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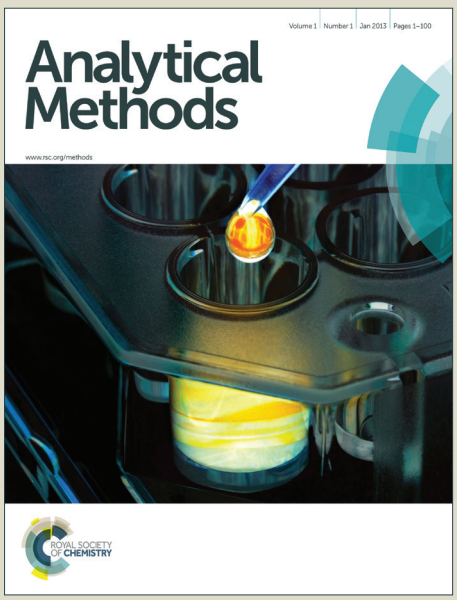

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PAPER

Chemiluminescent assay of phenol in wastewater using HRP-catalysed luminol oxidation with and without enhancers

Marina M. Vdovenko,[a] Vladislav Papper,[b] Robert S. Marks,[b,c] and Ivan Yu. Sakharov*[a]

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Two methods for the phenol determination based on the suppression of chemiluminescence (CL) produced upon HRP-catalysed oxidation of luminol were developed. One method used an enhanced CL reaction with 3-(10'-phenothiazinyl)-propane-1-sulfonate and 4-morpholinopyridine as primary and secondary enhancers, respectively, and another one is free of the enhancers. Comparison of the phenol assay with enhancers showed much higher sensitivity and lower LDL value than the assay without enhancers. In addition, the amount of HRP used in the assay with enhancers is 40 times less than in the assay without them. The study of the specificity of the developed assays demonstrated that in the assay without enhancers several phenol compounds, for instance, unsubstituted phenol, suppressed the CL intensity, whereas other phenols, such as 4-chlorophenol and 2,4-dichlorophenol, increased the light output. Contrary to that assay, in the assay with enhancers all tested phenols suppressed the CL intensity. Upon the analysis of phenols in the water plant effluents using the assay without the enhancers, a high matrix effect was observed. On the other hand, the analysis of the same effluents using the assay with enhancers did not show any matrix effect, and the recovery values from the spiked samples were found to be 92-96%.

Introduction

Phenol and its derivatives are extensively used in the manufacture of a wide variety of chemical products, such as polymers, fertilizers, adhesives, paints, pesticides and explosives.[1,2] They are formed in many industrial processes (petroleum refining, paper and soap manufacturing, and dye tanning). Phenols are highly toxic and difficult to degrade biologically.[3] Eleven common phenols belong to the US Environmental Protection Agency priority pollutants list [4], which need to be closely monitored to ensure acceptable environmental levels of phenols. This has led to an extensive development of highly sensitive assays for determination of phenols in wastewater, with the present work contributing to the area with a new methodology.

Gas chromatography (GC) is a commonly used technique for detection of phenols, however, its drawback is the presence of hardly volatile compounds in water samples that can clog and destroy the chromatographic columns. Other disadvantages include the requirement for sample pre-concentration and matrix elimination in order to detect the relatively low analyte concentrations. For this, a solid-phase extraction is highly effective, but time consuming and expensive.[5]

As an alternative to the aforementioned technique, a chemiluminescence-based interference assay is proposed. The chemiluminescence (CL) technique may be a good alternative to chromatography, as it is highly sensitive, rapid, easy to perform, enabling high throughput multiple-sample analysis with automation. One of the most efficient CL systems is a luminol–hydrogen peroxide mixture.[6-8] Oxidation of luminol with hydrogen peroxide produces 3-aminophthalate ion in an excited state (3-aminophthalate*), which upon relaxation to the ground state. This reaction proceeds effectively under alkaline conditions. However, at mild alkaline conditions (pH 8-9) the reaction rate markedly drops and, hence, a catalyst should be used. The usage of some native peroxidases and their mimetics, metal ions and nanoparticles as catalysts for luminol oxidation has been reported. [8,10-13] The most active catalyst is a horseradish peroxidase (HRP, EC 1.1.11.7). Consequently, the reaction of luminol and hydrogen peroxide catalysed by HRP is widely used in development of various CL analytical methods.[14-17]

