

Biomimetic Oxidation of Ibuprofen with Hydrogen Peroxide Catalysed by Horseradish Peroxidase (HRP) and 5,10,15,20-Tetrakis-(2',6'-dichloro-3'-sulphonatophenyl)porphyrinatoiron(III) and Manganese(III) Hydrates in AOT Reverse Micelles

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Abstract—The oxidation of ibuprofen with H_2O_2 catalysed by Horseradish peroxidase (HRP), $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH)}_2$ and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ in AOT reverse micelles gives 2-(4'-isobutyl-phenyl)ethanol (**5**) and p-isobutyl acetophenone (**6**) in moderate yields. The reaction of ibuprofen (**2**) with H_2O_2 catalysed by HRP form carbon radicals by the oxidative decarboxylation, which on reaction with molecular oxygen to form hydroperoxy intermediate, responsible for the formation of the products **5** and **6**. The yields of different oxidation products depend on the pH, the water to surfactant ratio (Wo), concentration of $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH)}_2$ and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ and amount of molecular oxygen present in AOT reverse micelles. The formation of 2-(4'-isobutyl phenyl)ethanol (**5**) may be explained by the hydrogen abstraction from ibuprofen by high valent *oxo*-manganese(IV) radical cation, followed by decarboxylation and subsequent recombination of either free hydroxy radical or hydroxy iron(III)/manganese(III) porphyrins. The over-oxidation of **5** with high valent *oxo*-manganese, Mn(IV) radical cation intermediate form **6** in AOT reverse micelles by abstraction and recombination mechanism. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Ibuprofen and related aryl propionic acids are important non-steroidal anti-inflammatory drugs.¹ They inhibit selected cyclooxygenase reactions in the biosynthesis of prostaglandins from archidonate.^{2–5} Cyclooxygenase catalyses the conversion of archidonic acid to PGG_2 and reduction of PGG_2 to PGH_2 . Both reactions are catalysed by formation of transient higher oxidation states of the heme enzymes. Ibuprofen and related aryl propionic acids inhibit cyclooxygenase reaction but not the peroxidase activity.^{6,7} The reaction of Horseradish peroxidase (HRP) with hydrogen peroxide forms a high valent *oxo*-iron(IV) radical cation which is reduced to high valent *oxo*-iron(IV) intermediate by accepting electrons from substrate leading to formation of substrate radicals. The coupling, disproportionation and other reactions of substrate radicals are responsible for the formation of final products.^{8,9} Reverse micelles are simplest models of natural membrane and they form variable reaction media depending on the ratio of water to surfactant and governs the efficiency of chemical and

enzymatic reactions.^{10,11} Reverse micelles act as functional modulators and accelerate the reactivity of heme peroxidase and other enzymes.^{12–17}

The oxidation of indole-3-acetic acid with hydrogen peroxide catalysed by 5,10,15,20-tetrakis-(2',6'-dichloro-3'-sulphonatophenyl)porphyrinatoiron(III)hydrates gives indole-3-carbinol and corresponding aldehyde in AOT reverse micelles¹⁸ in different reaction conditions. Herein, we report the biomimetic oxidation of ibuprofen with H_2O_2 catalysed by HRP and 5,10,15,20-tetrakis(2',6'-dichloro-3'-sulphonatophenyl)porphyrinatoiron(III) and manganese(III) hydrates in AOT reverse micelles in different reaction conditions, to elucidate the molecular mechanism of heme peroxidase and related enzymes.

Results

Formation of *type I* intermediate by the reaction of HRP with hydrogen peroxide (H_2O_2) and the subsequent catalytic reaction with ibuprofen (2**) in AOT reverse micelles**

Formation of reactive intermediate (*type I* of HRP) by the reaction of HRP with hydrogen peroxide (H_2O_2) has

