Chapter

5

Peroxidase-like and Photocatalytic Activities of Selected PS-MTPPS Systems

5.1 Introduction

As demonstrated in the previous chapter, the PS-MTPPS (M=Fe(III), Mn(III) and Co(III))systems developed are efficient catalysts to mimic catalase-like activity in the dismutation of H_2O_2. Similar to catalase, peroxidases are also a class of enzymes containing the heme moiety. Hence studies on the peroxidase-like activities of various PS-MTPPS systems are important in the enzyme model study. In addition to these enzyme-like modeling, the investigation on the photocatalytic activities of these immobilised porphyrins are also highly significant, especially in biomimetic studies.

Peroxidases which encompass the group of specific enzymes are known for their vital role in biological systems by way of catalytically oxidising a wide variety of electron donor substrate species bearing phenolic amine or acid functions aided by H_2O_2 or organic peroxides[1-4]. The core of these metalloenzyme, as discussed above, is the highly tunable heme moiety which are buried in a protein envelope. A few systems involving MPs have been reported with a view to model the enzymatic reaction of peroxidases[5-8]. However, the effect of polymeric environment on the peroxidase-like activity of metalloporphyrins is not seen investigated with much attention. In the first part of the present chapter we report the peroxidase-like activity of some selected PS-MTPPS systems. The change in

any catalytic property and some of the additional advantages of these supported metalloporphyrins over the unsupported systems are also discussed.

Porphyrins, having strong absorption characteristics in the visible region, are also known to exhibit interesting photophysical, photochemical and photoredox properties. The potential use of MPs have been actively investigated as photosensitisers in some of the photobiological applications such as photodynamic therapy and inactivation of microorganisms[9-11]. The ability for the photogenaration of singlet oxygen ($^1O_2$) is the most important catalytic application of such photosensitsers. Recently water soluble H_2TPPS and some of its metal complexes (eg ZnTPPS) are reported to photogenarate singlet oxygen[12]. Since, the polymer grafting is found to impart an appreciable modulation within the porphyrin frame-work, (and hence influence their electronic properties) we expect some kind of property variation in photosensitisation of immobilised porphyrin moieties. So in the second part of the present chapter we also discuss our preliminary effort to investigate the efficiency in singlet oxygen generation of PS-H_2TPPS, PS-ZnTPPS and PS-CdTPPS systems. The photosensitising ability of polystyrene grafted Rose-bengal is also included in this study for a comparison.

5.2 Experimental

5.2.1 Preparative details

Synthesis and characterisation of porphyrins (H_2TPPS/MTPPS) and functionalised polystyrene support (PS) are already discussed in detail in Chapter 3. Grafting of MTPPS on polystyrene (PS-MTPPS) is also discussed in the same chapter.

5.2.2 Preparation of polymer beads bonded to Rose-bengal

An aqueous solution of Rose bengal was prepared by dissolving 97.4mg ($1x10^{-4}$mole) in minimum amount of water. About 1g N-alkyl pyridinium functionalised chloromethylated polystyrene beads (PS) was added to this solution and heated on a water bath for 24h. During this time almost the entire Rose-bengal was found to

be immobilised on polymer surface as evidenced by the colour change observed for the solution. The modified beads were collected by filtration and were washed thoroughly with water and methanol.

5.2.3 Monitoring of peroxidase-like activity

The peroxidase-like activity of the immobilised MTPPS (PS-MnTPPS, PS-FeTPPS and PS-CoTPPS) was monitored through dye formation reaction between 4-amino antipyrine and phenol in presence of H_2O_2 [6]. In a typical reaction 12.5mg of the catalyst (containing $1.25x10^{-6}$ mole MTPPS) was added to 5ml of 1:1:3 mixture of phenol ($0.75x10^{-4}$ mole/ml, 4-amino antipyrine ($0.25x10^{-5}$ mole/ml) and the buffer solution. Hydrogen peroxide (1ml, $0.176x10^{-2}$N) was added to this and stirred slowly for about 30 min. The absorbance of the solution was then measured at 505nm (λ_{max} of quinoid dye) against the reagent blank which was negligibly low. The relative activities of the PS-MTPPS resins were then evaluated by comparing the absorbance of the quinoid dye formed with the value obtained in the case HRP under same conditions reported previously[6].