Although HRP effectively catalyses the reaction of luminol and hydrogen peroxide, a use of certain compounds, named "enhancers", can additionally increase the CL intensity. The enhanced chemiluminescence reaction (ECR) mechanism has been already described elsewhere[9, 17] In the first step of ECR,

[a] *Department of Chemistry, Lomonosov Moscow State University, Moscow, 119991, Russia. Fax: +7 495 9395417; Tel: +7 495 9393407; E-mail: sakharovivan@gmail.com*
[b] *School of Materials Science and Engineering, Nanyang Technological University, Singapore.*
[c] *Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

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HRP is oxidized with hydrogen peroxide with a formation of Compound I, which, in turn, reacts with the primary enhancer, which is a better substrate for HRP than luminol. The reaction is catalysed by HRP, according to the "ping-pong" mechanism, as shown in the following equations:

$$E + H_2O_2 \longrightarrow EI + H_2O \quad (1)$$

$$EI + SH \longrightarrow EII + S^{\bullet} \quad (2)$$

$$EII + SH \longrightarrow E + S^{\bullet} + H_2O \quad (3)$$

where SH is a primary enhancer, $S^{\bullet}$ is a radical product of one-electron oxidation of the enhancer, E is a resting form of the ferric enzyme, EI and EII – Compound I and Compound II, the peroxidase oxidized intermediates, which are by two and one oxidation equivalents above the ferric enzyme, respectively.

Then the formed radical product ($S^{\bullet}$), through its oxidative potential, reacts with luminol (see reaction 4). The aforementioned chemical process is complex and not clear so far. However, it is well known that the final product of this reaction is 3-aminophthalate:

$$S^{\bullet} + \text{Luminol} \rightleftharpoons SH + \text{3-Aminophthalate} + N_2 + \mathbf{h\nu} \quad (4)$$

Thus, the primary enhancers play a role of mediators in the peroxidase catalysis[18], and while not affecting the final product, they increase the CL intensity due to their high reactivity towards Compound I and Compound II, compared to luminol alone.

It has been recently demonstrated that 3-(10'-phenothiazinyl)-propane-1-sulfonate (SPTZ) and 3-(10'-phenothiazinyl)propionic acid (primary enhancers) in combination with 4-morpholinopyridine (MORPH, secondary enhancer) are the most efficient enhancers for plant peroxidases.[19-22] The mechanism of action of MORPH as a secondary enhancer has been recently reported.[23] Conditions of the HRP-catalysed luminol oxidation with these enhancers were optimized by a full factorial design.[21, 22]

Indeed, we describe herein the development of a highly sensitive, robust and cheap CL method for the determination of phenols. The method is based on the suppression of HRP-catalysed CL. The assay is performed in two formats for performance comparison: (1) with, and (2) without the addition of SPTZ/MORPH enhancers to the reaction mixture.

Materials and methods

Materials

Horseradish peroxidase (HRP, type VI-A, RZ 3.0), phenol, luminol and Tris were commercially available from Sigma (USA). Phenol derivatives (4-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,3,6-trichlorophenol, pentachlorophenol, 2-nitrophenol and 4-nitrophenol) were a gift of Dr. A. A. Farmanovsky (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russia). Sodium 3-(10'-phenothiazinyl)propane-1-sulfonate (SPTZ) was prepared as described previously.[19] 4-Morpholinopyridine (MORPH) was purchased from Aldrich (USA). H_2O_2 (30%) was from BDH PROLABO (France). H_2O_2 concentration was determined by monitoring A_{240}, using $\varepsilon = 43.6\ M^{-1}cm^{-1}$.[24] The required dilutions of H_2O_2 were prepared daily. Black polystyrene plates were obtained from Nunc (Denmark).