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been reported in aqueous solution.^{7,8} The reaction of H_2O_2 to HRP, at -40°C in AOT reverse micelles, results in the decrease of the Soret absorbance of HRP and formation of a new peak at 412 nm. The spectrum of this new species is analogous to *type I* form of Horseradish peroxidase, i.e. *oxo-iron(IV)* radical cation intermediate.² The new peak at 412 nm does not appear in the presence of BPH at low temperature in AOT reverse micelles. The reaction of ibuprofen (**2**) with hydrogen peroxide (H_2O_2) catalysed by HRP in AOT reverse micelles ($W_o=10$) at pH 4.0 in 1 h gives 2-(4'-isobutylphenyl)ethanol (**5**) and *p*-isobutyl-acetophenone (**6**) in 42 and 12% yields, respectively. The oxidation products of ibuprofen (**2**) with H_2O_2 depend on the pH and presence of molecular oxygen in AOT reverse micelles. The sterically hindered *p*-*tert*-butyl phenol (BPH) quenches the oxidation of ibuprofen with H_2O_2 –HRP system in AOT reverse micelles. The reaction of ibuprofen with H_2O_2 catalysed by HRP in AOT reverse micelles at pH 4.0 ($W_o=10$) is completely quenched by using BPH in 1:1 and 0.5:1 molar ratio with HRP. In case of HRP and quencher (0.25:1), **5** and **6** are formed in 7% and 1% yields respectively.

Formation of high valent radical cation intermediate by the reaction of hydrogen peroxide (H_2O_2) with $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH)}_2$ and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ and the subsequent catalytic reaction with ibuprofen (2**) in AOT reverse micelles at different pH**

The reaction of hydrogen peroxide with iron(III) and manganese(III) porphyrin in aqueous solution,²¹ AOT reverse micelles^{22,23} and latex^{24,37} have been studied. But the detailed kinetics of the reaction of $\text{Cl}_8\text{TPPS}_4\text{Mn(OH)}_2$ with H_2O_2 at different pH and the study of active intermediate at low temperature (-40°C) in AOT reverse micelles has not yet been studied. Spectra of **1** at different pH ($4 < \text{pH} < 11$) in presence of H_2O_2 at -40°C are recorded as shown in Figure 1. In the absence of oxidising agent, the manganese porphyrins are stable in aqueous solution. The absorbance maximum at 469 nm is the Soret of Mn(III) porphyrins. However, upon addition of H_2O_2 the absorption spectrum changes and a new species is formed at low temperature (-40°C). The absorption maxima at 469 nm decreases and a new band at 424 nm on addition of H_2O_2 becomes sharper and increased in intensity. The intensity of 424 nm band is higher at pH 9.2 as compared to at pH 7.0 and 4.0. Again at pH 11.0, the intensity of 424 nm band is lowered. Manganese(IV) porphyrin may be present in the form of a μ -*oxo*-dimer in which there is anti-ferromagnetic coupling between two manganese(IV) atoms. The formation of μ -*oxo*-dimers is well known in iron porphyrins as well as manganese(III) porphyrins in extremely alkaline solution.^{25,26} The peak at 424 nm may be assigned for the high valent *oxo*-manganese species, at low temperature in AOT reverse micelles. In the presence of BPH, there is no formation of the new species showing absorbance at 424 nm.

The reaction of $\text{Cl}_8\text{TPPS}_4\text{Mn(III)Cl}$ (**1**) with acidic aqueous buffer solution (pH 4.0) exchanges the axial chloride ligand forming $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$, (**1a**)

