Chapter

4

Catalase-like Activities of Selected PS-MTPPS Systems

4.1 Introduction

The enzyme-like catalytic activities of synthetic metalloporphyrins, as discussed earlier, is one of the most active research field in bioinorganic chemistry. It is the possibility of electronic and redox tuning of the porphyrin moiety by peripheral substitution and central metal variations which makes them versatile catalysts for such biomimetic studies[1-4]. As demonstrated extensively in Chapter 3, the polymer matrix could also contribute appreciable fine-tuning of the catalytic porphyrin site just as protein envelope adds efficiency to the enzyme core. It should also bear in mind that 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. Hence the catalytic activities of polymer supported metalloporphyrins especially in enzyme model studies are of great importance. However, only a limited number of articles are available concerning the enzyme like activities of polymer supported porphyrins, as discussed in Chapter 2.

In the present chapter and also in the following chapter we discuss the enzyme-like (catalase and peroxidase) activities of some selected polystyrene grafted MTPPS (M=Fe(III), Mn(III) and Co(III)) systems. First we discuss the catalase-like activity of PS-MTPPS catalysts and various factors contributing to the efficiency of these catalytic systems.

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 causing damage to hemoglobin through oxidation[5]. A few model systems have already been reported, among which Fe(III) macrocyclic systems are probed in some detail[6-10]. It is known that one major detrimental factor for Fe(III) porphyrin acting as an efficient catalase model is its tendency to from μ-oxo dimer species[11]. 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.

4.2 Experimental

4.2.1 Preparative details

The detailed procedure employed for the preparation and characterisation of metalloporphyrins and polymer support are already discussed in Chapter 3. The generation of various PS-MTPPS systems are also discussed in detail in this chapter. The uptake of MTPPS on polystyrene surface was adjusted to be $1x10^{-4}$ mole porphyrins/g of polymer

4.2.2 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, 20mg of catalyst (2.0×10^{-6} mol MTPPS) was added to 4ml buffer containing 7mg 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).

4.3 Results and Discussion

4.3.1 The catalase system-A brief outlook

Catalase (hydrogen-peroxide : hydrogen-peroxide oxidoreductase) is one of the first enzymes to be isolated, purified and crystallised[12]. Its function is the dismutation of hydrogen peroxide generated in various life processes as in:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Catalase occurs widely in animal tissues, microorganisms and fungi. The catalase activity of tissues varies greatly; it is highest in liver and kidney and low in connective tissue. In tissues it is mainly particle bound whereas it exists in a soluble state in erythrocytes[13]. The molecular mass of catalase is 230,000-240,000; the enzyme is made up of 4 identical subunit and each subunit contains one ferriprotoporphyrin group which corresponds to a protoheme content of 1.10% and an iron content of 0.09%[14]. Protoheme is the major structural component of the enzyme and indeed is the principal determinent of activity and specificity[15-18]. The protein structure around ferriheme groups in catalase system has also a vital role in the activity of the enzyme (discussed below). In catalase molecule from bovine liver (BLC), the heme group is linked to the protein via the O-atom of Tyr 357. The distal region contains His 34 and Asp147. The heme group is buried 2.0nm in the protein globule and communicates with the surface through the hydrophobic channel. The heme groups of the sub units are 3.12nm, 4.55nm and 3.46nm apart from one another.

The mechanisms of the catalase reaction have actively been investigated. The interaction of H_2O_2 with catalase (E) gives Compound I (E_1) which is an Fe-oxo species and has two oxidising equivalents. This oxidised species returns back to the enzyme form by interaction with a second molecule of H_2O_2[16].

$$E + H_2O_2 \longrightarrow E_1 + H_2O$$

$$E_1 + H_2O_2 \longrightarrow E + H_2O + O_2$$

'Compound I' which is an iron-oxo (Fe=O) species is a common intermediate for catalase and peroxidases (discussed in the next chapter). In 'Compound I' one oxidising equivalent is localised on the iron atom (ie. Fe^{IV}) and the other in the form of the cation radical of the porphyrin (ie. $por^{+\cdot}$)

The rapid occurrence of the compound I formation is favoured by the presence of a distal histidine and arginine residue in the protein envelope and the elementary act of formation of compound I is assisted by an imidazole group[15]. The compound I in catalase has stronger oxidizing properties than compound I in peroxidases. This causes the addition of another molecule of H_2O_2 to compound I in catalase rather than the oxidation of other donor substrates by compound I in peroxidases. The difference is due to the presence of a tyrosine residue in the coordination sphere of the heme-iron of catalase in distinction to the histidine residue in peroxidases.

