

Studies on a catalase model system based on immobilised metalloporphyrins on modified solid polystyrene support

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Abstract. Metalloporphyrins immobilised on solid polymer supports are developed as efficient model systems for catalyse enzyme, which dismutate hydrogen peroxide in many living organisms, saving them from several harmful oxidative processes. The metalloporphyrins employed are Mn(III), Fe(III) and Co(III) derivatives of anionic tetrasulphonated tetraphenylporphyrins (H_2 TPPS). The polymer support used was divinylbenzene crosslinked polystyrene (in bead form) which was first surface functionalised to chloromethyl derivative and then to N-alkyl pyridinium derivatives (PS). The negatively charged MTPPS were then appended on to the polymer support by ion exchange method. The catalytic efficiency of these immobilised MTPPS was found to be far superior to other supported systems reported earlier. The relative efficiency of the three supported metalloporphyrin systems was studied and compared in varying pH and temperature conditions. In acidic pH, the order of efficiency of the metalloderivatives was Fe(III) > Mn(III) > Co(III) while in basic pH, the order changed to Mn(III) \approx Co(III) > Fe(III). The maximum efficiency was found to be around pH 10.0 in all the cases. While the Fe(III) derivatives showed greater sensitivity to temperature, the Mn(III), on the contrary, showed the least sensitivity. The catalytic efficiency of the immobilised systems was found to be retained even after repeated use of the catalysts indicating a high degree of recyclability.

Keywords. Polystyrene support; metalloporphyrins; catalase model.

1. Introduction

Porphyrin is an essential moiety of several biological systems. A variety of synthetic porphyrins and their metalloderivatives have been demonstrated to be efficient model systems for many life processes and enzyme actions. The possibility of the redox tuning of these cyclic conjugated systems by peripheral substitution and central metal variations makes them versatile catalysts¹⁻⁴. It has been shown that their applicability can also be enhanced by immobilising them on solid polymer support⁵⁻⁷. The electronic energy levels of metalloporphyrins are found to be dependent on the type of the polymer backbone. Consequently, the nature of polymer support is also expected to play a major role in redox tuning the metalloporphyrin moiety and imparting a high degree of specificity in enzyme mimicking. Besides, such polymer supported metalloporphyrins possess several advantages over conventional homogeneous catalysts, such as, possibility of better and easy work up, recyclability and controllability of micro environments.

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The catalase enzyme, a heme protein with molecular weight around 240,000 is very vital for catalytic dismutation of hydrogen peroxide which gets accumulated in body fluids by various life processes and cause damage to haemoglobin through oxidation⁸. A few model systems have already been reported⁹, among which Fe(III) macrocyclic systems are probed in some detail¹⁰. It is known that one major detrimental factor for Fe(III) porphyrin acting as an efficient catalase model is its tendency to form μ -oxo dimer species¹¹. The present study is an attempt to develop more efficient metalloporphyrin-based catalyst model systems immobilised on modified polymer support which can function effectively in aqueous medium without the possibility of μ -oxo dimerisation. Along with Fe(III) porphyrins, their Mn(III) and Co(III) analogues are also developed and studied for their enzyme action.

2. Experimental

2.1 Preparations

Tetraphenylporphyrin (H_2 TPP) was prepared from pyrrole and benzaldehyde by the Adler's method¹² with some modifications followed by purification by column chromatography. It was sulphonated under controlled conditions and purified by reported procedure¹³ to get the tetrasulphonated derivatives (H_2 TPPS). The metallo-derivatives MTPPS (M = Fe(III), Mn(III), Co(III)) were prepared in aqueous conditions by reacting H_2 TPPS with metal salts, especially their chlorides. The solution was heated on a steam-bath until there was no spectrophotometric evidence for the free-base porphyrin. The solution was then evaporated to dryness, dissolved in a minimum amount of methanol and filtered. The process was repeated 2–3 times. The final filtrate was then passed through a silica gel (60–120 mesh) column using methanol as eluant.

The polymer support was prepared by reacting divinylbenzene (DVB) crosslinked polystyrene in bead form (5 gm) with chloromethyl methyl ether (30 ml) at 45–50°C. Anhydrous $ZnCl_2$ in THF was used as the catalyst. Heating was stopped after 8 h and the reaction mixture kept for one day. The beads were thoroughly washed first with dichloromethane, then acetone and finally with methanol before drying. This chloromethylated resin was further functionalised by heating with pyridine for about 8 h, followed by washing off excess pyridine by dichloromethane and acetone. The resulting polymer with N-alkyl pyridinium functions is then used as an effective polymer support (PS) for immobilising anionic MTPPS by the anion exchange method.

