin pellet, and placed in the sample holder of the spectrometer. The OMNIC software was used for the processing of the spectra.

XPS

An XPS study of the CoSalen-chitosan complexes was carried out using an XSAM-800 (Kratos) spectrometer with Al $K\alpha_{1,2}$ irradiation. The atomic ratios were determined from the integral peak

intensities by using the photoionization cross-sections for Al $K\alpha_{1,2}$ radiation.[10] The binding energies ($E_b$) were corrected to take into account the effect of sample charging using the C 1*s* signal (285.0 eV) as a standard.

Elemental Analysis

Metal contents in the samples were determined by the atom-emission spectroscopy in V.I. Vernadsky Institute of Geochemistry, Russian Academy of Sciences (The laboratory of substance analysis). The measurements of the contents for other elements were carried out in the laboratory of microanalysis No.18 of the A.N. Nesmeyanov Institute of rganoelement Chemistry, RAS.

**Catalytic Tests of CoSalen-chitosan Complexes in the Reaction of Catecholamine Oxidation**

CoSalen-chitosan complexes were tested as catalysts in catecholamine oxidation into corresponding quinones by molecular oxygen. The catalyst was loaded in a thermostatically controlled glass reactor with a magnetically stirred substrate aqueous solution (molar ratio substrate/catalyst = 5/1÷50/1) through which oxygen was purged at a rate of 120 ml/min for 3 h. The samples of the reaction mixture were taken periodically for the analysis. A control for the reaction proceeding was carried out by the change of the absorption band intensity in UV-Vis spectra ascribed to quinone products (Specord M-40 Carl Zeiss spectrometer, bands with maximum absorption at 475 nm and 480 nm for dopa-quinone and adrenochrome, respctively). Since the 3,4-dihydroxyphenylalanine oxidation product is unstable, it is impossible to draw the calibrating dependences for this compound. The change in the dopa-quinone concentration was controlled by the change of a directly proportional value –ln(T) (T - transmittance).

## Results and Discussion

At the initial stage of our research, the oxidation of substrates by molecular oxygen was carried out without a catalyst as well as in the presence of a homogeneous CoSalen aqueous solution. Kinetic curves of DOPA and L-adrenaline oxidation presented in Fig. 1 indicate that the rate of substrate oxidation sufficiently increases in the presence of CoSalen.

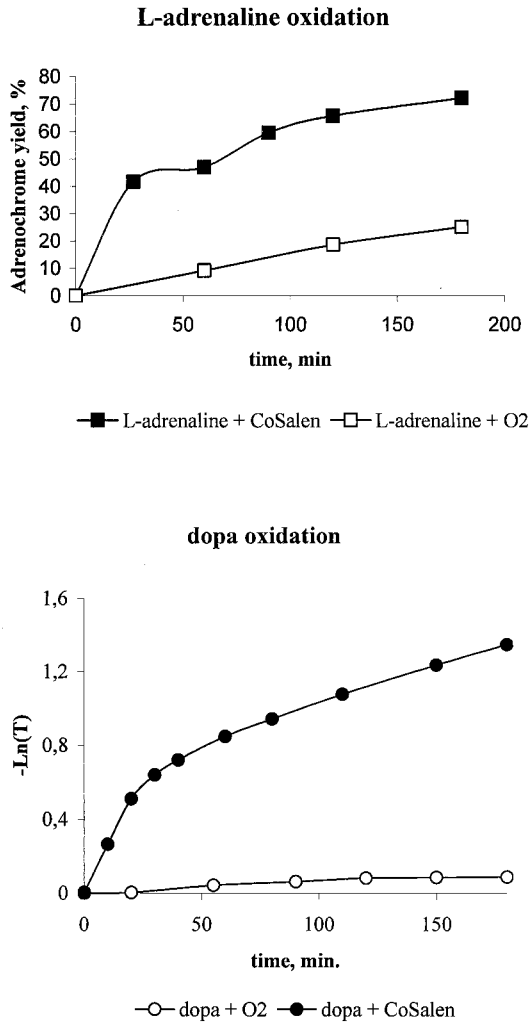



Fig. 1. Kinetic curves of dopa and L-adrenaline oxidation without a catalyst and in the presense of the CoSalen aqueous solution.

IR spectra of initial chitosan and CoSalen-chitosan complex prepared by the adsorption method are presented in Fig. 2. The absorption band which appears in the spectrum of the CoSalen-

chitosan complex at 576 $cm^{-1}$ could be ascribed to stretching vibrations of the Co-N bond [9]. The change in the intensity as well as broadening of the bands of the bending virations of the chitosan amino group (1600-1650 $cm^{-1}$) also testify to the formation of the CoSalen-chitosan complex.[10,11] An assumed structure of the catalytic site is presented in Fig. 3 In accordance with XPS data, the metal content in the polymer complex obtained is 1.13 wt. %.