Wastewater samples

Tap water samples were taken from the urban water network in Singapore. The samples of primary and secondary effluents were collected on Changi Water Reclamation Plant (CH2M HILL), which is the integral part of the Singapore Deep Tunnel Sewerage System (DTSS), and used to estimate the phenol concentration. Prior to the analysis, the effluents were centrifuged at 13,000 rpm for 5 min at room temperature and used without further purification.

Optimization of conditions for the HRP-catalyzed oxidation of luminol without enhancers

Full (2^4) factorial design was used to optimize the experimental conditions for the HRP-catalysed oxidation of luminol by hydrogen peroxide, such as concentration of luminol, hydrogen peroxide and Tris, and pH of the tested solution. The corresponding data is presented in Table 1. CL intensity was measured at room temperature on a Synergy H1 Hybrid Reader (BioTek, USA), with the CL intensity expressed in relative CL units (RLU). The reaction efficiency was evaluated as a ratio of the HRP-catalyzed CL to background. The obtained results were analyzed using Statgraphics© XV.I statistical analysis and data visualization software.

Inhibition of the HRP-catalyzed oxidation of luminol without enhancers

Inhibition of the HRP-catalyzed oxidation of luminol by phenols without enhancers was carried out under the conditions optimized by the full (2^4) factorial design (see above) as follows. A solution of 0.125 mM hydrogen peroxide and 3.75 mM luminol in 100 µL of 150 mM Tris-HCl (pH 8.3) was added to the black polystyrene plate wells, followed by the addition of 100 µL of phenol aqueous solution. A concentration of phenol in said aqueous solution was varied in the range of 8.0×10^{-7} to 2.5×10^{-3} M. The enzymatic oxidation of luminol was initiated by the introduction of a 50 µL solution of 9×10^{-10} M HRP in water. The CL intensity was measured at room temperature.

Table 1 The variable values used in the optimization of experimental conditions of luminol–H_2O_2 reaction catalyzed by HRP

Variables	Levels		
	Low (-1)	Centered (0)	High (+1)
X_1 - [luminol] , mM	0.05	1.53	3
X_2 - [H_2O_2] , mM	0.05	1.53	3
X_3 – [Tris], mM	20	60	100
X_4 - pH	7.8	8.3	8.8

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Inhibition of the HRP-catalyzed oxidation of luminol in the presence of enhancers

Inhibition of the HRP-catalysed oxidation of luminol by phenols in the presence of enhancers was carried out under the conditions previously optimized using the full (2^5) factorial design[21] as follows. A solution of 4.375 mM hydrogen peroxide, 0.425 mM luminol, 5.25 mM SPTZ and 21.88 mM MORPH in 100 µL of 200 mM Tris-HCl (pH 8.3) was added to the black polystyrene plate wells, followed by the addition of 100 µL of phenol aqueous solution. Concentrations of phenol in said aqueous solutions were varied in the range of 8.0×10^{-7} to 2.5×10^{-3} M. The enzymatic oxidation of luminol was initiated by the introduction of 50 µL solution of 2.25×10^{-11} M HRP in water. The CL intensity was measured at room temperature.

Data Analysis

Calibration curves were obtained by plotting the light intensity against the logarithm of phenol concentration and fitted to a four-parameter logistic equation 6 using the OriginLab© Origin 7.5 data analysis and graphing software:

$$Y = (A-D) \div (1+(x/C)^B) + D, \quad \textbf{(5)}$$

where A is the asymptotic maximum (CL intensity in the absence of phenol), B is the curve slope at the inflection point, C is the x value at the inflection point, and D is the asymptotic minimum (background signal). The calibration curves of both developed assays had R^2 of 0,99.

Results and Discussion

Optimization of conditions for the peroxidase-catalyzed oxidation of luminol

We used the full 2^4 factorial design in order to optimize the experimental conditions for the HRP-catalysed oxidation of luminol without enhancers. Previously this method was successfully applied to optimize the HRP-catalysed oxidation of luminol in the presence of SPTZ/MORPH enhancers.[21] In the present work, concentrations of luminol, hydrogen peroxide and Tris and pH value of the reaction medium were selected as independent variables. The corresponding data are presented in Table 1. The reaction efficiency was estimated as a ratio of the CL intensity to the background signal.

