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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

High chemiluminescent activity of Fe^{III}-TAML activator in aqueous-organic media and its use in determination of organic peroxides

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

High activity of Fe^{III}-TAML, peroxidase mimic, upon the performance of catalytic oxidation of luminol in aqueous-organic media (ethanol, isopropanol and acetonitrile) was determined. Using Fe^{III}-TAML the sensitive chemiluminescent assays for determination of benzoyl peroxide and tert-butyl hydroperoxide in the presence of organic solvents were constructed.

Peroxides are widely used as polymerization initiators, cross-linking agents, additives for rubber curing, whiteners, and oxidants.¹⁻³ They also occur as intermediates in processes involving oxidation of hydrocarbons with molecular oxygen. Additionally, in industry there are some peroxy by-products often present in many organic syntheses and in ageing processes of organic materials. This has promoted a development of analytical methods and sensors for peroxides monitoring.

Numerous analytical methods have been developed for determination of hydrogen peroxide.⁴⁻¹⁰ One of the most sensitive methods is the method based on a measurement of chemiluminescence (CL) which is formed upon a catalytic oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in aqueous medium.¹¹ Horseradish peroxidase (HRP) is usually used as a biocatalyst in this indicator reaction.¹²⁻¹⁴ But the high cost of the enzyme has stimulated a search of its alternatives.¹⁵ Some peroxidase mimetics such as hemin, metal-containing porphyrins and phthalocyanines and nanoparticles of different chemical nature were reported to be used as catalysts of the luminol oxidation by H₂O₂.¹⁵⁻¹⁹ Recently new mimetic, an iron containing tetraamido macrocyclic ligand (Fe^{III}-TAML, Fig. 1) was shown to be also an effective catalyst in CL assay of hydrogen peroxide.²⁰ Moreover, HRP mimetics have higher thermal stability than the native enzyme.

Contrary to hydrogen peroxide, the number of publications concerning the methods of determination of organic peroxides is very limited. The feature of such methods is that many of organic peroxides have a poor solubility in aqueous solutions and, hence, their determination should be carried out in organic or aqueous-organic media. It is well known that in the presence of organic solvents HRP is quickly inactivated and misses its catalytic activity.^{21,22} Contrary to HRP, some of its mimetics remain their catalytic ability in the presence of organic solvents and, hence, are good alternatives of HRP in assays of organic peroxides.^{23,24}

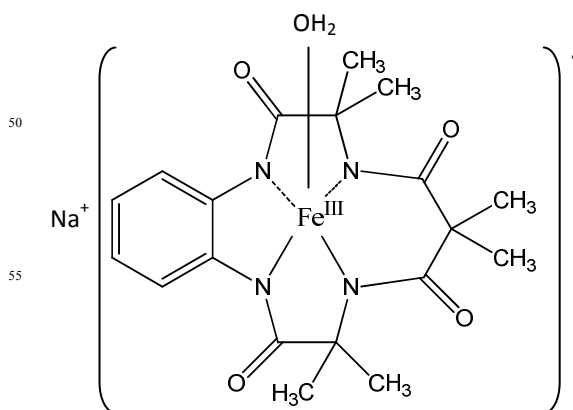


Figure 1. Chemical structure of Fe^{III}-TAML

Herein we describe the study of catalytic activity of Fe^{III}-TAML in the luminol oxidation upon its performance in the presence of different organic solvents (ethanol, isopropanol, and acetonitrile). Molecular, spectral and catalytic properties of Fe^{III}-TAML have been reported previously in detail.^{25,26} High catalytic ability of Fe^{III}-TAML allowed a development of sensitive CL assays for the determination of benzoyl peroxide (BP) and tert-butyl hydroperoxide (TBH) in aqueous-organic media.