4.3.2 Catalase-like activity of PS-MTPPS systems developed

The ease of formation of the hypervalent iron-oxo species (compound I) by one molecule of H_2O_2 and its consequent reduction back into its initial state causing the dismutation of another molecule of H_2O_2 necessitates a stable frame-work of ligand which can act as an electronic sink. The protoporphyrin frame-work is highly suitable in this context. Just like in its biological analogue, the FeTPPS also has highly redox tunable cyclic conjugated π- framework which is quite resistant to oxidation and reduction. It may be noted that the generation of stable H_2TPP^{1-} and H_2PP^{2-} or H_2TPP^{+} and H_2TPP^{2+} have been well established. Even though we have tried to monitor the catalase and/or peroxidase like activities with various PS-MTPPS, it was found that only Fe(III), Mn(III) and Co(III) species show appreciable catalytic activities. This indicates that besides the redox resistance of the porphyrin frame-work, the nature of central metal ion also is a critical factor. Since Fe(III), Mn(III) and Co(III) have another easily accessible redox state (metal centered) the activity exhibited by their metalloporphyrins could be easily understood in this context. As demonstrated extensively in Chapter 3, the

polymeric matrix PS also contributes appreciable fine-tuning of the catalytic MTPPS (M=Fe, Mn and Co) site just as protein envelope adds efficiency to the enzyme core.

The catalase activity can be measured by following either the decomposition of H_2O_2 or the liberation of O_2. The decomposition H_2O_2 can easily be monitored by titrimetric method and this technique is used throughout in the present study.

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 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) ≈ Co(III) > Fe(III). The details are shown in **Fig. 4.1**.