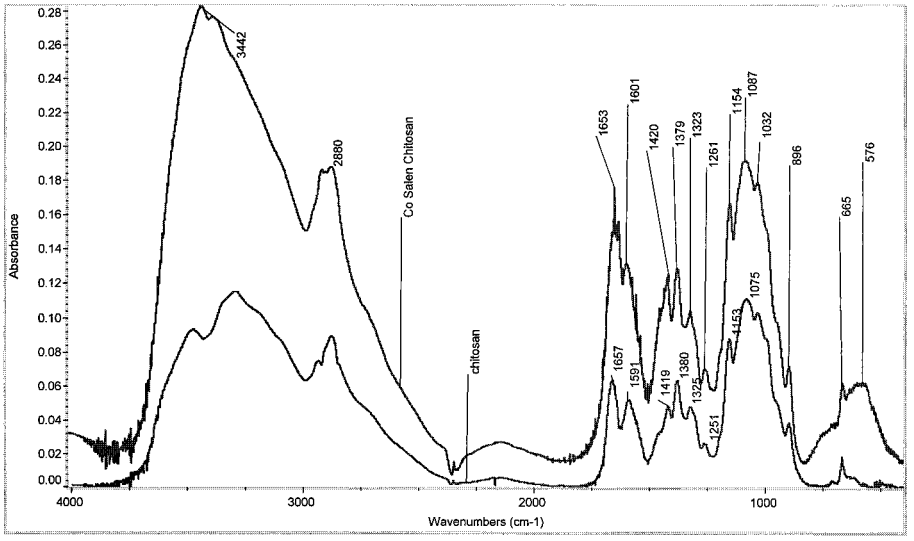



Fig. 2. IR spectra of initial chitosan and adsorption CoSalen-chitosan complex.

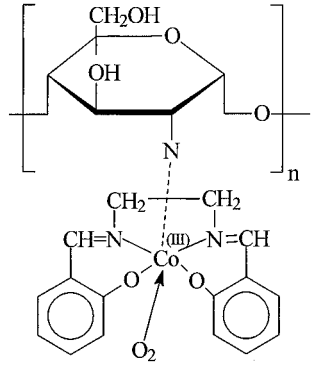



Fig. 3. An assumed structure of the active site of the CoSalen-chitosan complex.

Using the abovementioned complex as a catalyst, it was found that the reaction rate of catecholamine oxidation significantly decreases as compared with homogeneous conditions for the substrates used. Furthermore, leaching of the Co complex from the polymeric support in a reaction solution was detected.

To increase the coordination ability of the chitosan amino group, the polymer was modified with 4-pyridinecarboxaldehyde. IR spectra of initial chitosan, modified chitosan, and CoSalen complex with modified chitosan are presented in Fig. 4. IR data on the modified polymer are in a good agreement with the literature data.[10]

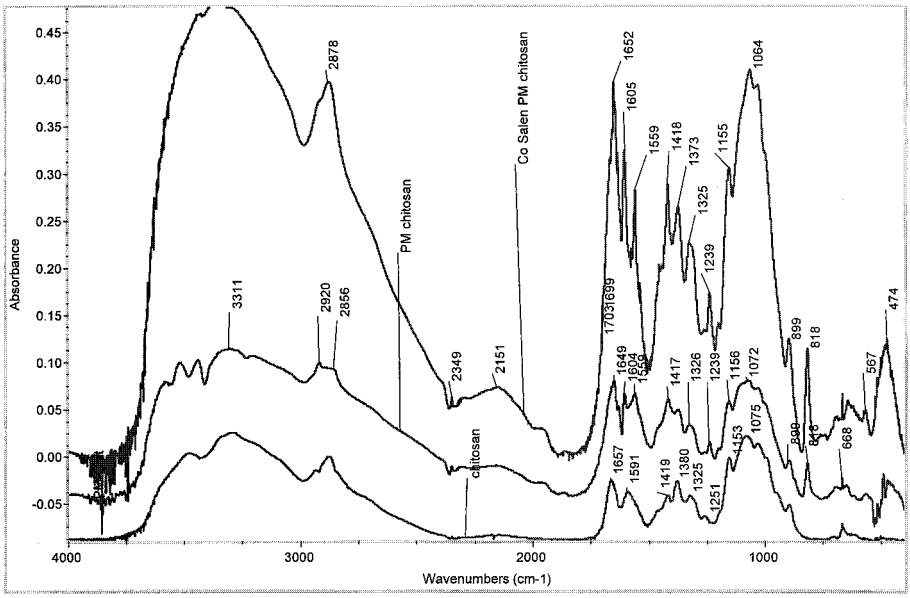



Fig. 4. IR spectra of initial chitosan, chitosan modified with 4-pyridinecarboxaldehyde (4-pyridinemethylidene chitosan), and CoSalen complex with the modified polymer.