In previous work we developed the sensitive chemiluminescent Fe^{III}-TAML-catalyzed assay of H₂O₂ in aqueous buffered medium.²⁰ For quantification of organic peroxides Fe^{III}-TAML has to remain the catalytic activity in organic or aqueous-organic media. To estimate the ability of Fe^{III}-TAML to catalyze the luminol oxidation in the presence of organic solvents the concentration effect of 4 miscible solvents (acetonitrile, dimethylsulfoxide, ethanol, and isopropanol) in 20 mM carbonate buffer, pH 9.9 with 50 mM Tris on CL intensity was studied. The buffer mentioned above was shown to be optimal for Fe^{III}-TAML-catalyzed oxidation of luminol in aqueous solution.²⁰

As seen in Fig. 2, for all the studied solvents increasing of the solvent concentration in the reaction solution diminished a light output and at concentration of the solvents equal to 50% (v/v) the catalytic activity of Fe^{III}-TAML was no more than 5%. Interestingly, the influence of different solvents on CL intensity was not similar. The lowest catalytic activity of Fe^{III}-TAML was observed in the presence of dimethylsulfoxide. By this reason di-

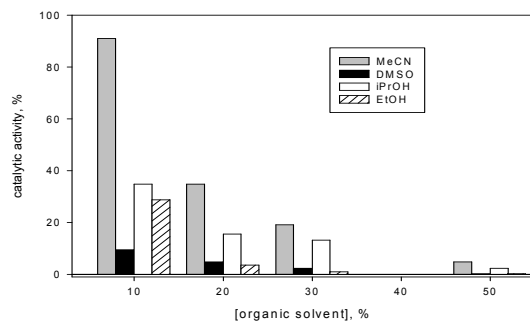


Figure 2. Effect of organic solvents on Fe^{III}-TAML-catalyzed chemiluminescence. Experimental conditions: 20 mM carbonate, pH 9.9 containing 50 mM Tris, 27 μM luminol, 0.1 mM H₂O₂ and organic solvent; [Fe^{III}-TAML] = 10⁻⁸ M. The activity of Fe^{III}-TAML measured in absence of organic solvent was expressed as 100%.

methysulfoxide was not used by us in the further work. On the other hand, in the presence of acetonitrile Fe^{III}-TAML showed the highest catalytic activity (Fig. 2). In the reaction solutions with ethanol and isopropanol Fe^{III}-TAML was also active, although its activity was lower than that in solutions with acetonitrile.

Since Fe^{III}-TAML was studied by us as alternative to HRP, we compared the inactivation of Fe^{III}-TAML and HRP with acetonitrile. As seen in Fig. 3, HRP is labile even in the presence of low concentrations of acetonitrile. Only 18% and 2.5% of its initial activity remained in 10% and 20% solutions of this solvent, respectively. In contrast, Fe^{III}-TAML is significantly more stable catalyst. So, in 10%, 20% and 30% (v/v) acetonitrile Fe^{III}-TAML showed 91%, 35% and 19% of initial activity, respectively. Therefore, unlike HRP, Fe^{III}-TAML may be used as the effective catalyst in luminol oxidation in aqueous-organic solutions.

The aforementioned experiments have been carried out using the buffer previously optimized for Fe^{III}-TAML catalysis in

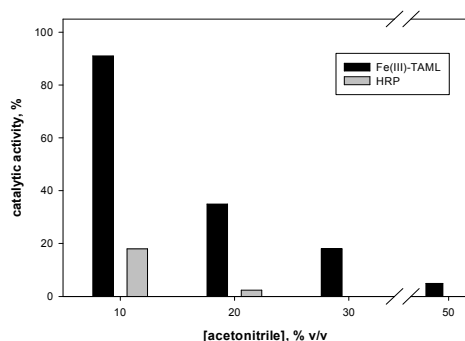


Figure 3. Effect of acetonitrile concentration on activity of Fe^{III}-TAML and HRP. Experimental conditions for Fe^{III}-TAML catalysis: 20 mM carbonate, pH 9.9 containing 50 mM Tris, 27 μM luminol, 0.1 mM H₂O₂ and organic solvent; [Fe^{III}-TAML] = 10⁻⁸ M. Experimental conditions for HRP catalysis: 60 mM Tris, pH 8.3, containing 1.5 mM luminol, 0.1 mM H₂O₂ and organic solvent; [HRP] = 1.8 × 10⁻¹⁰ M. The activity of Fe^{III}-TAML measured in absence of organic solvent was expressed as 100%.

