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ScienceDirect
Mendeleev Commun., 2011, **21**, 97–98
Mendeleev Communications

Hydrophilic ionic liquids as reaction media for the determination of guaiacol using horseradish and soybean peroxidases

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DOI: 10.1016/j.mencom.2011.03.013

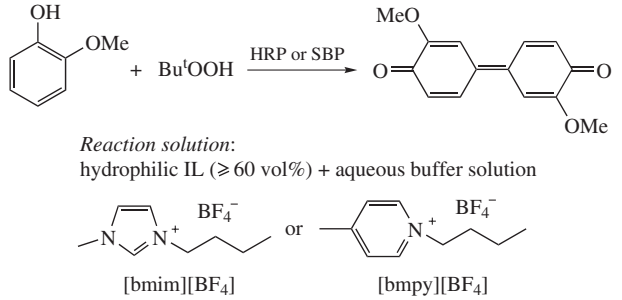
The advantages of hydrophilic ionic liquids – 1-butyl-2-methylimidazolium and *N*-butyl-3-methylpyridinium tetrafluoroborates – over conventional polar solvents (DMSO and acetonitrile) for the enzymatic determination of 0.05–3 mM guaiacol in the reaction media containing 20–40 vol% H_2O are shown as the result of a comparative study of the kinetics of guaiacol oxidation by Bu^{t}OOH catalyzed by native horseradish and soybean peroxidases.

Ionic liquids (ILs) are solvents widely used in chemical analysis.[1] In biotechnology, hydrophobic ILs are mainly applied for carrying out organic synthesis employing lipases.[2] The kinetic studies of reactions catalyzed by native hem-containing proteins (hemoglobin and myoglobin) and oxidases (peroxidase, laccase and tyrosinase) in hydrophilic ILs have been reported.[2,3] Note that a significant drawback of molecular polar organic solvents is their denaturing effect on enzymes,[4] especially native peroxidases,[5] which are commonly used in chemical analysis. This fact limits the applicability of biochemical methods to the analysis of aqueous-organic solutions, organic extracts and samples with low water concentrations.

Guaiacol is a classic peroxidase substrate, which is poorly soluble in water but readily soluble in polar organic solvents. The development of rapid and simple procedures for the determination of guaiacol in various samples is an important and promising challenge.

The aim of this work was to study the analytical possibilities of hydrophilic ILs {1-butyl-2-methylimidazolium ([bmim]) and *N*-butyl-3-methylpyridinium [bmpy] tetrafluoroborates}, and polar organic solvents (DMSO, acetonitrile) as reaction media for the determination of a model phenolic substrate (guaiacol) using its oxidation with Bu^{t}OOH catalyzed by horseradish (HRP) and soybean (SBP) peroxidases (Scheme 1), and to develop enzymatic procedures for the determination of guaiacol in organic solutions. It was reasonable to compare the efficiency of guaiacol transformation in ILs and polar organic solvents under the influence of commercial cationic HRP and anionic SBP (EC 1.11.1.7). Acetonitrile and DMSO are often applied as solvents for phenolic compounds[6] and diluents for their extraction from different samples.[7]

To solve the above problems, we studied the dependence of the rate of the indicator reaction of guaiacol oxidation catalyzed by peroxidases on the volumetric concentration of an organic solvent in the mixture. The reaction rate was controlled by spectrophotometry (see Online Supplementary Materials). The optimal order of the introduction of the components into the indicator system was IL – Bu^{t}OOH – buffer solution – guaiacol – enzyme because the dynamic viscosity of IL is higher than that of DMSO or acetonitrile.



Scheme 1

To oxidize guaiacol in the presence of ⩾25 vol% IL, organic buffer solutions (Table 1) should be added to the indicator reaction. The components of a phosphate buffer solution, which provides the high catalytic activity of peroxidases in aqueous solutions and mixtures with DMSO (acetonitrile), get salted out in IL. The same situation was observed in the reaction catalyzed by HRP in a phosphate buffer solution in the presence of hydrophilic ILs of a different nature.[8] The proper choice of an optimal buffer solution, which is a co-solvent of IL, allowed us to study the kinetics of guaiacol oxidation catalyzed by peroxidase in the presence of ⩾60 vol% of hydrophilic IL.

The indicator reaction catalyzed by HRP did not proceed in the presence of 60 vol% [bmpy][BF_4] in all of the buffer systems, while the relative activity of SBP was high and decreased in the following order of buffer solutions: imidazole–HCl ⩾ collidine–HCl > Tris–HCl (Table 1). In imidazole–HCl and collidine–HCl buffer mixtures, the values of SBP relative activity were comparable; however, the reproducibility of the results in the collidine–HCl buffer was better ($s_r \leqslant 0.11$, $n = 3$) as compared to the imidazole–HCl buffer ($s_r \leqslant 0.26$; $n = 3$). In the solution of [bmim][BF_4] – imidazole–HCl (80:20 vol%), the relative catalytic activity of SBP was 2.5 times higher than that in [bmim][BF_4] –

Table 1 Relative catalytic activity (a/a_0)[a] of HRP and SBP in the guaiacol oxidation with Bu^{t}OOH depending on the nature of a buffer solution – co-solvent of IL (concentrations: SBP, 8.0 nmol dm^{-3}; HRP, 9.0 nmol dm^{-3}; Bu^{t}OOH, 120 mmol dm^{-3}; guaiacol, 1 mmol dm^{-3}; 0.05 M buffer solutions, pH 7.0).