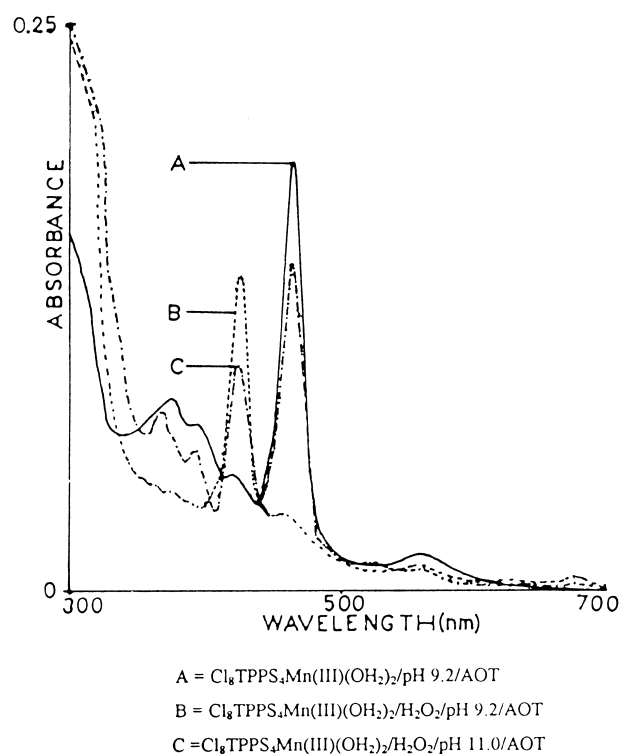


Figure 1. Formation of high valent *oxo*-manganese(IV) radical cation from the reaction of H_2O_2 with $\text{Cl}_8\text{TPPS}_4\text{Mn(III)Cl}$ (**1**) in AOT reverse micelle at different pH. All the reactions were performed at -40°C in phosphate buffer (pH 7.0, 9.2 and 11.0). $\text{Cl}_8\text{TPPS}_4\text{Mn(III)Cl}$, 1.8×10^{-7} mol; H_2O_2 , 1.0×10^{-5} mol; AOT, 3 cm^3 .

whereas in basic buffer solution, it forms $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$.^{29,30} The reaction of **2** with H_2O_2 catalysed by $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH)}_2$ in AOT reverse micelle ($W_o=12$) at pH 4.0 in 1h gives **5** and **6** in 45 and 12% yields, respectively. Further the reaction of **2** with H_2O_2 catalyzed by $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ (**1a**) gives **5** and **6** in 40% and 10% yields, respectively. The reaction of **2** with H_2O_2 catalysed by **1a** in aqueous acetate buffer pH 4.0 gives **5** and **6** only in 2 and 0% yields, respectively. The yields of the oxidation products increases with increase of W_o upto 12, then decreases with further increase in W_o at pH 4.0 in acetate buffer. The dependence of W_o in the formation of **5** is shown in Figure 2. The formation of 2-(4'-isobutylphenyl)ethylchloride (2%) is observed with the reaction of ibuprofen (**2**) in saturated sodium chloride with hydrogen peroxide (H_2O_2) catalysed by $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ in AOT reverse micelles. The sterically hindered *p*-*tert*-butyl phenol (BPH) also quenches the oxidation of ibuprofen with H_2O_2 – $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ system in AOT reverse micelles. The reaction of ibuprofen with H_2O_2 catalysed by $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ in AOT reverse micelle at pH 4.0 ($W_o=10$) is completely quenched by using BPH in 1:1 and 0.5:1 molar ratio with porphyrin whereas the reaction is partially quenched by using BPH in 0.25:1 molar ratio with porphyrin. In case of $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH)}_2$ **5** and **6** are formed in 10 and 1% yields, respectively, while in $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$, **5** and **6** are formed in 12 and 1% yields, respectively. Similar reactions have been performed at

pH 7.0, 9.2, 12.0 and results are presented in Figure 2. All the products have been characterized by comparison with authentic samples and their HPLC retention time. The reaction of ibuprofen ester (**7**) with H_2O_2 catalyzed by $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ at $\text{Wo}=12$ in AOT reverse micelle at pH 4.0, 7.0 and 9.2 does not give any decarboxylated product.

The incorporation and UV–visible spectroscopic study of $[\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2]$ (**1a**), ibuprofen (**2**) and *p*-tert-butyl phenol (BPH) in AOT reverse micelle have been done by following the literature procedures.^{19,20} The comparison of UV-visible spectra in phosphate buffer and methanol of **1a** indicate that **1a** resides in the interphase of AOT reverse micelles. Ibuprofen and *p*-tert-butyl phenol (BPH) resides in the interphase of AOT reverse micelles, inferred by the comparison of its UV-visible spectra in reverse micelles with those in phosphate buffer (pH 7.0), methanol and chloroform. The maximum absorbance of ibuprofen was obtained at water to surfactant ratio, $\text{Wo}=24$ of AOT reverse micelles.