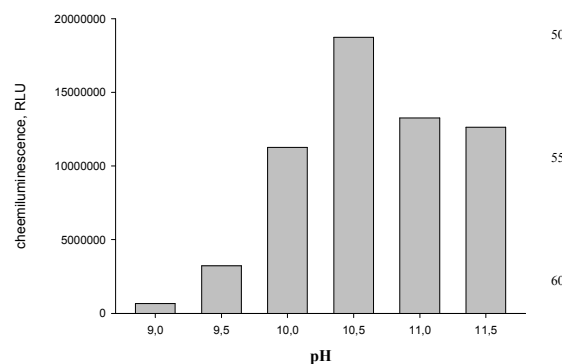
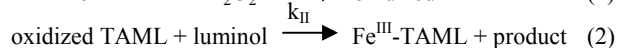
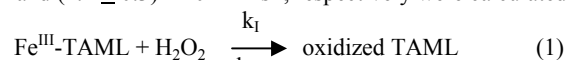


Figure 4. Dependence of light output formed upon Fe^{III}-TAML-catalyzed luminol oxidation in the presence of isopropanol as a function of pH of the reaction buffer. Experimental conditions: 20 mM carbonate containing 50 mM Tris, 27 μM luminol, 0.1 mM H₂O₂ and 10% (v/v) isopropanol; [Fe^{III}-TAML] = 10⁻⁸ M.

the aqueous solution. However, it is well known that an introduction of organic solvents to aqueous solutions may affect an ionization state of soluble molecules, thereby changing pH-optimum of the reaction of interest. By this, we estimated pH-optimum for Fe^{III}-TAML-catalyzed oxidation of luminol in presence of isopropanol. The obtained results demonstrated that the addition of isopropanol to the reaction solution leads to shifting of the pH-optimum of Fe^{III}-TAML from 9.9 to pH 10.5 (Fig. 4). The identical results were obtained by adding of ethanol and acetonitrile (data not shown).

Previously to evaluate the catalytic efficiency of Fe^{III}-TAML in aqueous medium the dependence of the concentrations of luminol and H₂O₂ on an initial rate of luminol oxidation was studied. Based on the mechanism proposed by Chahbane et al.²⁷ (eqs. 1,2), the values of the rate constants k_I and k_{II} of $(2.2 \pm 0.3) \times 10^3$ and $(1.1 \pm 0.3) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively were calculated.¹⁹



$$\frac{d[\text{luminol}]}{dt} = \frac{k_I k_{II} [\text{Fe}^{\text{III}}\text{-TAML}] [\text{peroxide}] [\text{luminol}]}{k_I [\text{peroxide}] + k_{II} [\text{luminol}]} \quad (3)$$

Similar study for Fe^{III}-TAML-catalyzed oxidation of luminol was carried out in 20 mM carbonate buffer, pH 10.5 containing 50 mM Tris and 20% (v/v) acetonitrile (Fig. 5). The initial rate values have been fitted to equation 3 to calculate the rate constants k_I and k_{II} of $(3.4 \pm 0.5) \times 10^3$ and $(2.0 \pm 0.1) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Comparison of the values of the rate constants calculated under optimized conditions in aqueous medium (20 mM carbonate buffer, pH 9.9 with 50 mM Tris) and the buffer-acetonitrile mixture demonstrated that Fe^{III}-TAML did not miss its catalytic activity in the presence of the organic solvent. The decrease of Fe^{III}-TAML activity in the presence of acetonitrile mentioned above (Fig. 2) was likely connected with a change of pH value of the reaction solution due to the addition of the organic solvent. Moreover, the kinetic constants of Fe^{III}-TAML were shown to be slightly improved at the addition of acetonitrile to the reaction solution.

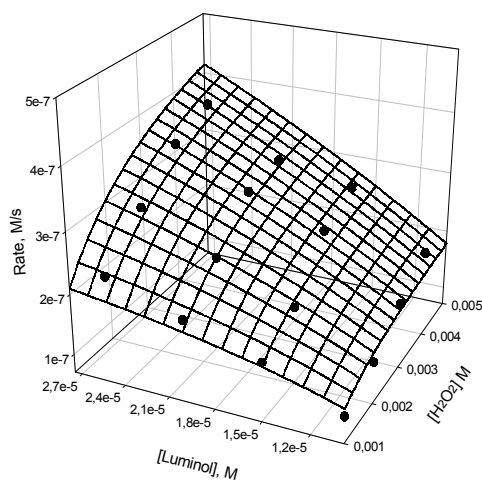


Figure 5. Effect of concentrations of luminol and hydrogen peroxide on initial rate of Fe^{III} -TAML-catalyzed oxidation of luminol performed in the presence of acetonitrile. Conditions: 20 mM carbonate buffer, pH 10.5 with 50 mM Tris and 20% (v/v) acetonitrile, $[\text{Fe}^{\text{III}}\text{-TAML}] = 10^{-8}$ M.