5.2.4 Detection of singlet oxygen

The detection of singlet oxygen was performed by RNO bleaching assay [13]. The sensitisers (30mg) (porphyrins or Rose-bengal) were exposed to light in the presence of imidazole (0.01M) and RNO (0.05M) in a 0.05 M phosphate buffer (pH=7.4).

5.3 Results and Discussion

5.3.1 A brief out look on peroxidases

Peroxidases catalyze the oxidation of electron donor molecular species (DH_2) by H_2O_2 in various biological systems as in:

$$DH_2 + H_2O_2 \xrightarrow{\text{Peroxidase}} 2H_2O + D$$

Various electron donors may serve as substrates for peroxidases: phenols, amines, alcohols, acids, inorganic ions, etc. Just like to catalase enzyme, peroxidases also contain the prosthetic group ferriprotoporphyrin IX. Catalase and peroxidases have strong similarities, not only in function but also in structure and mechanism of action[4].

The most studied peroxidase is horseradish peroxidase(HRP), which is present in high concentration in root of the horseradish plant. Others of interest are cytochrome c-peroxidase, chloroperoxidase, myeloperoxidase, lactoperoxidase, thyroid peroxidase and glutathione peroxidase[1].

HRP contains a polypeptide of molecular weight 33890, some carbohydrate and the protoporphyrin IX. Two calcium ions are also present, probably for the stabilization of protein conformation. The 5th coordination position of Fe^{III} in HRP is filled by an imidazole group, while the 6th may be vacant[1,4].

Upon reaction with H_2O_2 or organic peroxides, the Fe(III) prosthetic group of peroxidases undergo a two-electron oxidation to generate a green compound, 'Compound-I' which then may undergo a one-electron reduction to give a red species 'Compound II', along with the oxidation of a donor species (DH_2) (**Scheme 7**). 'Compound II' oxidizes the second donor molecule or causes the first molecule to be fully oxidized and thereby getting reduced to the native enzyme[1-4].

$$\text{Enzyme} + H_2O_2 \longrightarrow \text{CompoundI} + H_2O$$

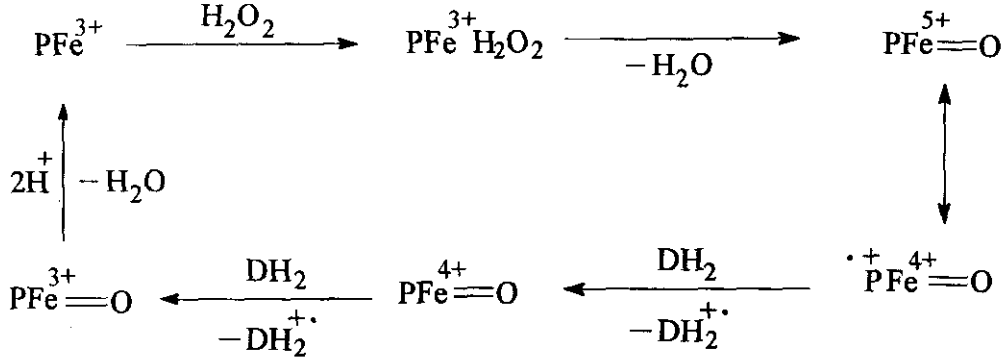
$$\text{CompoundI} + DH_2 \longrightarrow \text{CompoundII} + DH_2^{+\bullet}$$

$$\text{CompoundII} + DH_2 \longrightarrow \text{Enzyme} + DH_2^{+\bullet}$$

Scheme 7

The radical products generated in these processes will react further (for example with O_2) to give superoxide ion[3]. The compound I is now proved to be a hypervalent Fe^{IV}=0 with porphyrin π- cation radical and compound II is an Fe^{IV}=0 species[1].

The possible mechanisms of the chemical reaction steps of peroxidase reactions could be summarised as below (**Scheme 8**)[4] where PFe^{3+} is the ferriheme moiety and DH_2 is the donor species.