2.2 Physical measurements

The characterisation of porphyrin and their metalloderivatives was carried out by electronic and IR spectral measurements using Shimadzu UV-160A and IR-470 spectrophotometers. The electronic spectra (in solution) of the ionic porphyrins were measured either in water or methanol. IR spectra were recorded in solidstate in the form of KBr pellets. The quantitative estimation of grafted MTPPS was done by monitoring absorbance decrement of Soret peak of the MTPPS solution. The electronic spectra of supported porphyrins were measured in solidstate by grinding the solid PS - MTPPS with nujol and spreading the fine paste obtained uniformly on a strip of Whatmann 41 filter paper. The reference strip contained the paste made from the porphyrin-free polymer support.

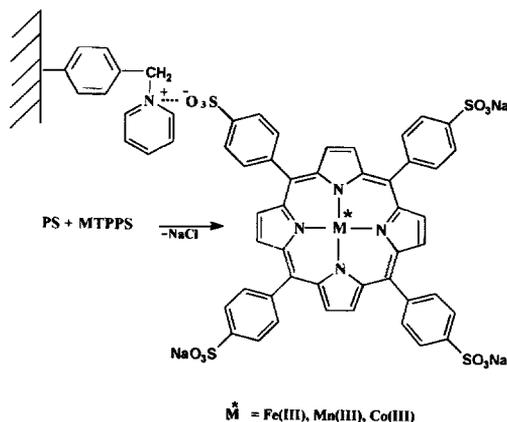
2.3 Catalytic reaction monitoring

The catalase activity was monitored by noting H_2O_2 decomposition in presence of solid PS-MTPPS in aqueous medium. In a typical reaction, 20 mg of catalyst (2.0×10^{-6} mol MTPPS) was added to 4 ml buffer containing 7 mg H_2O_2 (taken in solution form) and stirred continuously. After the specified time, the non-decomposed H_2O_2 was determined titrimetrically using KMnO_4 . A blank was also carried out with catalyst-free polymer beads. Catalytically decomposed H_2O_2 was then calculated from the difference obtained. Buffers used in this study were 0.1 N H_2SO_4 (pH 1), acetate buffer (pH 4) carbonate buffer (pH 10) and 0.1 M NaOH (pH 13).

3. Results and discussion

3.1 Characterisation

The functionalisation of DVB-crosslinked polystyrene was necessary to graft metalloporphyrins on the support. For this, the solid polymer was subjected to chloromethylation by reacting with chloromethyl methyl ether. The functionalisation (CH_2Cl) was confirmed by chemical analysis and IR measurements (appearance of $\nu_{\text{C-Cl}}$ bands at 668 and 1420 cm^{-1}). The CH_2Cl function of the support was further converted to N-alkyl pyridinium chloride function by treating with excess pyridine to get cationic resins (PS). The extent of N-alkyl pyridinium function was found out by estimating the ionic chlorides (using Volhard's method) which gave a value of 3.8 meq/g of the resin. This value, in fact, corresponds to total Cl content in the resin. The metalloporphyrins which are converted to anionic form (MTPPS) are essentially the sodium salt of tetrasulphonated metalloporphyrins. All the MTPPS were characterised by electronic spectra. The immobilisation of MTPPS on the N-alkyl pyridinium functionalised polymer support (PS) mentioned above, was done by a simple ion-exchange method in aqueous condition. Once ionically bonded to the support, these porphyrins are found to be not exchangeable easily and the support system is seen to be very stable both in polar and nonpolar solvents. The spectral measurements confirmed the presence of the appended porphyrin. The PS-MnTTPPS gave absorption peaks at 405, 475, 577 and 615 nm; PS-FeTTPPS at 420, 573, 615 nm and PS-CoTTPPS at 433 and 532 nm, respectively. The nature of the immobilised porphyrin is shown in scheme 1.



Scheme 1.