The obtained results presented in Table 2 were analyzed using Statgraphics© XV.I statistical analysis and data visualization software. This software allows the determination of the ratio of the CL intensity to the background signal (Y) as a function of the luminol (X_1), hydrogen peroxide (X_2) and Tris (X_3) concentrations, and pH (X_4).

$$Y = 10.5 + 2.4X_1 - 11.9X_2 + 3.5X_3 - 4.9X_4 - 10.9X_1^2 - 1.6X_1X_2 - 0.2X_1X_3 - 2.3X_1X_4 + 27.4X_2^2 - 3.7X_2X_3 + 4.6X_2X_4 - 7.1X_3^2 - 3.0X_3X_4 - 9.3X_4^2 \quad \textbf{(6)}$$

The ANOVA (analysis of variance) showed that a significance level is more or equal to 95% (with a confidence interval p-value ≤ 0.05). This model (although with a lack-of-fit) represents the data reasonably well, including significant contribution of linear [H_2O_2] and square ([H_2O_2] × [H_2O_2]) concentration effects. This fact is also confirmed by a reasonable coefficient of the determination (R^2 = 73%).

Equation 6 allowed a calculation of a combination of concentrations of the reacting substances to obtain the maximum value of S/N ratio. The calculated conditions were the following: 60 mM Tris, pH 8.3, containing 1.5 mM luminol, and 0.05 mM H_2O_2. Under these optimized conditions, the background signal was observed to be very low (2-4 RLU).

Chemiluminescence assay for the determination of phenol

The dependence of the light output on the phenol concentration in an HRP-catalysed oxidation of luminol without enhancers was determined under the optimized conditions. As seen in Fig. 1, the

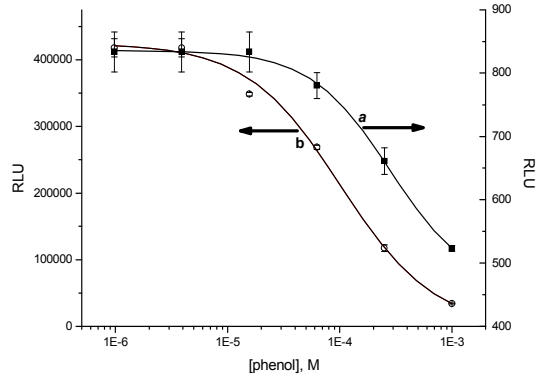

Fig. 1 Calibration curves for phenol determination using the HRP-based luminol oxidation method (a) without and (b) with SPTZ and MORPH. Experimental conditions: a) 60 mM Tris, pH 8.3, containing 1.5 mM luminol, and 0.05 mM H_2O_2, [HRP] = 180 pM; b) 80 mM Tris, pH 8.3, containing 0.17 mM luminol, 2.1 mM SPTZ, 8.75 mM MORP and 1.75 mM H_2O_2, [HRP] = 4.5 pM. Chemiluminescence intensity was recorded 5.0 min after the start of the reaction. Each point represents the mean of the duplicates. Vertical bars indicate ±S.D about the mean.

increase of the phenol concentration results in the decrease of the light output intensity.

This is likely because both phenol and luminol are substrates of HRP, and they compete with each other for Compounds I and Compound II, the active intermediates of peroxidase. Moreover, phenol can react with the luminol radicals produced upon the HRP-catalyzed oxidation of luminol that also leads to CL quenching. Analysis of the resulting calibration curve (Fig. 1, curve a) shows that the lower detection limit (LDL) (3σ) and working (linear) range of the CL assay were 1.2×10^{-4} M and 3.2×10^{-4} to 7.8×10^{-3} M, respectively. The slope value in the linear range reflecting the assay sensitivity is found to be 199 a.u. (arbitrary units). The coefficient of variation (CV) for the phenol concentration within the working range of the assay varies from 0.8 to 3.2% for intra-assay and was not more than 2.8% for inter-assay.