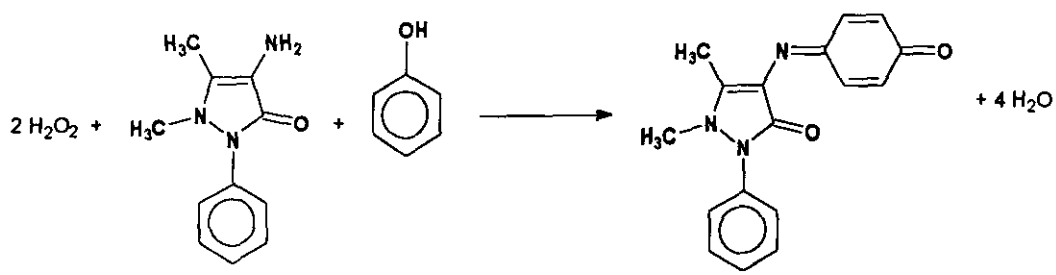


Scheme 8

It is generally believed that the presence of the highly active 5th ligand (imidazole group) and also the possibility of the formation of H-bonds in the region of distal histidine groups moiety would tune the ferriheme prosthetic group in its catalytic cycle.

5.3.2 Peroxidase- like activity of selected PS-MTPPS systems

The activities of peroxidases (eg. HRP) are sometimes monitored by quinoid dye formation through cooxidation reaction as given in **Scheme 9** [6]. We also employed this reaction for comparing the efficiencies of PS-MnTPPS, PS-FeTPPS and PS-CoTPPS in the peroxidase-like model study.



Scheme 9

The possible steps involved in the above reaction could be summarised below [14], where $AmNH_2$ is 4-aminoantipyrine, PhOH is phenol and P_1 and P_2 are some side products.

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

$$E_1 + PhOH \longrightarrow E_2 + PhO^{\bullet}$$

$$E_1 + PhOH \longrightarrow E + PhO^{\bullet}$$

$$E_1 + AmNH_2 \longrightarrow E_2 + Am\dot{N}H$$

$$E_2 + AmNH_2 \longrightarrow E + Am\dot{N}H$$

$$AmNH_2 + PhO^{\bullet} \longrightarrow Am\dot{N}H + PhOH$$

$$Am\dot{N}H + PhO^{\bullet} \longrightarrow AmNH{-}OPh$$

$$AmNH{-}OPh + H_2O_2 \longrightarrow Am{-}N{=}\langle\bigcirc\rangle{=}O\ (P)$$

$$PhO^{\bullet} + PhO^{\bullet} \longrightarrow P_1$$

$$Am\dot{N}H + Am\dot{N}H \longrightarrow P_2$$

As discussed above (**Scheme 8**) the first step involving the uptake of H_2O_2 by porphyrin to form a hypervalent metal-oxo species has been demonstrated to be common for both catalase and peroxidase. While this reactive intermediate acts on and dismutate directly a second molecule of H_2O_2 in catalase, in peroxidase it acts on the donor DH_2, getting back itself to the initial state. This results in two different competitive reactions. So in the present study we tried to mitigate the complication by using optimum amount of H_2O_2 which is preferably added only as a last reagent in the reaction mixture.

Before investigating the activities of the solid PS-MTPPS, the peroxidase-like activity of the unsupported and water-soluble MTPPS (M = Fe^{III}, Mn^{III} and Co^{III}) were monitored for evaluating the relative efficiencies of the homogeneous and heterogeneous species. Expectedly the relative efficiency of the peroxidase-like reaction with the soluble catalysts gave difficulties in evaluating the product formation because of the absorption overlap of the porphyrin moieties with that of the quiniod dye formed. However, we tried to circumvent this as much as possible by using a blank solution (reference) containing the same amount of MTPPS, $AmNH_2$ and PhOH without H_2O_2. The soluble MTPPS seem to exhibit a moderate peroxidase-like activity as shown in **Table 5.1**.

Table 5.1 The relative efficiencies of various metalloporphyrins in peroxidase-like reactions (pH 8.2).

