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High chemiluminescence activity of an Fe^{III}–TAML activator in aqueous–organic media and its use in the determination of organic peroxides†

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

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. In addition, in industry there are some peroxy by-products often present in many organic syntheses and in ageing processes of organic materials. This has promoted the development of analytical methods and sensors for peroxide 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 the measurement of chemiluminescence (CL) which is formed upon a catalytic oxidation of luminol (5-amino-2,3-dihydro-l,4-phthalazinedione) in aqueous medium.[11] Horseradish peroxidase (HRP) is usually used as a biocatalyst in this indicator reaction.[12–14] However, the high cost of this enzyme has stimulated a search for its alternatives. Some peroxidase mimetics such as hemin, metal-containing porphyrins and phthalocyanines and nanoparticles of different chemical nature were reported to be used as catalysts for the luminol oxidation by H_2O_2.[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 the CL assay of hydrogen peroxide.[20] Moreover, HRP mimetics have higher thermal stability than the native enzyme.

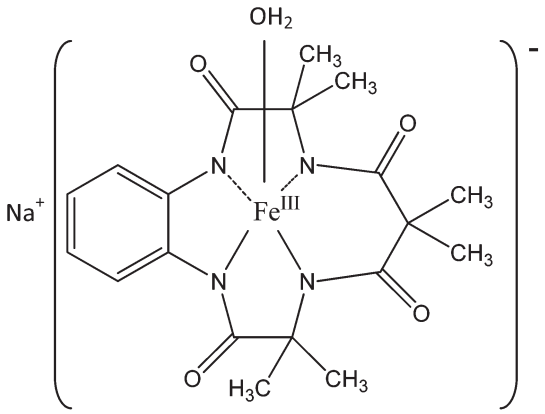

Fig. 1 Chemical structure of Fe^{III}–TAML.

In contrast 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 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 loses its catalytic activity.[21,22] In contrast to HRP, some of its mimetics retain their catalytic ability in the presence of organic solvents and, hence, are good alternatives of HRP in assays of organic peroxides.[23,24]

Herein we describe the study of the catalytic activity of Fe^{III}–TAML in luminol oxidation and 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 the 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 had developed the sensitive chemiluminescence Fe^{III}–TAML-catalyzed assay of H_2O_2 in aqueous

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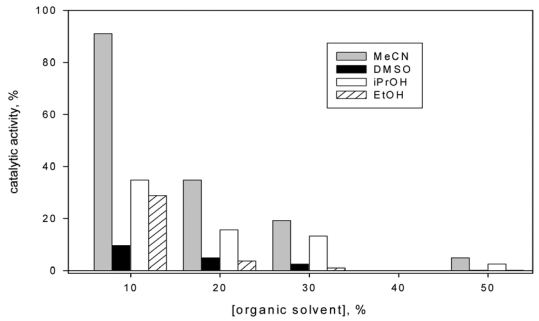

Fig. 2 Effect of organic solvents on Fe^{III}–TAML-catalyzed chemiluminescence. Experimental conditions: 20 mM carbonate, pH 9.9 containing 50 mM Tris, 27 μM luminol, 0.1 mM H_2O_2 and organic solvent; [Fe^{III}–TAML] = 10^{-8} M. The activity of Fe^{III}–TAML measured in the absence of an organic solvent was expressed as 100%.

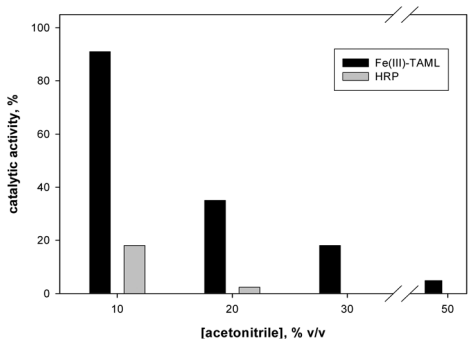

Fig. 3 Effect of acetonitrile concentration on the activity of Fe^{III}–TAML and HRP. Experimental conditions for Fe^{III}–TAML catalysis: 20 mM carbonate, pH 9.9 containing 50 mM Tris, 27 μM luminol, 0.1 mM H_2O_2 and an organic solvent; [Fe^{III}–TAML] = 10^{-8} M. Experimental conditions for HRP catalysis: 60 mM Tris, pH 8.3, containing 1.5 mM luminol, 0.1 mM H_2O_2 and an organic solvent; [HRP] = 1.8×10^{-10} M. The activity of Fe^{III}–TAML measured in the absence of an organic solvent was expressed as 100%.