The immobilisation, is in fact, such that two or more of the sulphonate groups of a single MTPPS moiety exchange Cl^- from PS. The extent of the appended porphyrin was estimated spectrophotometrically by measuring the absorbance decrement of the Soret peak of the MTPPS solution after treating it with a known amount of solid PS. The estimation gave 0.1 meq of MTPPS uptake per g of resin. This unexpectedly low value could be attributed to the bulky nature of the MTPPS moiety and its ability to exchange even four anions of the resin by its single unit. It was found that the electronic spectra of the MTPPS get slightly altered on appending, indicating some electronic modification within MTPPS.

3.2 Catalase activity

The reaction of catalase in living organisms is essentially represented by

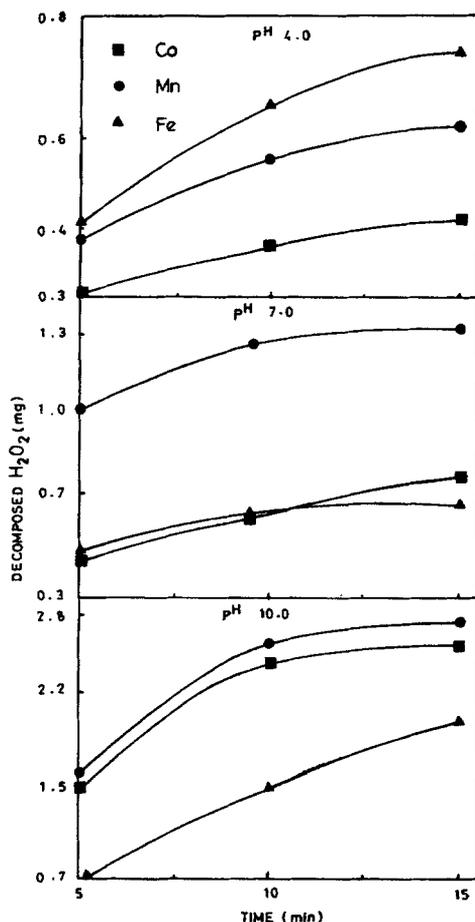
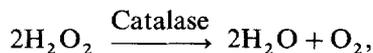


Figure 1. Dependency of metal ion on the catalase-like activity of PS-MTPPS at different pH conditions. (▲-PS-FeTPPS, ●-PS-MnTPPS, ■-PS-CoTPPS, Temperature-30°C)

in which the dismutation of hydrogen peroxide is achieved by a high degree of efficiency. All water soluble metalloporphyrins (MTPPS) developed in the present study were found to exhibit a moderate catalase-like activity in the unsupported form, as was evident from some amount of H_2O_2 decomposition detected. However, it was not possible to quantify this as the end point during the titration and it could not be properly detected because of the colour interference of $KMnO_4$ and dissolved MTPPS. An attempt by the spectrophotometric method also met with failure because of the absorption overlap of $KMnO_4$ and the porphyrins. However, in the case of all the polymer supported PS-MTPPS systems, we could easily monitor the catalytic activity, as the catalyst could be easily removed by filtration when required, while determining the extent of H_2O_2 decomposition. As mentioned earlier, the amount of MTPPS present in the modified support was very low and they all showed a high degree of catalytic efficiency.

It was found that the three metalloderivatives have varying efficiencies with respect to the dismutation reaction. In neutral condition, the most effective system was that of Mn(III), and the least efficient—the Fe(III) system. In acidic condition the order gets changed to Fe(III) > Mn(III) > Co(III); while in basic condition it is Mn(III) \approx Co(III) > Fe(III). The details are shown in figure 1.

The effect of the amount of the catalyst and the concentration of initial H_2O_2 on the catalase-like activity was also studied. From the kinetic measurements, the decomposition of H_2O_2 was observed to obey a first order kinetics of the concentration of the metalloporphyrins and of H_2O_2 (figures 2 & 3).

Similar observations were reported earlier for water soluble monomeric porphyrins¹⁰ and for some polymer supported macrocycles¹⁴. Based on the