Catalyst	Absorption at 505(nm)	Amount of dye formed ($x10^{-6}$)mol.	Relative activity
HRP *	0.799	0.61	100
MnTPPS	0.350	0.26	44
CoTPPS	0.350	0.20	44
FeTPPS	0.250	0.19	31
PS-MnTPPS	0.560	0.43	70
PS-CoTPPS	0.785	0.59	95
PS-FeTPPS	0.445	0.34	56

In the case of solid PS-MTPPS catalysts the reaction monitoring was easy and error-free as the polymer beads (bearing MTPPS) could be simply removed by filtration from the reaction mixture. As shown in **Table 5.1**, we find these polymer supported systems to be very efficient, even as comparable to original HRP. The extra efficiency of the supported MTPPS could be attributed partially to the structural and electronic modifications brought about in the porphyrin system by the polymer network (see Chapter 3 for details). In this context, it may

also be noted that since the catalysts could easily be removed from the reaction mixture, the cooxidised product (antipyrilquinoneimine) could be isolated very easily in pure form. The μ-oxo dimerisation of MTPPS which is one of the detrimental factors for the enzyme activity (as discussed in the previous chapter) could also be prevented due to polymer immobilisation.

It is seen in our experiment that all the PS-MTPPS systems exhibit appreciable peroxidase-like activity but with varying efficiency. At the optimum pH chosen (8.2), the PS-CoTPPS system was the most efficient while PS-FeTPPS was the least. PS-MnTPPS showed intermediate activity. It is interesting to the note that the Co species instead of the Fe (which is present in natural systems) exhibited the highest peroxidase-like activity in the synthetic porphyrin frame-work.

For all PS-MTPPS systems, the dye formation reaction was almost completed within 60 min as indicated in **Fig. 5.1**. Beyond this interval, some decrease in absorbance value (dye concentration) was observed for both PS-CoTPPS and PS-MnTPPS, which may be attributed to the absorption of some of the dye formed on to the resin. This was physically verified.

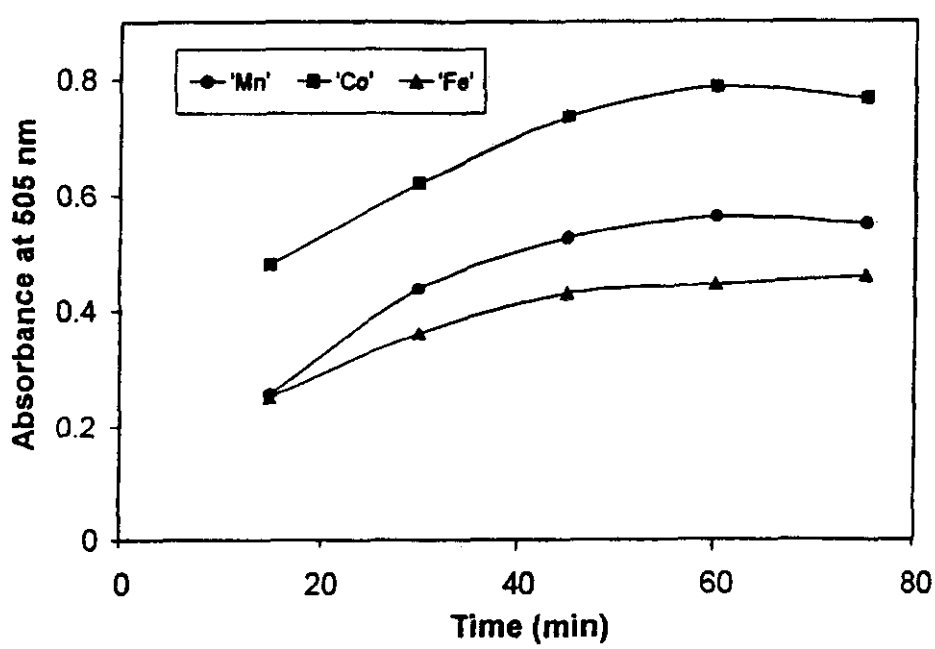


Fig. 5.1. Amount of antipyrilquinoneimine formed against the incubation time (pH- 8.2, temp 30^0C).

The pH dependency on the product formation was monitored in the range 1.0-13.0 which showed remarkable variation on the enzyme-like reaction. Buffers employed in the present work to bring about the pH range are varying concentrations of dil. H_2SO_4, acetate buffer, phosphate buffer, carbonate buffer and dil. NaOH respectively. We find the maximum efficiency in the pH range 8.0 to 8.6 (peaking at 8.2). Above and below this narrow pH range, the enzyme activity was seen negligible. The details are shown in **Fig. 5.2**.