buffered medium.[20] For the quantification of organic peroxides Fe^{III}–TAML has to retain its catalytic activity in organic or aqueous–organic media. To estimate the ability of Fe^{III}–TAML to catalyze 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 shown in Fig. 2, for all the studied solvents increasing of the solvent concentration in the reaction solution diminished CL intensity 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. Because of this reason dimethylsulfoxide was not used by us for further studies. 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 an alternative to HRP, we compared the inactivation of Fe^{III}–TAML and HRP with acetonitrile. As shown 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 a significantly more stable catalyst. So, in 10%, 20% and 30% (v/v) acetonitrile Fe^{III}–TAML showed 91%, 35% and 19% of its initial activity, respectively. Therefore, unlike HRP, Fe^{III}–TAML may be used as an 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 aqueous solution. However, it is well known that introduction of organic solvents into aqueous solutions may affect the ionization state of soluble molecules, thereby changing the optimum pH of the reaction of interest. Through this, we

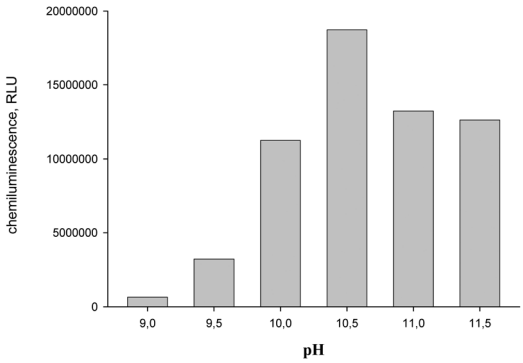

Fig. 4 Dependence of light output formed upon Fe^{III}–TAML-catalyzed luminol oxidation in the presence of isopropanol as a function of pH of the reaction buffer. Experimental conditions: 20 mM carbonate containing 50 mM Tris, 27 μM luminol, 0.1 mM H_2O_2 and 10% (v/v) isopropanol; [Fe^{III}–TAML] = 10^{-8} M.

estimated the optimum pH of Fe^{III}–TAML-catalyzed oxidation of luminol in the presence of isopropanol. The obtained results demonstrated that the addition of isopropanol to the reaction solution leads to shifting of the optimum pH of Fe^{III}–TAML from 9.9 to pH 10.5 (Fig. 4). Identical results were obtained by adding 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 H_2O_2 on an initial rate of luminol oxidation was studied. Based on the mechanism proposed by Chahbane *et al.*[27] (eqn (1) and (2)), the values of the rate constants k_I and k_{II} of $(2.2 \pm 0.3) \times 10^3$ and $(1.1 \pm 0.3) \times 10^5$ M^{-1} s^{-1}, respectively were calculated.[19]

$$Fe^{III} - TAML + H_2O_2 \xrightarrow{k_I} \text{oxidized TAML} \quad (1)$$

$$\text{oxidized TAML} + \text{luminol} \xrightarrow{k_{II}} Fe^{III} - TAML + \text{product} \quad (2)$$

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

A 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 rates were fitted to eqn (3) to calculate the rate constants k_I and k_{II} of $(3.4 \pm 0.5) \times 10^3$ and $(2.0 \pm 0.1) \times 10^5$ M^{-1} s^{-1}, respectively. A comparison of the values of the rate constants calculated under optimized conditions in an 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 lose its catalytic activity in the presence of an organic solvent. The decrease of Fe^{III}–TAML activity in the presence of acetonitrile mentioned above (Fig. 2) was likely due to a change of pH value of the reaction solution due to the addition of an organic solvent. Moreover, the kinetic constants of Fe^{III}–TAML were shown to be slightly improved after the addition of acetonitrile to the reaction solution.

The obtained knowledge about the high catalytic activity of Fe^{III}–TAML in aqueous–organic mixtures towards luminol allowed us to develop a Fe^{III}–TAML-based assay of benzoyl peroxide (BP) which is insoluble in water, but has a good solubility in organic solvents such as acetonitrile, isopropanol and ethanol. The quantification of BP is practically an important task, since this peroxide is widely used in the food industry. By this, there is a need for the 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 the Fe^{III}–TAML-based assay of H_2O_2 in aqueous medium.[20]

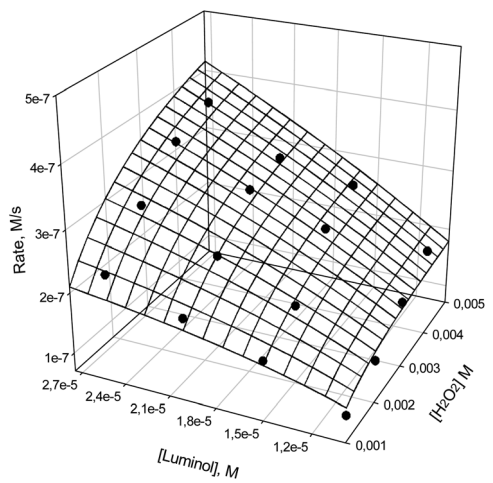

Fig. 5 Effect of concentrations of luminol and hydrogen peroxide on the initial rate of Fe^{III}–TAML-catalyzed oxidation of luminol performed in the presence of acetonitrile. Conditions: 20 mM carbonate buffer, pH 10.5 with 50 mM Tris and 20% (v/v) acetonitrile, [Fe^{III}–TAML] = 10^{-8} M.