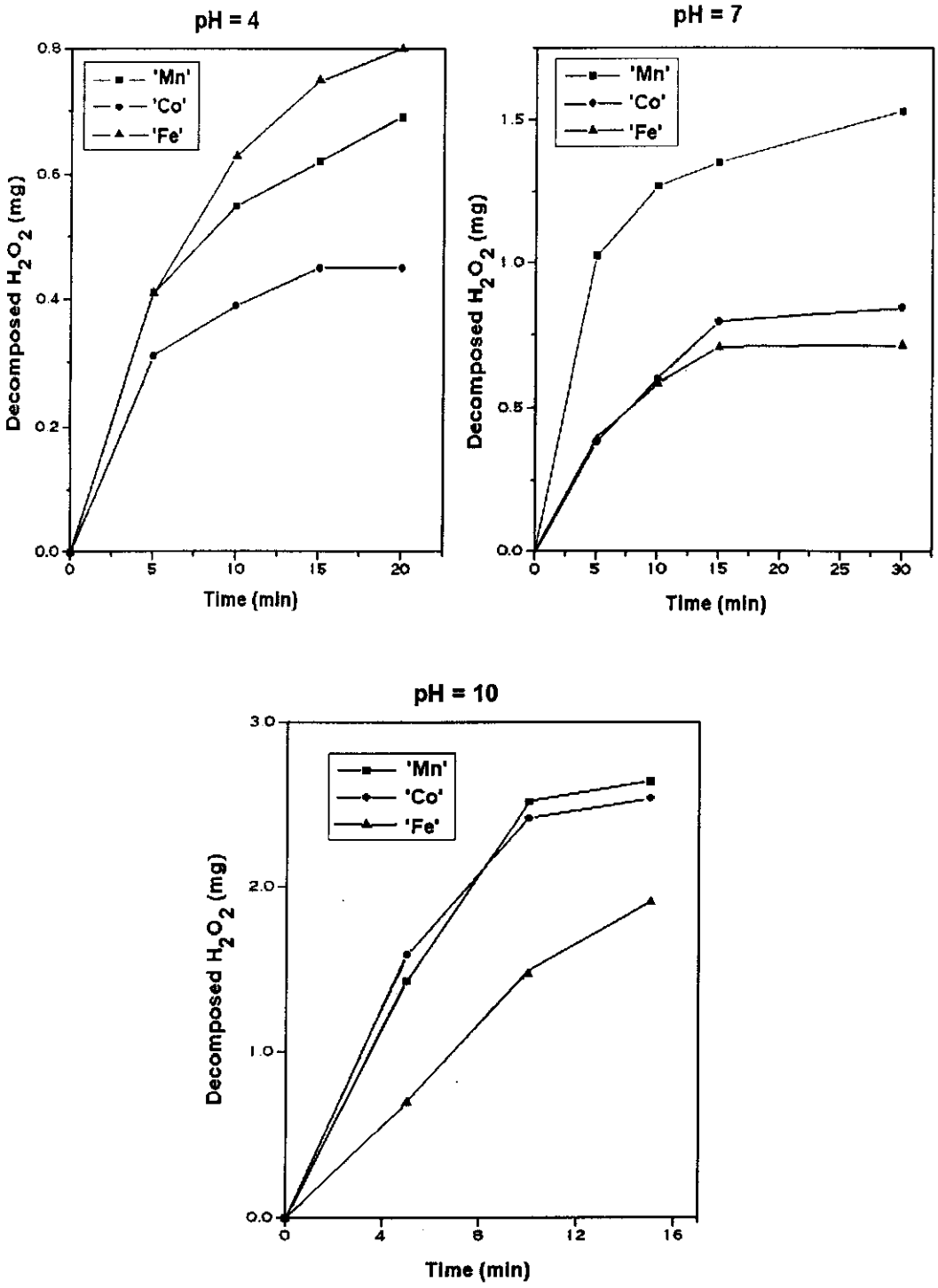


Fig. 4.1. Dependancy of Metal ion on the catalase-like activity of PS-MTPPS at different pH conditions (temperature – 30^0C).

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 H_2O_2 (**Fig. 4.2** and **4.3**).