At pH 8.2 the peroxidase activity shown by PS-CoTPPS reached about 95% of the activity exhibited by HRP under the similar experimental conditions. In an earlier report of MTPPS immobilised on Amberlite resin the maximum efficiency was seen at pH 7.9 and MnTPPS showed the maximum activity (i.e. efficiency) as in the order MnTPPS>FeTPPS>CoTPPS[6]. The observed difference in trend indicates the significance of the polymer matrix on enzyme tuning. The general nature of polymeric influence has been partially discussed in Chapter 3.

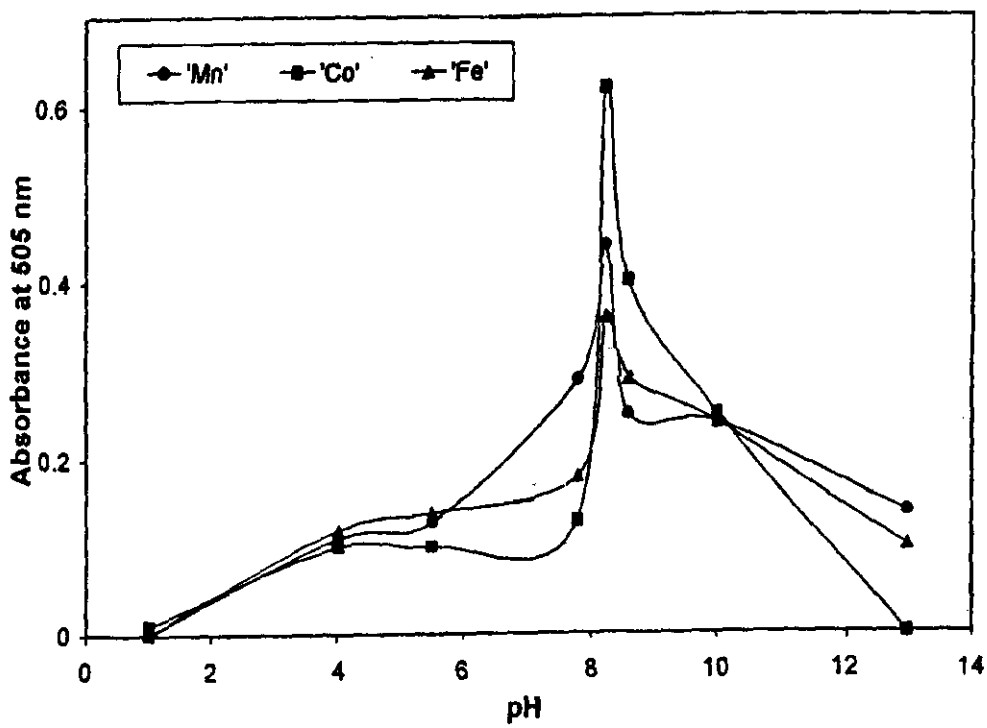


Fig. 5.2. Amount of antipyrilquinoneimine formed as function of pH (time 30 min, temp.30^{0}C).

As reported previously, the same PS-MTPPS system exhibited strongest catalase-like activity around pH-10.0 in the dismutation of H_2O_2. Hence the observed difference in the optimal pH for catalase and peroxidase-like activities (10.0 and 8.2 respectively) suggests that the PS-MTPPS are very sensitive to pH variations and that they can be used selectively for different catalytic/enzyme actions by choosing the appropriate pH.

The effect of temperature on the peroxidase activity of PS-MTPPS system was also studied (between 20-50^{0}C) maintaining the solution at pH 8.2 and keeping other reaction conditions same. In all the cases, the catalytic activity was found to be enhanced as the temperature was increased (**Fig. 5.3**). But the three metalloderivatives exhibited almost same degree of deviation with temperature variations. This is unlike to their catalase like activities where FeTPPS showed maximum sensitivity to temperature variation and MnTPPS the least.