The obtained knowledge about high catalytic activity of Fe^{III} -TAML in aqueous-organic mixtures towards luminol allowed us to develop Fe^{III} -TAML-based assay of benzoyl peroxide (BP) which is insoluble in water, but has a good solubility in such organic solvents as acetonitrile, isopropanol and ethanol. The quantification of BP is practically important task, since this peroxide is widely used in the food industry. By this, there is a need for a development of sensitive assays for the determination of BP.²⁸⁻³⁰

The determination of BP was carried out in the carbonate buffer, pH 10.5 containing 50 mM Tris and 10 or 20% of organic solvents. The concentration of luminol was 27 μM , as at higher concentrations of luminol a substrate inhibition of Fe^{III} -TAML was observed (data not shown). Note that the same concentration of luminol was found as optimal in Fe^{III} -TAML-based assay of H_2O_2 in aqueous medium.²⁰

The analytical parameters of Fe^{III} -TAML-based CL assay of BP in the presence of acetonitrile, isopropanol and ethanol are presented in Table 1. In 10% organic solvent solution the values of detection limit (defined as 3 standard deviation (3σ) of the blank) of BP were similar regardless, which solvent was used,

Table 1. The analytical parameters of Fe^{III} -TAML-based chemiluminescent assay of benzoyl peroxide in the presence of organic solvents.

Organic solvents	Concentration of organic solvents (v/v)			
	10%	20%	10%	20%
	Detection limit, nM		sensitivity, 10^{10} RLU M^{-1}	
acetonitrile	90	900	10.0 ± 0.5	4.0 ± 0.1
ethanol	70	100	25.4 ± 1.0	4.0 ± 0.2
isopropanol	100	100	3.0 ± 0.2	5.0 ± 0.1

Table 2. The analytical parameters of Fe^{III} -TAML-based chemiluminescent assay of tert-butyl hydroperoxide in the presence of organic solvents.

Organic solvents	Concentration of organic solvents (v/v)			
	10%	20%	10%	20%
	Detection limit, μM		sensitivity, 10^8 RLU M^{-1}	
acetonitrile	9	30	56 ± 3	32.0 ± 1.0
ethanol	3	90	19 ± 1	9.3 ± 0.6
isopropanol	50	50	28 ± 2	9.7 ± 0.6

and equal to $0.7\text{-}1.0 \times 10^{-7}$ M. The most sensitive BP assay (the assay sensitivity was defined as a slope of calibration curve) was developed in the presence of ethanol. Replacement of ethanol with isopropanol or acetonitrile decreased the assay sensitivity in 2.5 or 8.5 times, respectively. It should be noted that in the case of the use of H_2O_2 as an oxidant, the most favorable solvent was acetonitrile (Fig. 2).

Increasing of the organic solvent concentration up to 20% did not affect the values of detection limit of BP in the case of isopropanol and ethanol, but made worse this parameter by using of acetonitrile (Table 1). At the same time, in all solutions containing 20% organic solvent the sensitivity of BP assay was practically equal. Therefore, using Fe^{III} -TAML-catalyzed oxidation of luminol as an indicator reaction the sensitive assay of water-insoluble BP was developed, with the best analytical parameters being obtained in 10% ethanol solution. In the latter case the working (linear) range was $1 \times 10^{-7} - 5 \times 10^{-5}$ M ($R^2=0.98$) (Fig. 6).