Discussion

The reaction of H_2O_2 and HRP in AOT reverse micelles forms high valent radical cation (*type I*) intermediate, which accept one electron from the ibuprofen (**2**) at its heme edge without direct contact with the oxygen atom of the oxo-iron and it self-converts to *type II* intermediate of HRP. The ibuprofen radical cation loses H^+ to form the carboxylate radical (**3**) species which on subsequent decarboxylation leads to the ibuprofen

carbon radical (**4**). The carbon radical (**4**) reacts with molecular oxygen to form peroxy radical species (**10**) which abstracts H^+ from solvent or ibuprofen (substrate) leads to formation of hydroperoxide intermediate (**11**). The homolytic cleavage of hydroperoxide, form the alcohol **5** and elimination of H_2O gives the ketone **6** (Scheme 1). The formation of this type of product has been reported during the reaction of indole-3-acetic acid with HRP in aqueous phosphate buffer.³³

The yield of **5** becomes maximum at $\text{Wo}=10$ with H_2O_2 catalysed by HRP in AOT reverse micelles and then decreases at higher Wo . These results indicate that at water/surfactant, $\text{Wo}=10$, HRP has the right geometry for the maximum activity for the above oxidation reaction which is comparable with the maximum activity of HRP at $\text{Wo}=12$ in AOT reverse micelles during the oxidation of pyrogallol to purpurogallin.²⁷ In the absence of oxygen the formation of **5** and **6** is not observed in the AOT reverse micelles. The activity of HRP is quenched in the presence of BPH at $\text{Wo}=10$, in AOT reverse micelle.

The reactions of selected water soluble iron(III)porphyrins and H_2O_2 with organic substrates form the same products, obtained by the reactions of HRP and H_2O_2 in AOT reverse micelles in same reaction conditions.²⁸ The product formation (path A, Scheme 2) may be explained by abstraction of hydrogen radicals by high valent *oxo*-manganese(IV)porphyrins (**8a**) from ibuprofen (**2**) and subsequent decarboxylation from the radical species (**3**) form ibuprofen carbon radical (**4**). The recombination of **4** with chelated hydroxyl equivalent (**8b**) or free hydroxyl radical gave 2-(4'-isobutylphenyl)ethanol (**5**). Further, the over-oxidation of **5** with high valent radical cation (**8a**) form **6** by abstraction and recombination mechanism (Scheme 2). This kind of abstraction and recombination mechanism has been proposed for the oxidation of alcohols to