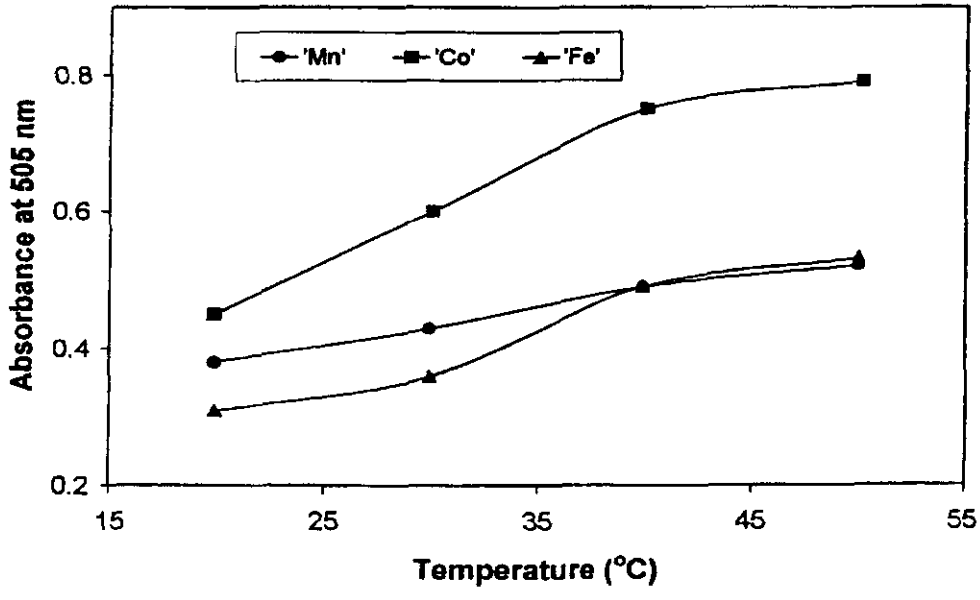


Fig. 5.3 Temperature dependency on the peroxidase-like activities of PS-MTPPS systems (pH 8.2, time 30 min).

The reusability of the PS-MTPPS systems was also studied at pH 8.2 for 10 cycles. The PS-CoTPPS system showed some deterioration in activity after the first cycle,

but without any further decrease. We attribute this to the adsorption of some amount of the product formed on to the resin. Mn and Fe systems, however, exhibited higher degree of recyclability without much poisoning. However, the overall efficiency of the Co(III) system was found to be superior to that of Mn(III) and Fe(III) throughout the reaction cycle. The result is shown in **Fig 5.4**.

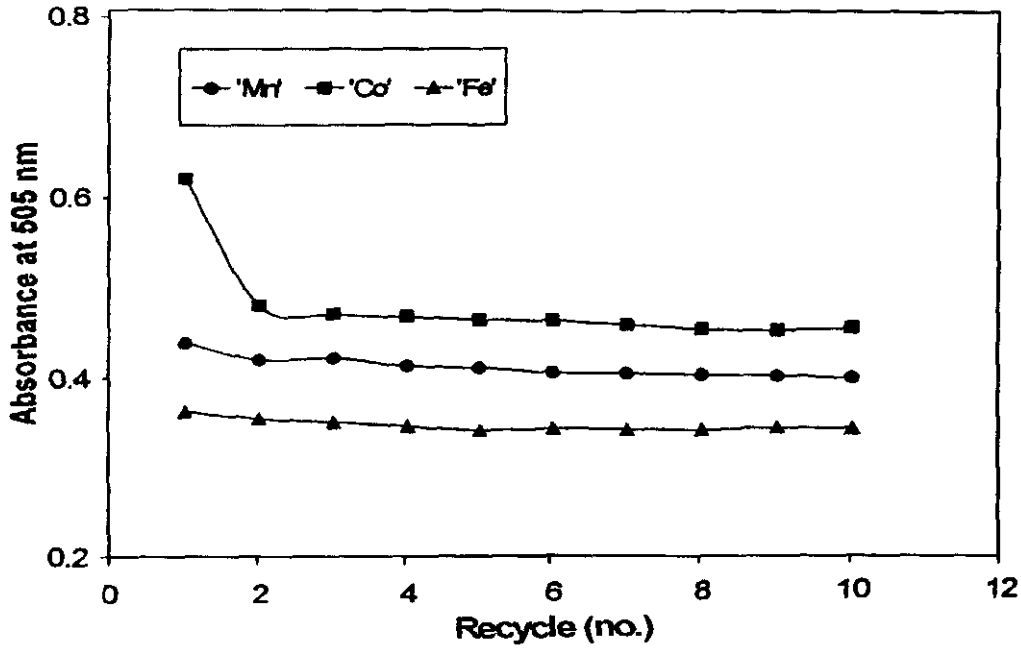


Fig 5.4. Recyclability of PS-MTPPS system (temp.30^{0}C, time 30min, pH.8.2).