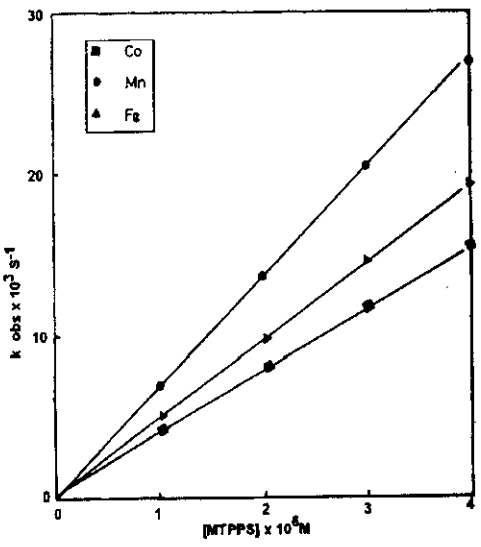


Fig. 4.2

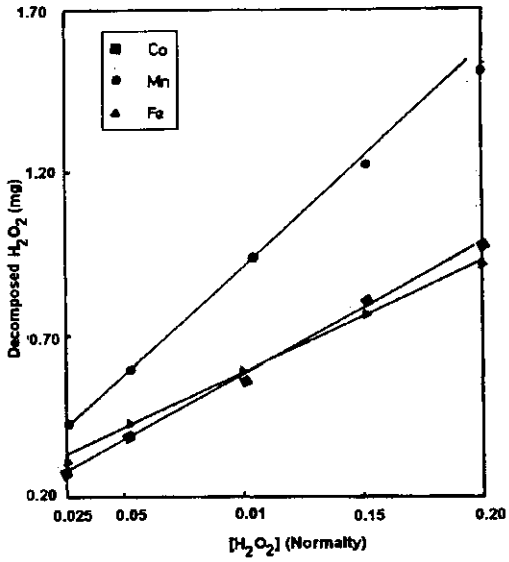
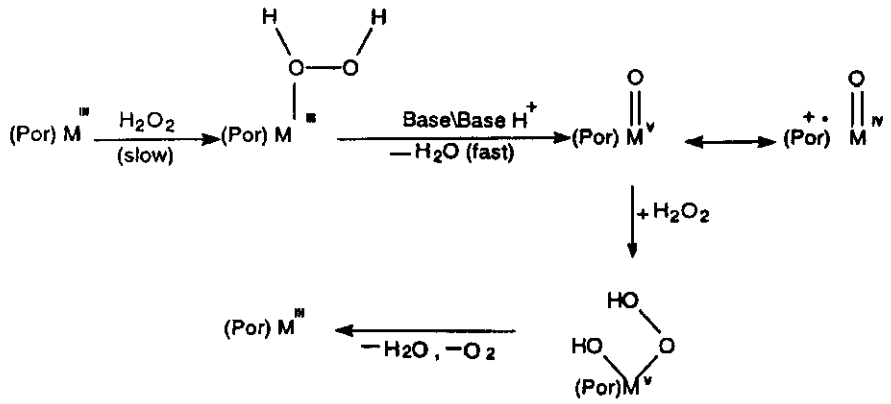


Fig. 4.3

Fig. 4.2. Observed first order rate constants for the decomposition of H_2O_2 as a function of the amount of catalyst (temperature-30^{0}C, pH-7, time-10min).

Fig. 4.3. Amount of decomposed H_2O_2 as a function of $[H_2O_2]$ (amount of PS-MTPPS-20mg, temperature-30^{0}C, pH-7, time-10min).

Similar observations were reported earlier for water-soluble monomeric porphyrins[10] and for some polymer supported macrocycles[19]. Based on the observed data, the mechanism for the decomposition of H_2O_2 by metalloporphyrins may be considered as follow (**Scheme 4**), in conformity with that indicated by Meunier[4].



Scheme 4

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 it 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 around pH 10.0, but decreasing thereafter (**Fig.4.4**). Thus, while modeling 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 .

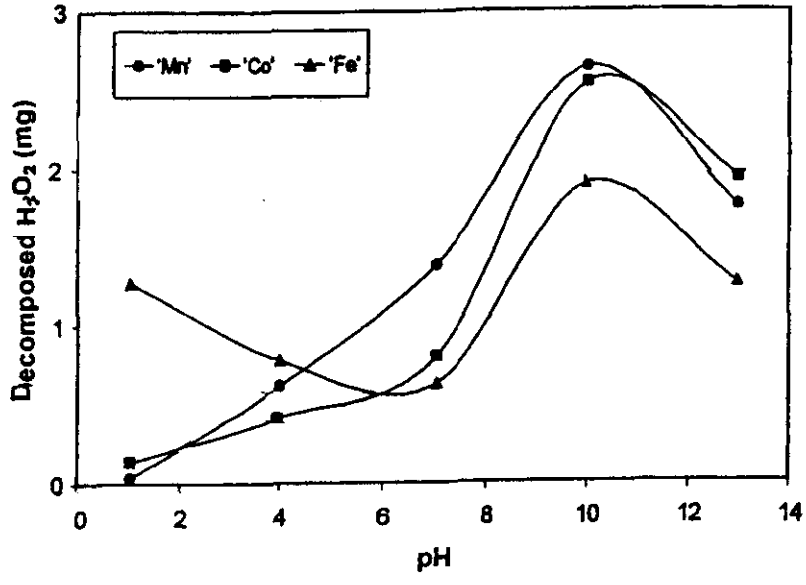
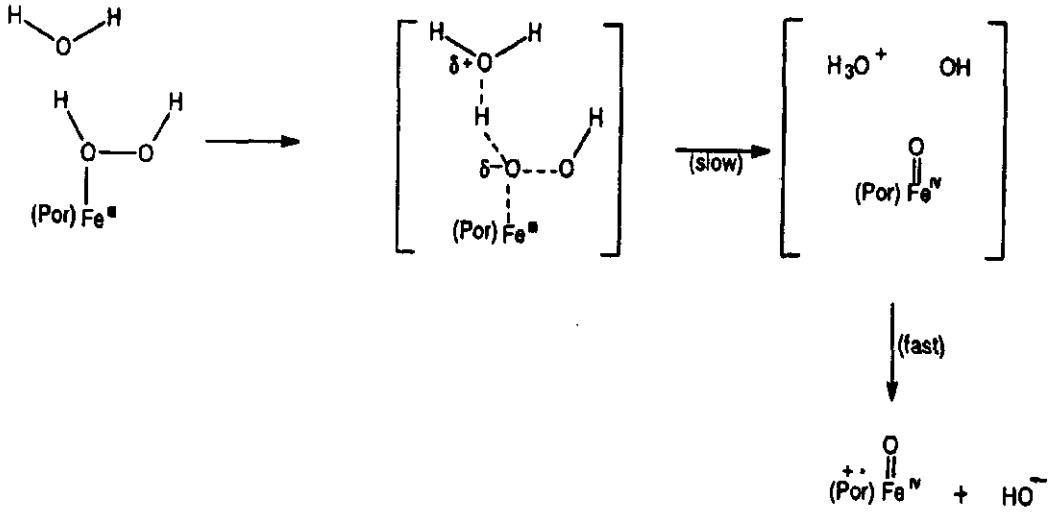