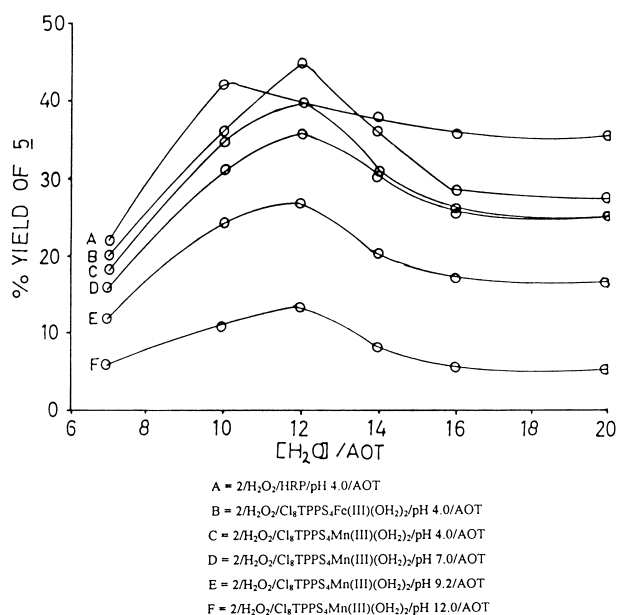
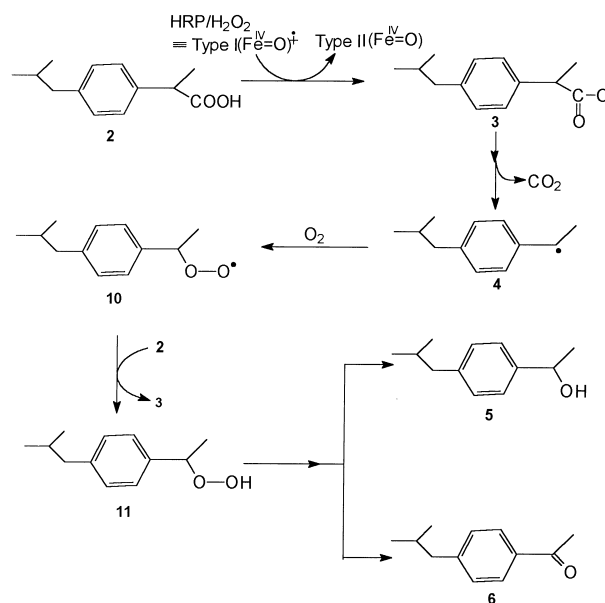
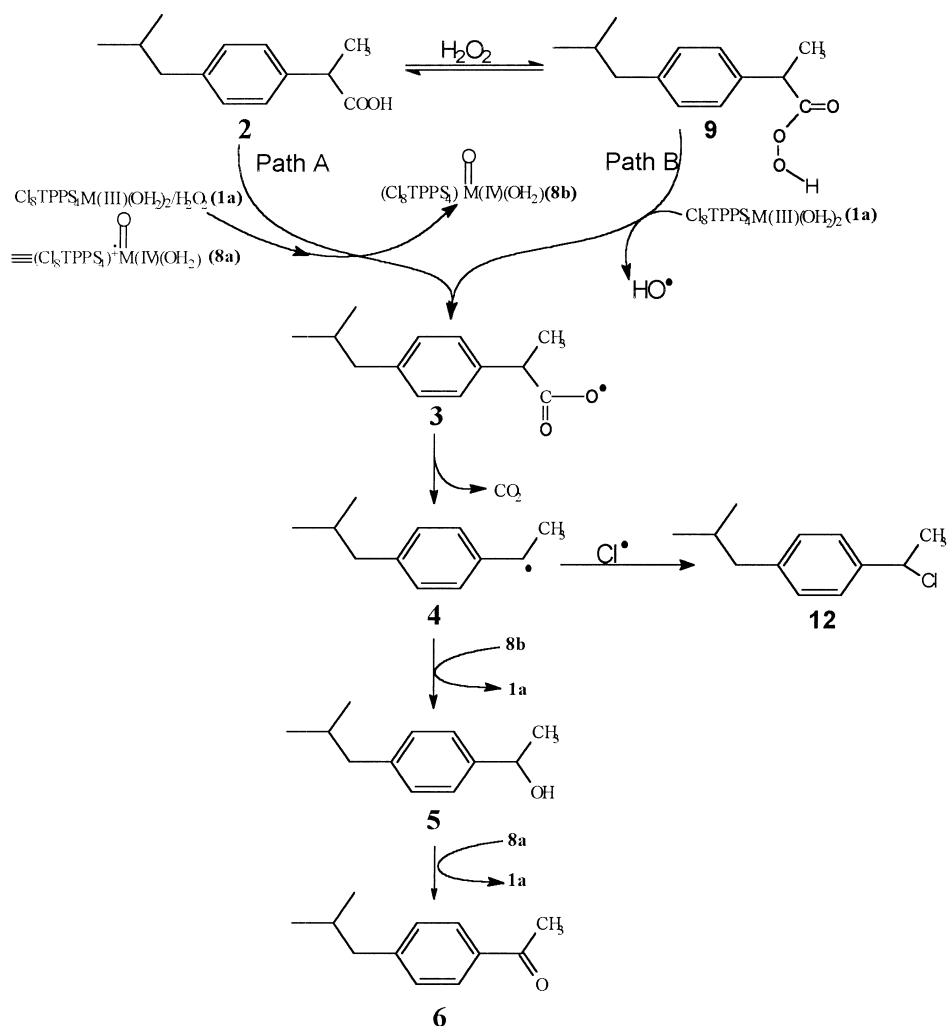


Figure 2. Formation of **5** from **2** and H_2O_2 catalyzed by HRP and water soluble porphyrins in AOT reverse miscelles at different pH and water:surfactant ratio, Wo . All the reactions were performed at room temperature in acetate buffer (pH 4.0), phosphate buffer (pH 7.0, 9.2 and 12). $\text{Cl}_8\text{TPPS}_4\text{Mn(III)Cl}$, 1.8×10^{-7} mol; $\text{Cl}_8\text{TPPS}_4\text{Fe(III)Cl}$, 1.9×10^{-7} mol; H_2O_2 , 1.0×10^{-5} mol; AOT, 3 cm^3 .



Scheme 1.