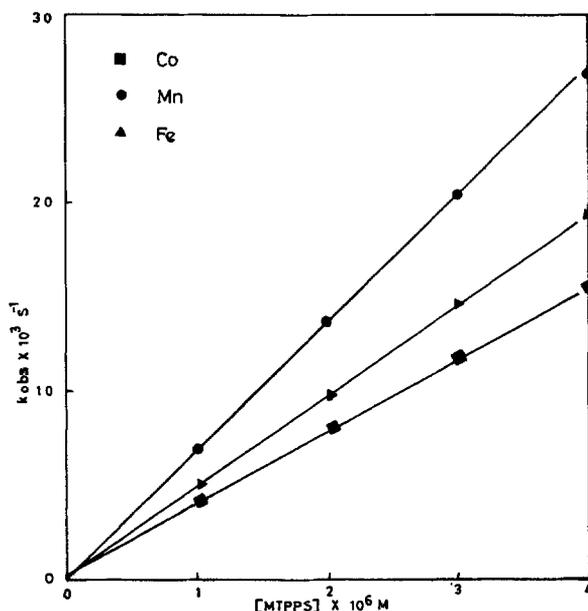


Figure 2. Observed first order rate constants for the decomposition of H_2O_2 as a function of the amount of catalyst. (▲-PS-FeTPPS, ●-PS-MnTPPS, ■-PS-CoTPPS, Temperature-30°C, pH-7, time - 10 min)

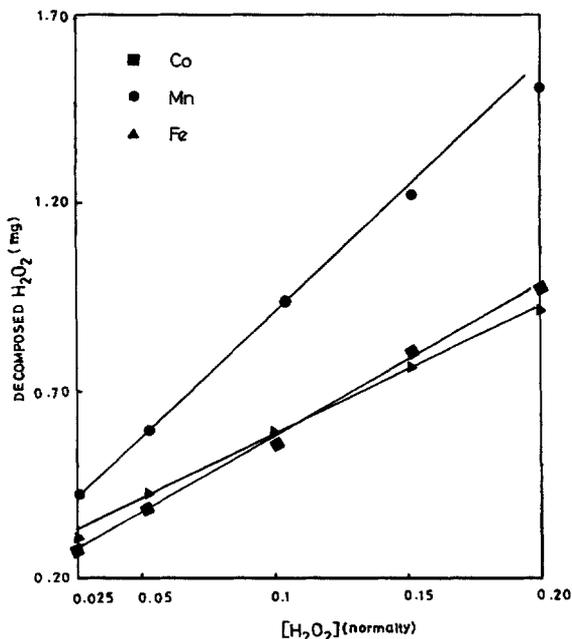
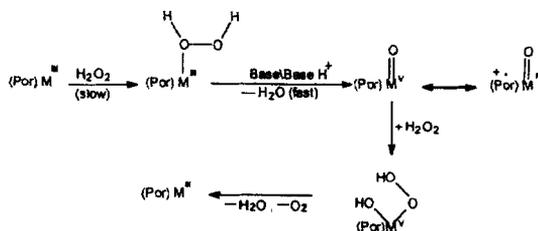


Figure 3. Amount of decomposed H₂O₂ as a function of [H₂O₂]. (▲)-PS-FeTPPS, (●)-PS-MnTPPS, (■)-PS-CoTPPS, amount of PS-MnTPPS-20 mg, Temperature-30°C, pH-7, time – 10 min)



Scheme 2.

observed data, the mechanism for the decomposition of H₂O₂ by metalloporphyrins may be considered as follows (scheme 2), in conformity with that indicated by Meunier¹⁵.

The pH condition of the reaction medium was also found to have a significant role in the catalase activity. In the case of supported CoTPPS, the efficiency was found to increase steadily up to pH 10.0 after which the efficiency slackened slightly. In the case of MnTPPS system also, the situation was found to be similar with maximum efficiency being exhibited around pH 10.0. But among these two systems, the Mn-system was found to be regularly better in enzyme activity than that of Co. Unlike the above two systems, the FeTPPS system exhibited significant activity at pH as low as 1.0, but on increasing the pH the efficiency was found to decrease with minimum at neutral pH. Above pH 7.0, the efficiency was found to further increase with the maximum

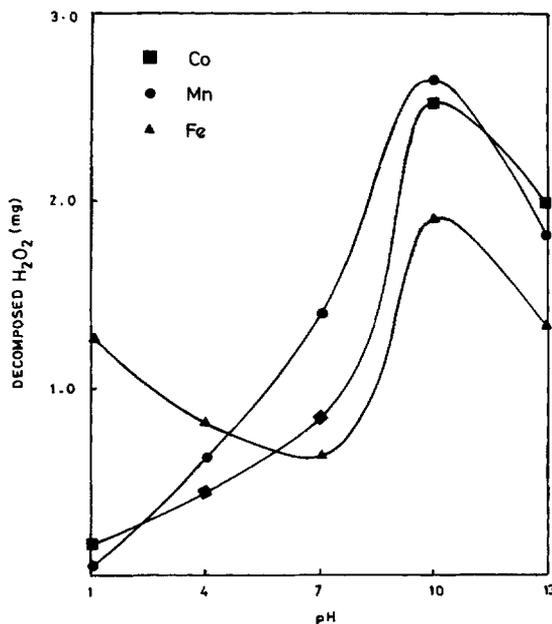


Figure 4. The effect of pH on the catalase activity of PS-MTPPS systems. (▲)-PS-FeTPPS, (●)-PS-MnTPPS, (■)-PS-CoTPPS, Temperature-30°C, time – 15 min)