Fig. 4.4. The effect of pH on the catalase activity of PS-MTPPS systems (temperature-30^{0}C, time-15min).

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

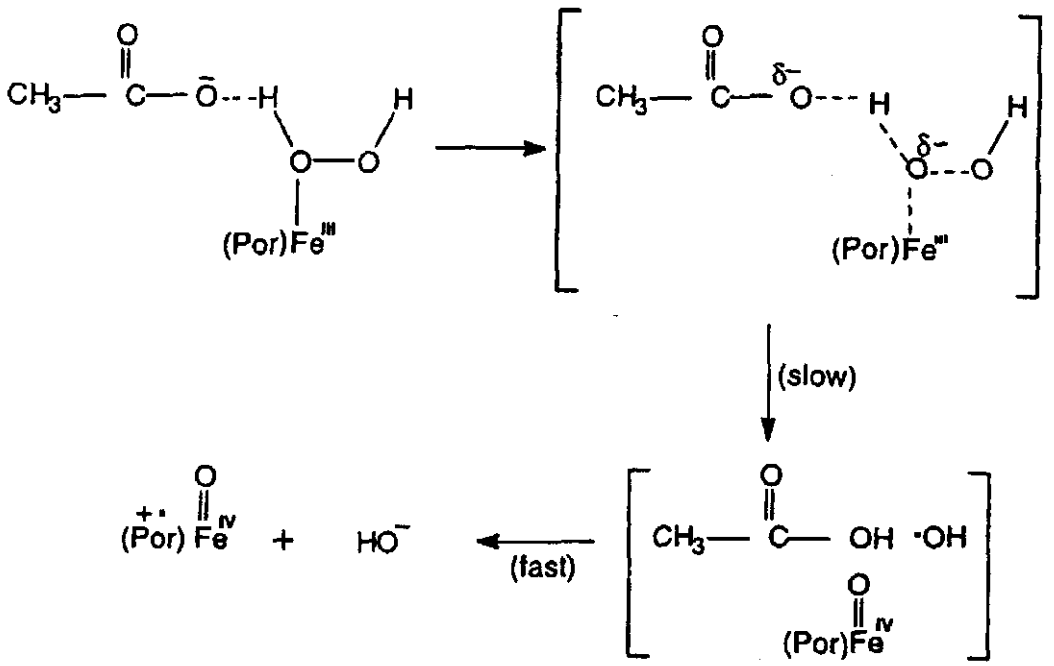
known that the increase of the rate of H_2O_2 decomposition with an increased in pH is an indication of the increased rates of O-O bond scission. From studies based on water-soluble monomeric porphyrin systems whose structure do not permit μ-oxo dimer formation, the reactive species at low pH are known 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 it is $(Por)M^{III}(H_2O)(OH)$ and HOO^- [10]. The observed decrease in the rate of decomposition at very high pH can therefore be due to the less favorable complexation of 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 acetic pH the pronounced catalytic activity observed for FeTPPS can be explained invoking homolytic O-O bond scission. **Scheme 5** 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 state[10].