This result confirms that the PS-MTPPS systems behave as efficient catalysts and the MPs are resistant to any oxidation by H_2O_2 when immobilised on polymer support.

5.3.3 Photogeneration of $^1O_2$

Polymer bound porphyrins are found to sensitise the photochemical production of singlet oxygen as observed by the bleaching of RNO. Photosensitised bleaching of RNO by PS-H_2TPPS followed spectrophotometrically at 440nm is given in **Fig.5.5**. The rate of bleaching of RNO by PS-H_2TPPS, PS-CdTPPS and PS-ZnTPPS as a function of irradiation time is shown in **Fig. 5.6**. Rate of RNO bleaching by PS-RB is also included in this figure. By taking the ratios of the

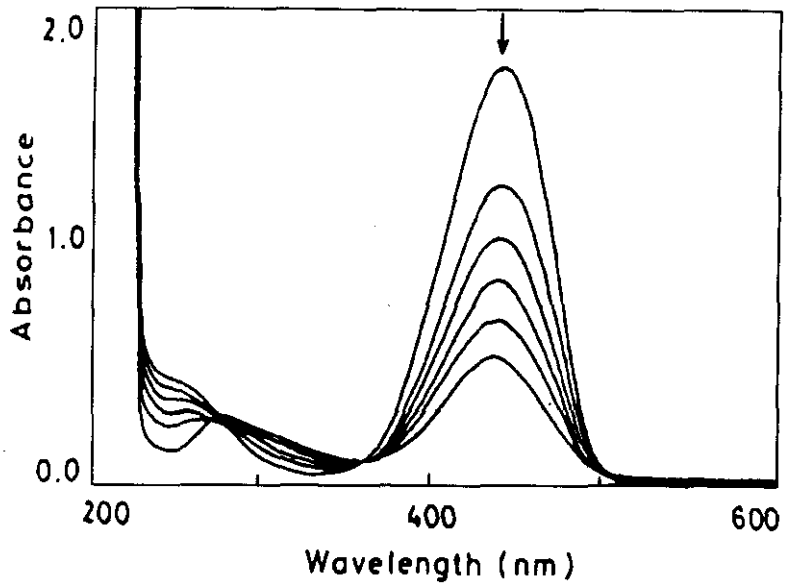


Fig. 5.5 Absorption spectra of RNO in the presence of imidazole (10mM) in 50 mM phosphate buffer (pH = 7.4) during photosensitised reaction of PS-H_2TPPS (30 mg) at each 3 min interval of irradiation time.

slope from **Fig. 5.6** and a known value for the quantum yield of singlet oxygen generation by illuminated Rose-bengal (0.76), the quantum efficiencies of $^1O_2$ by different polymer bound sensitisers were calculated. The relative efficiencies of generation of $^1O_2$ by different polymer bound sensitisers were calculated to be 0.29, 0.27 and 0.16 for PS-H_2TPPS, PS-ZnTPPS and PS-CdTPPS respectively.

In homogeneous phase, these unbound metal complexes are reported to generate $^1O_2$ upon photoillumination with the quantum yields of 0.62 and 0.81 for H_2TPPS and ZnTPPS respectively [12]. This shows that the binding of porphyrin to polymer has decreased its efficiency of $^1O_2$ generation. This is in accordance with the earlier observation for metallotetracarbethoxy-phthalocyanines bound to the amino groups of Amberlite IRA-93 [15]. The observed lowering has been attributed to aggregation and self-quenching. For more highly functionalised polymers, self-quenching becomes significant between sensitiser molecules on the polymer

chain. This self-quenching effect is very significant when the polymer is used as a heterogeneous sensitiser[13].