Scheme 2.

aldehyde/ketone with mono-oxygen donors catalysed by metalloporphyrins in organic^{22,23} and aqueous solution.³⁴ An alternative pathway (path B) is the reaction of H_2O_2 and ibuprofen (acid) form peroxy acid complex (**9**) reversible, which on homolytic cleavage in presence of metalloporphyrin form **3**. The decarboxylation of **3** form **4**, which is responsible for the formation of **5** and **6** (Scheme 2). Further the reaction of the peroxy acid (**9**) also reacts with metalloporphyrin, forms high valent *oxo*-radical cation heterolytically, which leads to final products in AOT reverse micelles. The formation of carbon radical after CO_2 elimination has been observed directly in homogeneous medium.³¹ The formation of 2-(4'-isobutylphenyl)ethylchloride (**12**) in 2% yield may be explained by the reaction of carbon radical with chlorine radical, which is generated by the reaction of Cl^- with $\text{HO}^\bullet$, in AOT reverse micelles.

This type of oxidative decarboxylation of selected acids has been reported by the reaction with iodosylbenzene catalysed by iron(III)porphyrin in organic solvents.^{31,32} The yield of **5** and **6** is maximum at $\text{Wo} = 12$ and again decreases with increasing Wo and pH in AOT reverse micelle. Hence the aqueous core of AOT reverse micelle ($\text{Wo} = 12$) has appropriate diameter for the incorpora-

tion of both ibuprofen (**2**) and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH)}_2$ (**1a**), for maximum interactions and high yields of different oxidation products. The AOT reverse micelles are more efficient reaction media than aqueous phosphate buffer in above oxidative decarboxylation and subsequent formation of oxidative products of ibuprofen in different reaction conditions.

Conclusion

The hydrogen abstraction followed by decarboxylation of ibuprofen and subsequent product formation by reaction of ibuprofen with hydrogen peroxide catalysed by HRP and 5,10,15,20-tetrakis(2',6'-dichloro-3'-sulphonatophenyl)porphyrinatoiron(III) and manganese (III) hydrates depend upon pH and water to surfactant ratio (Wo) and presence of oxygen in the AOT reverse micelles. The percentage yields of the products increases at lower pH as compared to higher pH in AOT reverse micelles. The high valent oxo-iron(IV) and manganese(IV) radical cation species are easily quenched by the sterically hindered *p*-*tert*-butyl phenol (BPH) in AOT reverse micelles and no oxidation products are formed in the presence of BPH. Although the same products are

formed by the reaction of ibuprofen and H_2O_2 catalysed by water soluble iron(III) and manganese(III)porphyrins, as formed by the reaction of ibuprofen with H_2O_2 catalysed by HRP, but their mechanism of formations are different. Thus the above chemical model systems, metalloporphyrins/ H_2O_2 mimic the formation of different products as obtained by the reaction of ibuprofen with H_2O_2 and HRP in aerobic organized media.

Experimental

Materials and Methods

UV-vis spectra were recorded using a Shimadzu UV-260 spectrophotometer. IR spectra were recorded using a Perkin-Elmer FTIR spectrum 2000 spectrophotometer. EIMS spectra were recorded on Jeol SX/102DA-6000 (6kV, 10 mA) spectrophotometer. The oxidation products of ibuprofen were identified and quantified by using Waters HPLC equipped with a photodiode array detector (Model 991) on a μ -Bondapak C_{18} column (3.9×300 mm) using methanol (100%) as eluent at a flow rate of 0.2 mL/min monitored at 265 nm and comparison of both UV spectra and retention time with that of authentic samples. ^1H NMR spectra were recorded on a Bruker 300 MHz spectrophotometer.