Scheme 5

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 proposed by Panicucci for soluble anionic Fe(III) porphyrin (**Scheme 6**).



Scheme 6

However the energy for desolvation of the oxy-anion base and electrostatic repulsion in the transition state could disfavour the mechanism of **Scheme 6** in comparison to **Scheme 5**.

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^{0}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 Mn>Co>Fe. Between 35-45^{0}C the order again gets changed to Co>Mn>Fe and above 45^{0}C and up to 60^{0}C the order again gets altered to Co>Fe>Mn. The temperature effect was more pronounced in the case of Fe(III) and less pronounced for Mn(III). The details are shown in **Fig 4.5**.

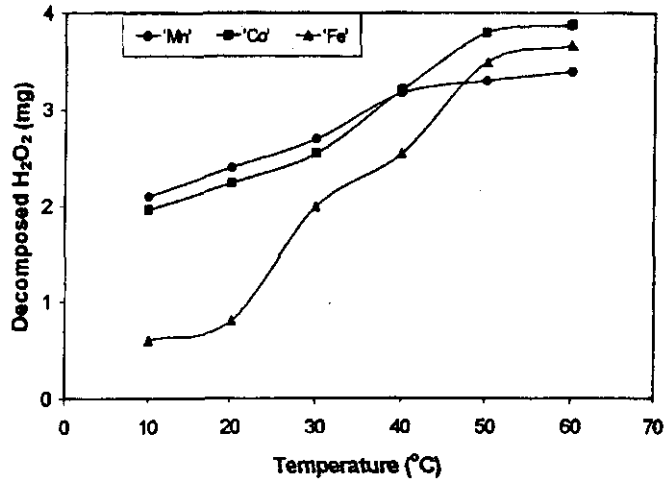


Fig. 4.5. The effect of temperature on the catalase activity of PS-MTPPS systems, (pH-10, time-15 min).

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 extent[20], explaining the anomalous behaviour of PS-Fe(III)TPPS system in the model study.

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 **Fig 4.6**.

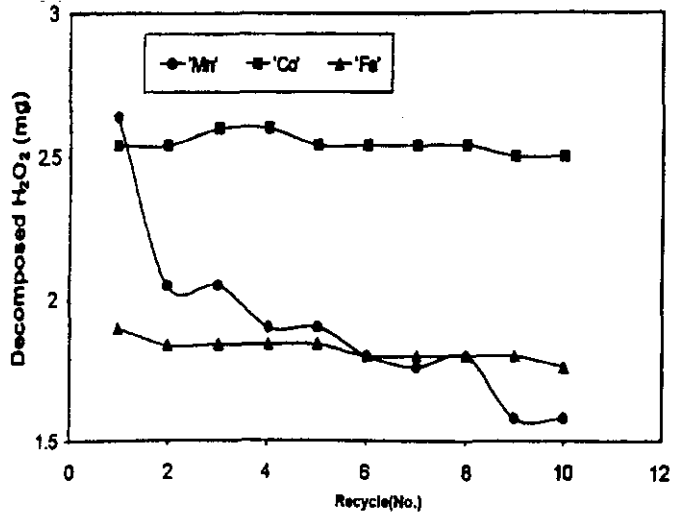


Fig. 4.6. Recyclability of the catalysts, (temperature-30^{0}C, pH-10, time-15 min).

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

4.4 References

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