In order to further develop the phenol assay, we conducted the HRP-catalysed oxidation of luminol in the presence of enhancers, such as SPTZ and MORPH. The optimized conditions for this reaction have been recently reported.[21] The HRP-catalysed oxidation of luminol in the presence of enhancers

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Table 2 Full factorial design used in the optimization of experimental conditions of luminol–H_2O_2 reaction catalyzed by HRP.

№	Variable Level				Ratio of HRP-induced CL to background
	X_1	X_2	X_3	X_4	
Main Block					
1	-1	-1	-1	-1	0.5
2	-1	-1	-1	+1	30.3
3	-1	-1	+1	-1	0.8
4	-1	+1	-1	-1	4.2
5	+1	-1	-1	-1	41.0
6	+1	+1	-1	-1	43.3
7	+1	-1	+1	-1	0.7
8	+1	-1	-1	+1	3.7
9	-1	+1	+1	-1	13.8
10	-1	+1	-1	+1	1.3
11	-1	+1	-1	+1	0.6
12	-1	-1	+1	+1	1.3
13	+1	+1	+1	-1	3.8
14	+1	+1	-1	+1	16.9
15	+1	-1	+1	+1	0.8
16	+1	+1	+1	+1	1.1
Additional Block					
17	-1	0	0	0	0.5
18	+1	0	0	0	3.4
19	0	-1	0	0	78.7
20	0	+1	0	0	1.9
21	0	0	-1	0	3.3
22	0	0	+1	0	8.3
23	0	0	0	-1	5.4
24	0	0	0	+1	1.8
25	0	0	0	0	3.3

shows a sharp increase of the CL intensity (more than 500 times) compared to the same reaction without enhancers. Interestingly, the enzyme concentration in the ECR was 40-fold lower than that in the reaction without enhancers.

As in the case of the reaction without enhancers, the introduction of phenol into the reaction mixture resulted in decrease of the CL intensity. The obtained calibration curve is presented in Fig. 1b. In case of the ECR, the LDL value and working range of the assay were calculated to be 1.1×10^{-5} M and 2.9×10^{-5} to 3.9×10^{-4} M, respectively. The obtained parameters were 1000-fold worse than those reported for phenol determination with HPLC.[25] However, the samples analyzed with HPLC are concentrated in 1000 times using adsorbents for solid-phase microextraction. The same concentration of the samples may be also applied in the CL assay. Therefore, the sensitivity of the CL assay with enhancers and HPLC are similar.

The coefficient of variation (CV) for phenol concentrations in the case of ECR within the working range of the assay was 0.8 - 4.3% for intra- and inter-assays. Moreover, in the presence of enhancers, the slope value in the linear range was found to be 252100 a.u., which is 1265-fold improvement over the sensitivity of the CL assay without enhancers.

Comparison of the two formats for the proposed phenol assay (with and without enhancers) demonstrates that the use of the enhancers, such as SPTZ and MORPH, in the HRP-catalysed oxidation of luminol significantly improves the analytical characteristics of the assay. Thus, the increased CL intensity in the presence of SPTZ and MORPH provides higher sensitivity of the assay.

Suppression of chemiluminescence with phenol derivatives

It is well known that plant peroxidases show a relatively sharp substrate specificity catalysing preferably the oxidation of phenols and anilines.[26, 27] Anilines are oxidized by peroxidases under acidic conditions[28, 29], whereas an optimal pH value for the oxidation of phenols lies under the mild alkaline conditions[30, 31], i.e. under the conditions, when HRP effectively oxidizes luminol, as described above. So far, we have used a number of phenol derivatives for the study of their effect on CL in both formats (with and without enhancers).

The results obtained from the CL measurements during the HRP-catalysed oxidation of luminol in the presence of the phenol compounds are summarized in Table 3. In case of the luminol oxidation without enhancers, several phenol compounds, such as for instance, unsubstituted phenol, suppress the CL intensity. However, other phenols, such as 4-chlorophenol and 2,4-dichlorophenol, increase the light output, and hence, can be considered CL enhancers themselves in an oxidation reaction without the aforementioned enhancers.