Sodium bis-(2-ethylhexyl)sulphosuccinate (Aerosol-OT) was purified by published procedure³⁵ before use. Ibuprofen was obtained from Ranbaxy, India and purified. The aqueous hydrogen peroxide (30% v/v) was obtained from CDH India and used without further purification. 5,10,15,20-tetrakis(2',6'-dichloro-3'-sulphonatophenyl)porphyrin (Cl_8TPPS_4) was prepared by sulphonation of Cl_8TPP with oleum at 130°C by following the literature procedure.³⁶ UV-visible λ_{max} nm (ϵ_{max} in methanol): 425.5 (0.74), 521.5 (0.03), 555.0 (0.01), 600.0 (0.01) and 658.0 (0.01). ^1H NMR (D_2O) δ ppm: 8.77 (s, SH, pyrrolic protons), 8.62 (in, 4H, aromatic protons) and 8.00 (m, 4H, aromatic protons). 5,10,15,20-tetrakis(2',6'-dichloro-3'-sulphonatophenyl)-porphyrinato-manganese(III)hydrate [$\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH}_2)_2$] (**1a**) was prepared by refluxing the water-soluble free base porphyrin with a 40-fold excess of manganese(II) chloride in DMF (10 mL) following the literature procedure.³⁷ UV-visible λ_{max} nm (ϵ_{max} in water): 370.2 (0.19), 395.6 (0.14), 418.2 (0.19), 464.0 (0.28), 560.2 (0.04). 5,10,15,20-tetrakis(2',6'-dichloro-3'-sulphonatophenyl)porphyrinatoiron(III)hydrate [$\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH}_2)_2$] (**1a**) was prepared by refluxing the water-soluble free base porphyrin with a 40-fold excess of ferrous(II) chloride in DMF (10 mL) following the literature procedures.^{21,28,38} UV-visible λ_{max} nm (ϵ_{max} in water): 400.0 (0.34), 416.5 (0.72), 466 (0.08) and 512.5 (0.06).

Reaction of ibuprofen (2) with H_2O_2 catalysed by HRP in AOT reverse micelle

To a solution of AOT in isoctane (0.05 M, 10 mL) was added the ibuprofen solution (6 μ mol) and the mixture was stirred vigorously till the ibuprofen was dissolved approximately 3 min. A solution of HRP in phosphate

buffer (pH 4.0, 10.0 μ L) was injected into the stirred solution followed by H_2O_2 (30%, 0.2 mmol, 10.8 μ L). The initially turbid reaction mixture gradually becomes clear in about 5 min, indicating the completion of the micellization process, was stirred for 1.5 h in the presence of dissolved oxygen atmosphere. The reaction mixture was concentrated and extracted with methanol and the extract was quenched by adding BPH and subjected to HPLC analysis for identification and quantification of products.

Oxidation of ibuprofen (2) with H_2O_2 catalysed by $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH}_2)_2$ and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH}_2)_2$ in AOT reverse micelles

The oxidation of ibuprofen in AOT reverse micelles was studied by minor modifications of the known methods.^{21,28,37} Ibuprofen (0.3 mol) was added to a solution of AOT in iso-octane (6 mL, $\text{Wo} = 12$) containing 10 μ L of $\text{Cl}_8\text{TPPS}_4\text{Fe(III)(OH}_2)_2$ and $\text{Cl}_8\text{TPPS}_4\text{Mn(III)(OH}_2)_2$ (3.6 mmol). Hydrogen peroxide (30%, 0.2 mmol, 10.8 μ L) was added to above solution and stirred for 1 h at room temperature in presence of inert atmosphere. The products were extracted with methanol (15 mL) and the extract was quenched by adding BPH and then subjected to HPLC analysis for identification and quantification of products. The same procedure was followed for reactions at different water to surfactant ratio (Wo) and at different pH. The results are presented in Figure 2. In an another experiment saturated NaCl solution (5.4 μ L) was injected to the POR/ H_2O_2 /AOT system and stirred for 1 h at room temperature. The products were extracted with methanol (15 mL) and the extract was subjected to HPLC analysis.

Oxidation of ibuprofen ester (7) with H_2O_2 catalysed by **1** in AOT reverse micelles

Ibuprofen ester (**7**) was prepared from ibuprofen (**2**) by refluxing in dry methanol for 3–4 h with catalytic amount of concd H_2SO_4 . Yield: (82%), FTIR ν (film)/ cm^{-1} 2955, 2871, 2361, 1904, 1739, 1612, 1512, 1377, 1334, 1207, 1165, 1068, 852, 798 and 727. ^1H NMR (CDCl_3); δ ppm 0.92 (d, 6H, $J = 6.5$ Hz), 1.43 (d, 3H, $J = 6.5$ Hz), 1.80 (in, 1H), 2.05 (m, M), 2.60 (d, 2H, $J = 7.2$ Hz), 3.70 (s, 3H) and 7.10 (m, 4H). EIMS (m/z): 220 (M^+), 205 ($\text{M}^+ - \text{CH}_3$), 161 (100%, $\text{M}^+ - \text{COOCH}_3$). The oxidation of ibuprofen ester was studied by the same procedure as ibuprofen.