Appearance of the bands with absorption maxima at 1649-1652 and 818 $cm^{-1}$ testifies to the inclusion of the pyridine ring in the structure of the polymeric matrix. According to elemental analysis data (Table 3), only 16.7% of free amino grous of initial chitosan are modified.

However, the catalytic testing of the polymer complex obtained also indicates its extremely low activity as a catalyst in DOPA oxidation. The reaction rate of oxidation of L-adrenaline remains

significantly lower as compared to the homogeneous catalyst, and the sufficient dependence of the reaction rate on the particle size of the heterogeneous catalyst was noticed (Fig. 5).

Table 3. Elemental analysis data for chitosan modified with 4-pyridinecarboxaldehyde.

| Element | Initial chitosan | Modified chitosan | Substitution degree of amino groups in modified polymer |
|---|---|---|---|
| C | 41.34 | 46.71 | 16.7% |
| H | 6.32 | 6.06 | |
| N | 7.36 | 8.18 | |

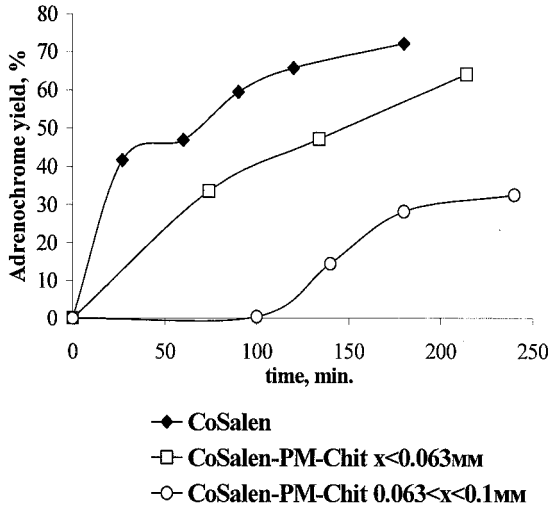



Fig. 5. Kinetic curves of L-adrenaline oxidation on the CoSalen homogeneous catalyst and heterogenized CoSalen-PM-chitosan complex with different particle size.

The heterogenized CoSalen-chitosan complex was synthesized by the coprecipitation method. It should be mentioned that, in contrast to the coprecipitated complex, its precursor, i.e. the homogeneous CoSalen-chitosan complex, demonstrates no activity in catecholamine oxidation. But surprisingly no sufficient differences were observed upon examination of the IR spectra of the CoSalen-chitosan complexes prepared by different methods (adsorption and coprecipitation).

The similar picture is reproduced in detailed IR and ESR studies of the structure of copper(II)-chitosan complexes prepared by different methods.[11]

Catalytic tests indicate that in the case of the coprecipitation method, the CoSalen complex is strongly retained by the chitosan matrix. No even trace amounts of the Co(II) complex were found in the reaction solutions on carrying out the quantitative experiments. The complex is rather inactive in L-adrenaline oxidation. The reaction rate of the heterogeneous dopa oxidation is higher than the reaction rate of the homogeneous process (Fig. 6).

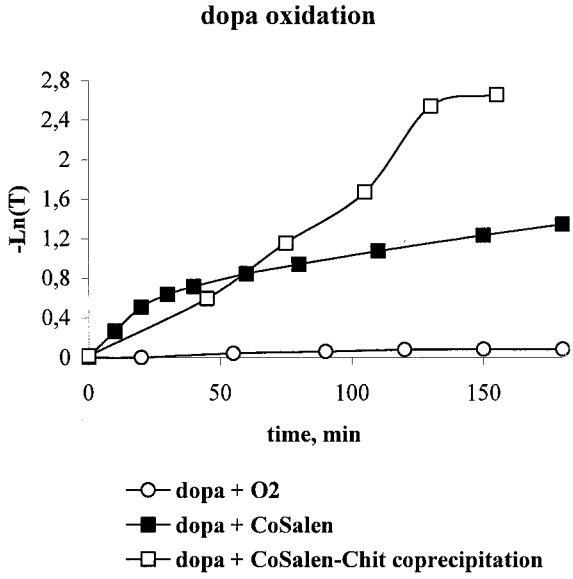



Fig. 6. Kinetic curves of dopa oxidation by molecular oxygen without a catalyst, in the presence of the homogeneous CoSalen complex and in the presence of the heterogeneous CoSalen-chitosan catalyst prepared by coprecipitation.