Similarly, CL Fe^{III} -TAML-based assay for the determination of *t*-BuOOH was developed. The analytical parameters of the assay are presented in Table 2. Like BP assay, the minimum value of detection limit of TBH was obtained in the carbonate buffer with 10% ethanol. Importantly, increasing of organic solvent concentration in the reaction solution resulted in the increase of

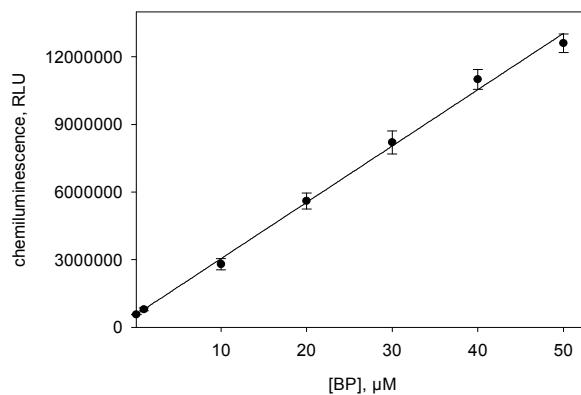


Figure 6. Calibration curve for the chemiluminescent determination of benzoyl peroxide using the luminol oxidation catalyzed by Fe^{III} -TAML in the presence of ethanol. Conditions: 20 mM carbonate buffer, pH 10.5 with 50 mM Tris and 10% (v/v) ethanol, $[\text{luminol}] = 27 \mu\text{M}$, $[\text{Fe}^{\text{III}}\text{-TAML}] = 10^{-8}$ M.

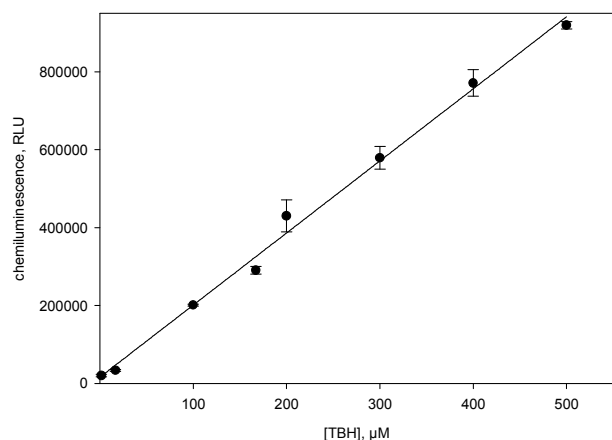


Figure 7. Calibration curve for the chemiluminescent determination of tert-butyl hydroperoxide using the luminol oxidation catalyzed by Fe^{III}-TAML in the presence of ethanol. Conditions: 20 mM carbonate buffer, pH 10.5 with 50 mM Tris and 10% (v/v) ethanol, [luminol] = 27 µM, [Fe^{III}-TAML] = 10⁻⁸ M.

detection limit value. It should be also noted that the detection limit for BP assay was 40-fold lower than that for TBH. The obtained results are in a good agreement with the data reported previously.²⁷

Contrary to the detection limit, the highest sensitivity of Fe^{III}-TAML-based assay of TBH was upon the performance of luminol oxidation in the presence of 10% acetonitrile. Replacement of acetonitrile with ethanol diminished the assay sensitivity in 3 times. Also, the sensitivity made worse at increasing of solvent concentration (Table 2). In the case, if the TBH assay was performed in the presence of 10% ethanol, the working range was $3 \times 10^{-6} - 5 \times 10^{-4}$ M ($R^2=0.98$) (Fig. 7).

Thus, in the present work we showed the high activity of Fe^{III}-TAML upon the performance of catalytic oxidation of luminol in aqueous-organic media. Calculations of values of rate constants k_f and k_{II} for the reaction of interest demonstrated that the addition of organic solvents to the substrate solution does not affect the efficiency of Fe^{III}-TAML. Using Fe^{III}-TAML we constructed sensitive CL assays for determination of BP and TBH in the presence of organic solvents with the detection limits lower than those for assays reported previously.³¹⁻³⁶ Therefore, the obtained results open up very promising perspectives for using Fe^{III}-TAML to develop analytical methods with high sensitivity of different peroxides in aqueous-organic media.

This work was supported by the Russian Foundation for Basic Research (13-04-00364 A).

Notes and references

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† Electronic Supplementary Information (ESI) available: experimental part. See DOI: 10.1039/b000000x/

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