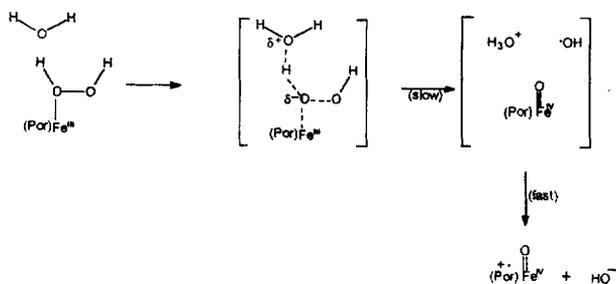
around pH 10.0, but decreases thereafter (figure 4). Thus, while modelling the enzyme it is worthy to note the high efficiency of Fe(III) at low pH and the enhanced efficiency of Mn(III) and Co(III) at high pH.

The catalytic efficiency of the supported metalloporphyrin systems in the present study is an indication of their resistance to form μ -oxo dimer species since such a dimerisation process is known to be detrimental to the enzyme-like action. It is known that the increase of the rate of H_2O_2 decomposition with an increase in pH is an indication of the increasing rates of O–O bond scission. From studies based on water soluble porphyrin monomeric systems whose structure does not permit μ -oxo dimer formation, the reactive species at low pH are known to be $(Por)M^{III}H_2O_2$ and H_2O_2 ; at intermediate pH $(Por)M^{III}(H_2O)(OH)$ and H_2O_2 and at high pH $(Por)M^{III}(H_2O)(OH)$ and HOO^- ¹⁰. The observed decrease in the rate of decomposition at very high pH can therefore be due to the less favorable complexation HOO^- to $(Por)M^{III}(H_2O)(OH)$ rather than the complexation of H_2O_2 to $(Por)M^{III}(H_2O)(OH)$, since in the former case, more negative charge is concentrated in the reaction site.

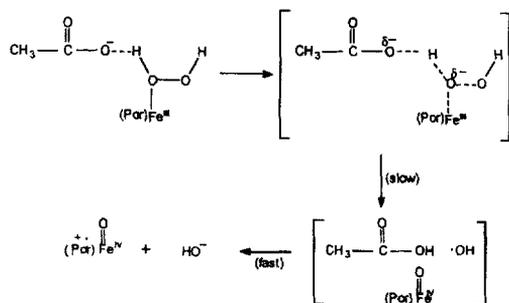
In the acidic pH the pronounced catalytic activity of FeTPPS can be explained invoking homolytic O–O bond scission. Scheme 3 shows the proposed mechanism at high acidic pH, indicating pre-association of solvent H_2O with $(Por)M^{III}(H_2O)(H_2O_2)$ and partial proton transfer to H-bonded H_2O_2 in the transition state¹⁰.

At pH around 4.0 (acetate buffer), CH_3COO^- species present could behave similar to H_2O in the above mechanism which could be similar to the one proposed by Panicucci¹⁰ for soluble anionic Fe(III) porphyrin (scheme 4).

However the energy for desolvation of the oxy-anion base and electrostatic repulsion in the transition state could disfavour the mechanism of scheme 4 in comparison to



Scheme 3.



Scheme 4.

scheme 3. The observed decrease in the catalytic activity of PS-FeTPPS as pH increased from 1.0 to 7.0 could, therefore, be attributed to this factor.

The effect of temperature on catalase activity of the PS-MTPPS system was also studied (between 10–60°C) maintaining the solution at pH 10.0. This pH was chosen because the maximum activity of the MTPPS was found to be in this basic condition. In all the cases, the activity was found to be enhanced as the temperature was increased. At room temperature and below ambient temperature, the order of efficiency was found to be $\text{Mn} > \text{Co} > \text{Fe}$. Between 35–45°C the order gets changed to $\text{Co} > \text{Mn} > \text{Fe}$ and above 45°C and up to 60°C the order again gets altered to $\text{Co} > \text{Fe} > \text{Mn}$. The temperature effect was more pronounced in the case of Fe(III) and less pronounced for Mn(III). The details are shown in figure 5.