In contrast, in ECR all phenols seem to inhibit the CL intensity (Table 3). The IC_{50} value (defined as a 50% suppression of the maximal CL signal) for a large number of the tested phenol compounds, including unsubstituted phenol, are in the range of 1.0×10^{-4} to 7.0×10^{-4} M. Only 4-chlorophenol, 2,4,5-trichlorophenol and pentachlorophenol are found to be poor inhibitors.

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Table 3 Inhibitory effect of phenolic compounds in the assays with and without enhancers.

Phenol compounds	IC_{50}, M	
	The assay without enhancers	The assay with enhancers
Phenol	2.0×10^{-3}	1.0×10^{-4} M
4-Chlorophenol	Activation*	$> 1.0 \times 10^{-3}$
2,4-Dichlorophenol	Activation*	7.0×10^{-4}
2,4,5-Trichlorophenol	$> 1.0 \times 10^{-3}$	$> 1.0 \times 10^{-3}$
2,3,6-Trichlorophenol	1.8×10^{-4}	2.3×10^{-4}
Pentachlorophenol	$> 1.0 \times 10^{-3}$	$> 1.0 \times 10^{-3}$
2- Nitrophenol	5.0×10^{-4}	5.3×10^{-4}
4- Nitrophenol	3.0×10^{-4}	2.7×10^{-4}
3- Bromophenol	4.2×10^{-4}	4.0×10^{-5}
4-Bromophenol	Activation*	3.5×10^{-4}
4-Iodophenol	Activation*	3.5×10^{-4}

*These phenolic compounds are activators, but not inhibitors.

Wastewater samples analysis

We have estimated the phenol concentration in wastewater samples using both formats of the above described phenol assay (with and without enhancers). Initially, we analysed the tap water samples, collected from the urban water network in Singapore, for phenols, but found none. Using local tap water, we prepared spiked samples with phenol concentrations of 0.06–1 mM. In both assay formats (with and without enhancers), the values of recovery and CV were found to be in the range of 95–98% and 0.2–1.0%, respectively.

The samples of the primary and secondary effluents were collected at the Changi Water Reclamation Plant, which is the integral part of the Singapore Deep Tunnel Sewerage System (DTSS), and analysed. The collected samples show different results obtained in the assay format with and without enhancers. In the developed phenol assay with the enhancers, the CL intensity was not affected by addition of the effluent samples into the reaction, which means that the tested water samples do not contain phenols. In contrast, in the assay without enhancers, we observed the CL quenching, which, in turn, depends on the sample dilution (data not shown).

To explain the above controversy, the same samples were analysed using GC-MS. Since no traces of phenols are found in the effluent samples analysed by GC-MS, we can conclude that the results obtained from the developed phenol assay with enhancers are correct. On the other hand, the same assay performed without enhancers is likely to suffer from the matrix effect of the effluents, which contain high concentrations of different compounds including polymeric and cyclic siloxanes used in water repelling windshield coatings, lubricants, food additives and some soaps, as well as phthalates used in a large variety of products, from enteric coatings of pharmaceutical pills and nutritional supplements to viscosity control agents, stabilizers, dispersants, lubricants, emulsifying agents, adhesives, etc.

Using the primary and secondary effluent samples, the spiked samples were prepared with the phenol concentration of 0.042, 0.11 and 0.25 mM. In the phenol assay with enhancers, the values of recovery and CV obtained from the spiked samples of the primary effluent were in the range of 94–96% and 0.25–2.3%, respectively. The values of recovery and CV obtained from the spiked samples of the secondary effluent were in the range of 92–94% and 2.1–5.8%, respectively.

Conclusions

We have developed two formats for the phenol assay based on an inhibitory effect of the phenols on chemiluminescence produced by the enzymatic oxidation of luminol. Both assay formats are based on the HRP-catalysed luminol oxidation with hydrogen peroxide. One of them uses enhancers, such as SPTZ and MORPH, and another one is free of them. When compared, the phenol assay with enhancers shows much higher sensitivity and lower LDL value than the assay without enhancers. In addition, an amount of HRP used in the assay with enhancers is 40 times less than in the assay without them. This fact has significant impact on the assay commercial value.