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

	Concentration of organic solvents (v/v)			
Organic solvents	10% Detection limit/nM	20%	10% Sensitivity, 10^{10} RLU M^{-1}	20%
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

The analytical parameters of a 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 the detection limit (defined as 3 standard deviation (3σ) of the blank) of BP were similar regardless of which solvent was used, and were equal to 0.7–1.0 × 10^{-7} M. The most sensitive BP assay (the assay sensitivity is 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 by 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 the organic solvent concentration up to 20% did not affect the values of the detection limit of BP in the case of isopropanol and ethanol, but worsened this parameter on using acetonitrile (Table 1). At the same time, in all solutions containing 20% organic solvent the sensitivity of the 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 × 10^{-7}–5 × 10^{-5} M (R^2 = 0.98) (Fig. 6).

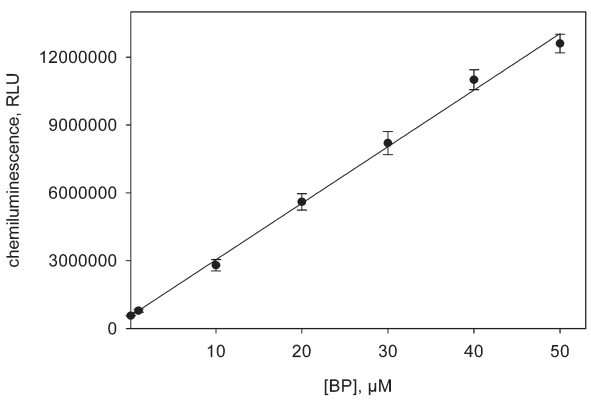

Fig. 6 Calibration curve for the chemiluminescence determination of benzoyl peroxide using the luminol oxidation catalyzed by Fe^{III}–TAML in the presence of ethanol. Conditions: 20 mM carbonate buffer, pH 10.5 with 50 mM Tris and 10% (v/v) ethanol, [luminol] = 27 μM, [Fe^{III}–TAML] = 10^{-8} M.

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

	Concentration of organic solvents (v/v)			
Organic solvents	10% Detection limit/μM	20%	10% Sensitivity, 10^8 RLU M^{-1}	20%
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

Similarly, a 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 the BP assay, the minimum value of the detection limit of TBH was obtained in the carbonate buffer with 10% ethanol. Importantly, increasing the organic solvent concentration in the reaction solution resulted in the increase of the detection limit value. It should also be noted that the detection limit for the BP assay was 40-fold lower than that for TBH. The obtained results are in good agreement with the data reported previously.[27]

In contrast to the detection limit, the highest sensitivity of the Fe^{III}–TAML-based assay of TBH was based on the performance of luminol oxidation in the presence of 10% acetonitrile. Replacement of acetonitrile with ethanol diminished the assay sensitivity by 3 times. Also, the sensitivity worsened on increasing solvent concentration (Table 2). In the case when the TBH assay was performed in the presence of 10% ethanol, the working range was 3×10^{-6}– 5×10^{-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 rate constants k_I 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. By 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.

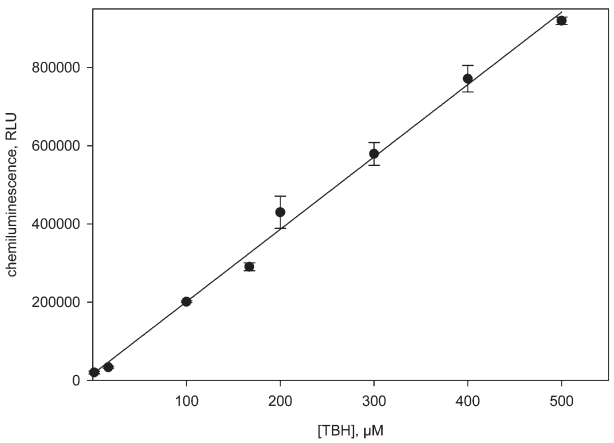

Fig. 7 Calibration curve for the chemiluminescence determination of *tert*-butyl hydroperoxide using the luminol oxidation catalyzed by Fe^{III}–TAML in the presence of ethanol. Conditions: 20 mM carbonate buffer, pH 10.5 with 50 mM Tris and 10% (v/v) ethanol, [luminol] = 27 μM, [Fe^{III}–TAML] = 10^{-8}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

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