The result is interesting especially in the case of the FeTPPS system. We believe that the temperature dependent spin cross-over could be a dominant factor for the anomalous behaviour. It is known that while Fe(III) complexes exhibit a strong affinity for such spin-state transition, Mn(III) and Co(III) show this only to a moderate extent¹⁶.

The stability of PS-MTPPS system towards the catalase activity was also studied to check the reusability of the supported systems. This was carried out at pH 10.0 for 10 cycles. The result is shown in figure 6.

While the Co-system showed practically no deterioration in activity, the Mn-systems were found to get poisoned marginally with 4 cycles. However, it showed constant activity thereafter. Fe-systems also were found to exhibit a higher degree of recyclability without much loss of activity.

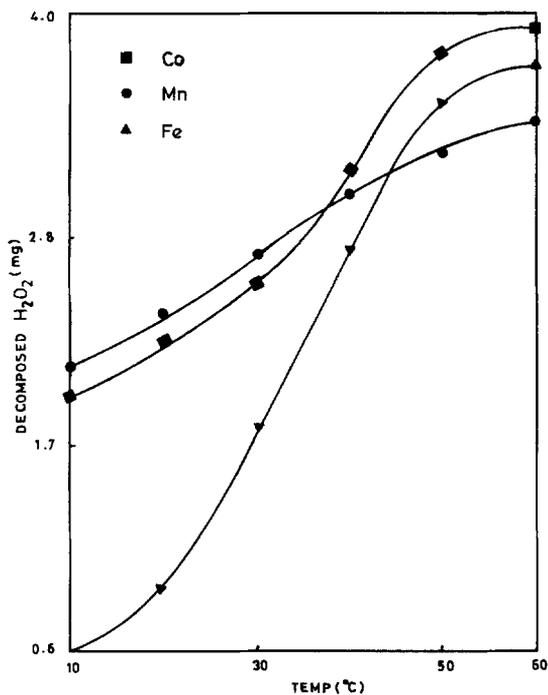


Figure 5. The effect of temperature on the catalase activity of PS-MTPPS systems. (▲-PS-FeTPPS, ●-PS-MnTPPS, ■-PS-CoTPPS, pH-10, time – 15 min)

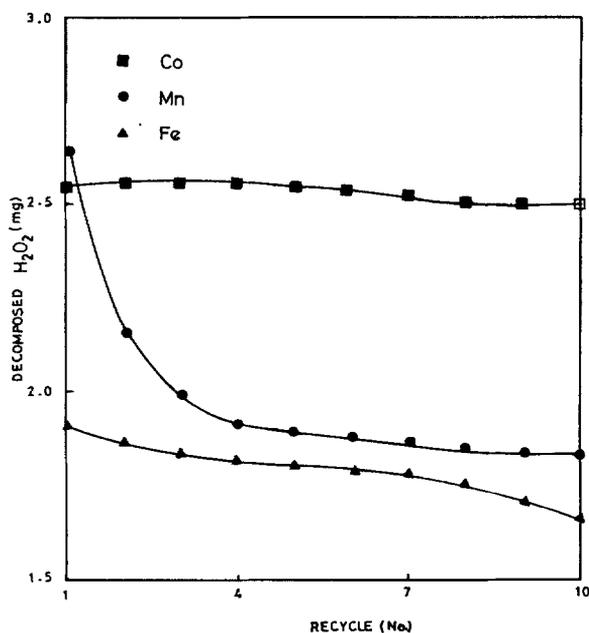


Figure 6. Recyclability of the catalysts. (▲-PS-FeTPPS, ●-PS-MnTPPS, ■-PS-CoTPPS, Temperature-30°C, pH-10, time – 15 min)

4. Conclusions

The polymer supported metalloporphyrin systems (PS-MTPPS) developed were found to be efficient catalysts for the dismutation of H_2O_2 and shown to mimic catalase-like activity quite effectively. The catalytic activity was dependent on pH and temperature of the reaction medium and the nature of the central metal atom of the porphyrin ring. All catalyst systems were found to be recyclable without much loss of activity.

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