We have also studied the specificity of the developed assay, and found that in the assay without enhancers several phenol compounds, for instance, unsubstituted phenol, suppressed the CL intensity, whereas other phenols, such as 4-chlorophenol and 2,4-dichlorophenol, increased the light output. Contrary to that assay, in the assay with enhancers, all tested phenols inhibited the CL intensity.

Both formats of the proposed assay were applied to estimate the phenol concentration in tap water and in the effluents from the water plant. The results clearly show that both modifications of the developed assay can be used to determine the phenol concentration in tap water with high accuracy and sensitivity. However, upon the analysis of phenols in the water plant effluents using the assay format without enhancers, a high matrix effect was observed. It made impossible to determine the analyte in the spiked samples. On the other hand, the assay of the same water plant effluents in the presence of the enhancers did not show any matrix effect, and the recovery values from the spikes samples are found to be 92–96%.

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References

1. J.A. Jonsson, T. Berhanu, in: *Handbook of Water Analysis*. (Ed.: L.M.L. Nollet), CRC Press, Boca Raton, 2007, pp. 409-435.
2. J.W. Moore, S. Ramamoorthy, in: *Organic Chemicals in Natural Waters*, Springer, New York, 1984, 289 pp.
3. J. Michałowicz, W. Duda, *Polish. J. Environ. Stud*., 2007, **16**, 347-362.
4. US Environmental Protection Agency, http://water.epa.gov/scitech/methods/cwa/pollutants. cfm
5. L. Lang, X. Qun, C. Jing, J. Rohrer, *LC-GC Europe*, 2007, **20**, 10-11.
6. P. Yang, Y. Li, P. Wang, L. Wang, *Luminescence***,** 2006, **22**, 46-52.
7. O. Nozaki, H. Kawamoto, *Luminescence*, **2**000, **15**, 137-142.
8. S. Xu, W. Liu, B. Hu, W. Cao, Z. Liu, *J. Photochem. Photobiol. A: Chemistry,* 2012, **227**, 32-37.
9. C.A. Marquette, L.J. Blum, *Bioanalysis*, 2009, **1**, 1259-1269.
10. I.S. Alpeeva, I.Yu. Sakharov, *J. Agric. Food Chem*., 2005, **53**, 5784-5788.
11. S.-L. Xu, H. Cui, *Luminescence*, 2007, **22**, 77-87.
12. T. Takayanagi, Y. Inaba, H. Kanzaki, Y. Jyoichi, S. Motomizu, *Talanta*, 2009, **79**, 1089-1093.
13. M.M. Vdovenko, A.S. Demiyanova, K.E. Kopylov, I.Yu. Sakharov, *Talanta***,** 2014, **125**, 361–365.
14. F. García Sánchez, A. Navas Díaz, C. Delgado Téllez, M. Algarra, *Talanta*, 2008, **77**, 294-297.
15. A.R.T.S. Araujo, F. Maya, M.L.M.F.S. Saraiva, J.L.F.C. Lima, J.M. Estela, V. Cerdà, *Luminescence*, 2011, **26**, 571-578.
16. L. Zhao, L. Sun, X. Chu, *Trends Anal. Chem*., 2009, **28**, 404-414.
17. A. Roda, M. Guardigli, *Anal. Bioanal. Chem*., 2012, **402**, 69-76.
18. I.S. Alpeeva, V.S. Soukharev, L. Alexandrova, N.V. Shilova, N.V. Bovin, E. Csöregi, A.D. Ryabov, I.Yu. Sakharov, *J. Biol. Inorg. Chem*., 2003, **8**, 683-688.
19. E. Marzocchi, S. Grilli, L. Della Ciana, L. Prodi, M. Mirasoli, A. Roda, *Anal. Biochem*., 2008, **377**, 189-194.
20. M.M. Vdovenko, L. Della Ciana, I.Yu. Sakharov, *Anal. Biochem*., 2009, **392**, 54-58.
21. M.M. Vdovenko, A.S. Demiyanova, T.A. Chemleva, I.Yu. Sakharov, *Talanta,* 2012, **94**, 223-226.
22. I.Yu. Sakharov, A.S. Demiyanova, A.V. Gribas, N.A. Uskova, E.E. Efremov, M.M. Vdovenko, *Talanta,* 2013, **115**, 414-417.
23. I.Yu. Sakharov, M. M. Vdovenko, *Anal. Biochem.,* 2013, **434**, 12-14.
24. R.J. Kulmacz, *Arch. Biochem. Biophys*., 1986, **249**, 273-285.
25. R.-S. Zhao, X. Wang, J.-P. Yuan, X.-D. Wang, *J. Sep. Sci*., 2009, **32**, 630 – 636.
26. L.K. Folkes, L.P. Candeias, *FEBS letters*, 1997, **412**, 305-308.
27. H.B. Dunford, in: *Peroxidase in Chemistry and Biology*. (Eds.: J. Everse, K.E. Everse, M.B. Grisham), CRC Press, Boca Raton, FL, 1991, pp.1–24.
28. W. Lui, J. Kumar, S. Tripathy, K.J. Senecal, L. Samuelson, *J. Amer. Chem. Soc*., 1999, **121**, 71-78.
29. A.V. Caramyshev, V.M. Lobachov, D.V. Selivanov, E.V. Sheval, A.Kh. Vorobiev, O.N. Katasova, V.Yu. Polyakov, A.A. Makarov, I.Yu. Sakharov, *Biomacromolecules*, 2007, **8**, 2549-2555.
30. L. Zhang, W.Z. Ma, G. Nie, Y. Cui, *Eur. Polymer J*., 2012, **48**, 580-585.
31. K.J. Baynton, J.K. Bewtra, N. Biswas, K.E. Taylor, *Biochim. Biophys. Acta*, 1994, **1206**, 272-278.