As was shown in our earlier paper devoted to structure investigations and catalitic testing of copper(II)-chitosan complexes, egg-shell systems with macroporous $SiO_2$ immobilized thin film of the Cu-chitosan complex are the most effective catalysts in oxidative processes.[11]

Such an approach allows one to increase significantly the catalyst surface area thereby increasing

the number of accessible active sites. The development of egg-shell systems was realized by two different ways. On the one hand, the homogeneous CoSalen-chitosan complex was immobilized on the surface of silica gel ([CoSalen-chitosan]$SiO_2$). On the other hand, chitosan was immobilized on the surface of silica gel, and then the support prepared was impregnated with an aqueous solution of the Co(II) complex (CoSalen[chitosan-$SiO_2$]).

In the case of egg-shell systems, no matter what is the preparation method, the rate of the reaction of catecholamine oxidation is significantly higher than in the case of corresponding bulky complexes or a homogeneous CoSalen solution.

The diagrams presented in Figs. 7 and 8 makes it possible to obtain the overall comparative picture for all the catalysts used in DOPA and L-adrenaline oxidation processes.

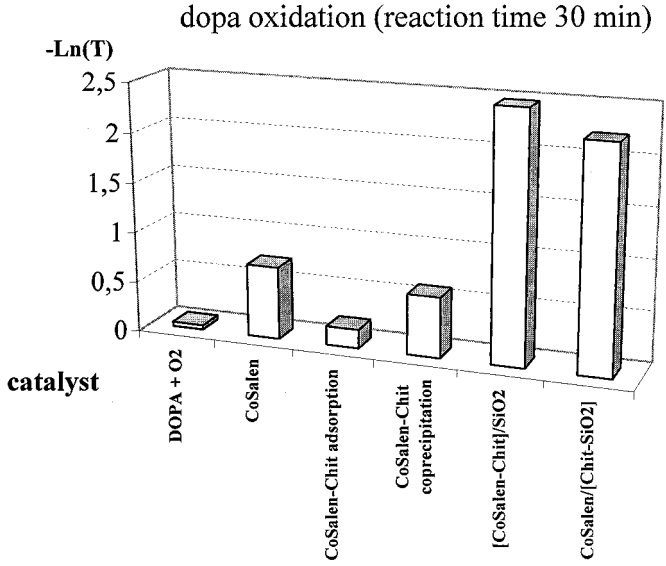



Fig. 7. Diagram of the catalyst efficiency of DOPA oxidation.

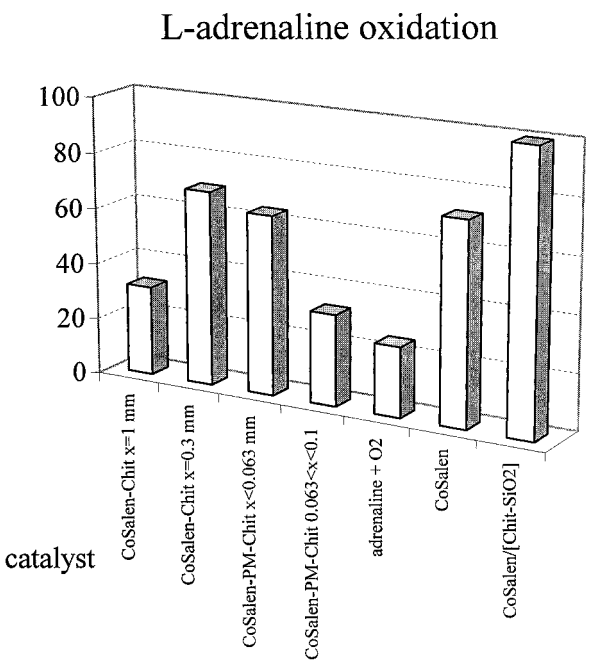



Fig. 8. Diagram of the catalyst efficiency of L-adrenaline oxidation.

However the question about the nature of the observed catalyst selectivity toward the substrates used remains unclear. It seems to be a topic of further investigations.

## Conclusions

1. A principal possibility of the utilization of the CoSalen complex immobilized on the unmodified and modified chitosan in catecholamine oxidation was demonstrated;

2. The dependence of CoSalen-chitosan complex activity on the preparation method was established;

3. It was found that egg-shell systems (CoSalen-chitosan complex immobilized on the $SiO_2$ with a developed surface area) are most effective as catalysts in the catecholamine oxidation.

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