Synthesis of *p*-isobutyl-acetophenone (**6**)

The isobutyl benzene was acylated by Ac_2O and anhydrous AlCl_3 by following the literature procedure³⁹ to give the title compound. In a typical experiment, a mix of Ac_2O (0.01 M, 1.0 mL) and isobutylbenzene (0.01 M, 1.3 mL) was added to anhydrous AlCl_3 in dry CH_2Cl_2 at -5°C to $+5^\circ\text{C}$ in ~ 4 h, and the mixture was stirred for 30 min, allowed to warm to $25-8^\circ$, and worked up to give *p*-isobutyl-acetophenone (**6**). Yield $\sim 70\%$, FTIR ν (film)/ cm^{-1} : 3063, 2923, 2360, 1685, 1599, 1449, 1359, 1266, 1180, 1078, 1024, 955, 760 and 690. ^1H NMR (300 MHz, CDCl_3) δ ppm: 0.89 (d, 6H, $J = 6.6$ Hz), 1.84 (m, 1H), 2.54 (d, 2H, $J = 7.3$ Hz), 2.62 (s, 3H), 7.24 (d, 2H, $J = 8.9$ Hz),

7.36 (d, 2H, $J=8.9\text{ Hz}$); EIMS (m/z): 176 (M^+), 161 (100%, M^+-CH_3).

Synthesis of 2-(4'-isobutylphenyl)ethanol (5)

The 2-(4'-isobutyl phenyl)ethanol (**5**) was prepared by the hydrogenation of *p*-isobutylacetophenone (**6**) in presence of 10% Pd/C in dry methanol by following the literature procedure⁴⁰ under 100 psig of H at 30°C for 1h with 95% conversion. FTIR ν (film)/ cm^{-1} : 3401, 2956, 2923, 2852, 2361, 1606, 1510, 1463, 1377, 1266, 1163, 909, 848 and 720. ^1H NMR (300 MHz, CDCl_3) δ ppm: 0.90 (d, 6H, $J=6.5\text{ Hz}$), 1.85 (m, 1H), 1.95 (d, 3H, $J=6.5\text{ Hz}$), 2.65 (d, 2H, $J=7.2\text{ Hz}$), 4.27 (q, 1H, $J=6.5\text{ Hz}$), 7.26 (d, 2H, $J=8.8\text{ Hz}$), 7.39 (d, 2H, $J=8.8\text{ Hz}$); EIMS (m/z): 179 ($M^+ + I$), 178 (M^+), 160 (100%, M^+-H_2O).

Synthesis of 2-(4-isobutyl phenyl)ethylchloride (12)

The 2-(4'-isobutyl phenyl)ethylchloride (**12**) was prepared by the chlorination of 2-(4'-isobutyl phenyl)ethanol using thionyl chloride. Pyridine (1.0 mL) and 2-(4'-isobutyl phenyl)ethanol (0.01 M, 1.8 mL) was stirred for 15 min at room temperature followed by addition of thionyl chloride (1.2 mL) drop by drop for 1 h. Then the resulting solution was refluxed for another 2 h, in which pyridinium hydrochloride separates and the excess thionyl chloride was evaporated with repeated azeotropic distillation in benzene under reduced pressure, which gives the oily layer of 2-(4'-isobutyl phenyl)ethylchloride (**12**) in 87% conversion. FTIR ν (film)/ cm^{-1} : 2956, 2926, 2054, 1579, 1475, 1380, 1268, 1194, 903, and 753. ^1H NMR (300 MHz, CDCl_3) δ ppm: 0.90 (d, 6H, $J=6.3\text{ Hz}$), 1.85 (m, 1H), 2.00 (d, 3H, $J=6.3\text{ Hz}$), 2.65 (d, 2H, $J=7.2\text{ Hz}$), 4.40 (q, 1H, $J=6.3\text{ Hz}$), 7.26 (d, 2H, $J=8.8\text{ Hz}$), 7.39 (d, 2H, $J=8.8\text{ Hz}$); EIMS (m/z): 196 (M^+), 161 (100%, $M^+-^{35}\text{Cl}$), 159 ($M^+-^{37}\text{Cl}$).

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