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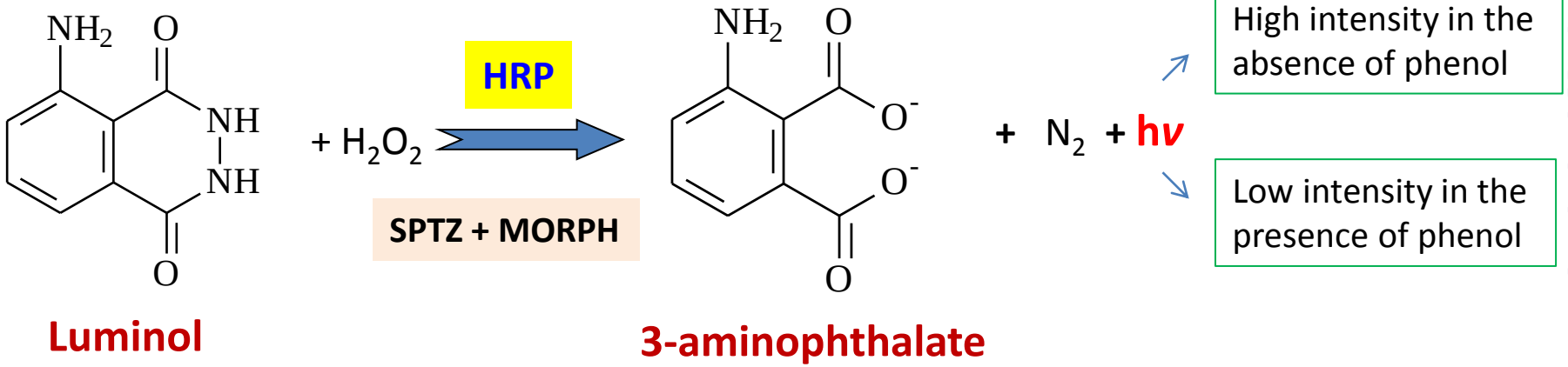

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