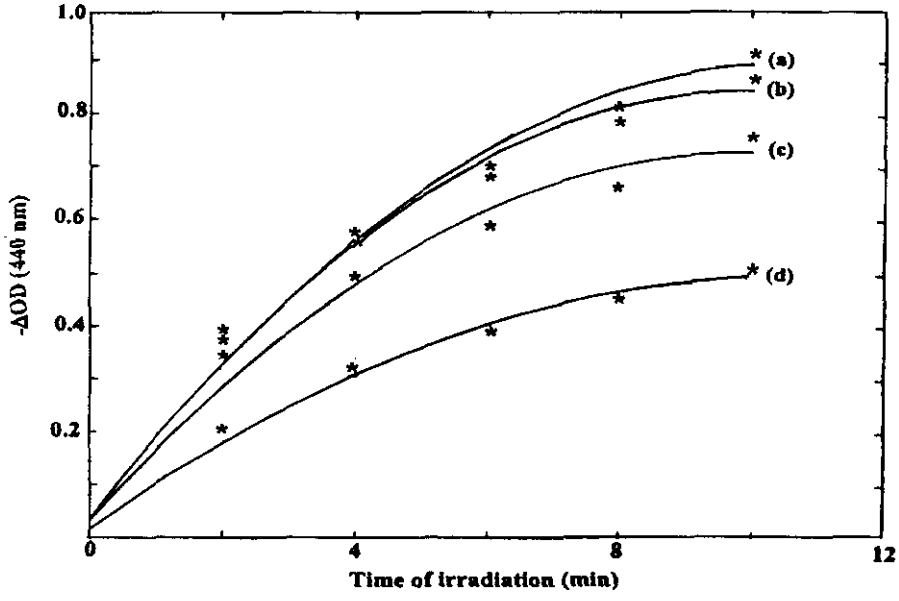


Fig. 5.6. Photosensitised RNO bleaching measured at 440nm in the presence of imidazole (10mM) in 50mM phosphate buffer (pH = 7.4) with (a) PS-H_2TPPS (b) PS-RB (c) PS-CdTPPS and (d) PS-ZnTPPS as a function of illumination time in min.

However, in our PS-H_2TPPS/PS-MTPPS systems, the self-quenching due to aggregation is less probable because the porphyrin molecules are well separated on polymer surface. Hence, in the present system, the low efficiency of $^1O_2$ generation could mainly be attributed to the structural modification of porphyrin moieties as discussed in Chapter 3, brought about by the polymer support. The structural deformation of porphyrin systems could decrease their aromatic character considerably, which in turn might inhibit their photocatalytic activities. The relative positions of the electronic energy levels of some MPs (eg. Cd and Zn) suitable for a stable photoexcited state is also highly perturbed due to this structural modification of porphyrin frame-work. As demonstrated in Chapter 3, Zn and Cd derivatives of porphyrins exhibit more electronic modulation than metal free porphyrin systems. The comparatively pronounced lowering of the activity observed for PS-ZnTPPS and PS-CdTPPS systems than that seen for PS-H_2TPPS could be justified by this factor. Another reason causing a decrease in

$^1O_2$ generation is the relative disposition of porphyrin moieties towards the incoming light source. In homogeneous conditions the photosensitisers are well distributed within the reaction medium and all the molecules would get photoexcited by the incoming light from the specified direction at a given time. But when the same number of such sensitiser molecules are immobilised on a polymer bead surface having a spherical shape, only half of them will be photoactivated at a given time because only half of the polymer surface would be exposed to the direction of the incoming light. Therefore, the amount of light quanta absorbed by the sensitiser molecule at a given time would be low (nearly half) in such hetrogeneous conditions when comparing the homogeneously dispersed conditions. The combined effect of these two factors might cause the lowering of $^1O_2$ generation (calculated value) in the case of PS-MTPPS/ PS-H_2TPPS.

Inactivation of a variety of bacteria and viruses using $^1O_2$ could be achieved by employing the porphyrin sensitisers in aqueous conditions. However, development of water or waste-water disinfection procedure employing dissolved or homogenised porphyrins has the drawback of undesirable contamination by the sensitiser molecule, the removal of which from the treated water is difficult. At the same time, inspite of their low activity, the solid polymer grafted porphyrins are very useful in such applications due to their heterogeneous characteristics. The antibacterial and antiviral properties of these PS-H_2TPPS/PS-MTPPS systems have been investigated as a test trial by carrying out the photokilling of *E.Coli* in the presence of light and oxygen. The results are highly encouraging as these polymer grafted systems are found very efficient in inactivation of *E. Coli* cells. Detailed investigation of photokilling of *E.Coli* and other micro organisms are planned as the future studies in these systems.

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