System (vol%)	Enzyme	a/a_0 (%)		
		Imidazole–HCl	2,4,6-Collidine–HCl	Tris–HCl
[bmpy][BF_4]–buffer solution (60:40)	HRP	—	—	—
	SBP	39	34	12
[bmim][BF_4]–buffer solution (80:20)	HRP	6	8	3
	SBP	23	9	—

[a] a/a_0 is the ratio between peroxidase catalytic activities in the presence and absence of organic solvent, respectively.

2,4,6-collidine–HCl and higher than the relative catalytic activity of HRP in the collidine–HCl buffer by a factor of 4. In the indicator reaction in the presence of [bmpy][BF_4] and [bmim][BF_4], the components of optimal buffer solutions (collidine–HCl and imidazole–HCl, respectively) contained the structural elements of IL. In the absence of IL, the catalytic activity of the plant peroxidases increased in the following order of buffer solutions: Tris–HCl < collidine–HCl < imidazole–HCl according to the decrease of pK_a[9] of the major components of these buffers [Tris (8.08) > 2,4,6-collidine (7.43) > imidazole (6.95)].

Regardless of IL, SBP was found to have the greatest catalytic activity (Table 1) and substrate specificity towards guaiacol characterized by the effective rate constant k_{eff}, which was calculated according to the 'ping-pong' mechanism.[10] The values of k_{eff} in the presence of 80 vol% [bmim][BF_4] and 60 vol% [bmpy][BF_4] were 2.6×10^3 and 2.2×10^3 dm^3 mol^{-1} s^{-1}, respectively. The efficiency of guaiacol transformation in aqueous DMSO and acetonitrile (their concentrations were 20 and 25 vol%, respectively), was higher in the case of SBP by factors of 2 and 1.5, respectively, as compared to HRP. In our opinion, the high catalytic activity of SBP at pH 2–11 (against HRP, which is active at pH 4–8)[11] along with its high conformational flexibility and thermal stability in aqueous solutions[12,13] are responsible for more efficient catalysis with SBP in the presence of IL along with DMSO and acetonitrile, as compared to HRP.

In the presence of ⩾30 vol% DMSO and acetonitrile in the indicator system, the enzymatic reaction did not proceed. At the same time, in the presence of IL and optimum buffer solutions, the relative catalytic activity of SBP remained at a level of about 20–30%. Thus, the use of hydrophilic IL instead of DMSO and acetonitrile provided efficient peroxidase catalysis in the presence of the polar solvents. The polarity was characterized by the logarithm of octanol–water partition coefficient log P {log P increased in the following order: acetonitrile (–0.33)[4] < DMSO (–1.30)[4] < [bmim][BF_4] (–2.44)[14] < [bmpy][BF_4] (–2.64)[15]}. Note that the thermodynamic parameters (Johns–Doul B-coefficients of viscosity and the structural volumes) characterizing the ability of cosmotropic cations [bmim]$^+$ and [bmpy]$^+$ towards hydration did not differ essentially from each other.[16]

Under the optimal conditions (concentrations of IL, organic solvents and buffer solutions), the rate of the indicator reaction (2–3 units of tg$\alpha\times10^2$) could be precisely determined spectrophotometrically; the residual catalytic activity of peroxidases was at least 10% of its value in water. The optimal concentrations of the enzymes, Bu^{t}OOH, and guaiacol (Table 2) were ascertained as a result of the consecutive study of the concentration dependence of the reaction rates.

Procedures were developed using SBP for the determination of guaiacol in concentration ranges of 0.05–1 and 0.1–3 mmol dm^{-3} in the presence of [bmpy][BF_4] and [bmim][BF_4], respectively [the calibration equations: $y = (22\pm10)x + (23\pm14)\times10^{-5}$ and $y = (8\pm1)x + (26\pm14)\times10^{-5}$, where y is tgα, absorbance units per minute, x is the guaiacol concentration, mmol dm^{-3}; $s_r \leqslant 0.06$, $n = 5$, $P = 0.95$]. It is more preferable to use [bmim][BF_4] due to wider applicable concentration range of guaiacol in it than that in [bmpy][BF_4], though the concentration of [bmim][BF_4] in the reaction was 20 vol% higher. As the result of the insignificant absorption of water by the dried IL, the rate of the indicator reaction in the presence of 80 vol% dried [bmim][BF_4] in the first 3 h was no more than 1.5 times higher than the rate of the reaction carried out in 80 vol% non-dried initial IL, but the determination limit of guaiacol did not change. Thus, it was sufficient to dry IL once a day before carrying out the experiments.

The developed procedure for guaiacol determination using SBP in the presence of 80 vol% [bmim][BF_4] was tested in the analysis of the water-insoluble dental preparation Guaiaphen no. 3 (Omega, Russia) which contained guaiacol, phenol, formaldehyde and dexamethasone. The guaiacol concentration in 100 g of the sample found by the addition method was (31±2) g (certified value, 30 g; $n = 5$, $P = 0.95$).

Table 2 Optimal conditions for the guaiacol oxidation with Bu^{t}OOH catalyzed by HRP and SBP in water–organic media.

System (vol%)	Concentration		
	Enzyme/ nmol dm^{-3}	Bu^{t}OOH/ mmol dm^{-3}	Guaiacol/ mmol dm^{-3}
[bmpy][BF_4]–0.05 M 2,4,6-collidine–HCl, pH 7.0 (60:40)	60 (SBP)	145	1
[bmim][BF_4]–0.05 M imidazole–HCl, pH 7.0 (80:20)	45 (SBP)	170	3
DMSO–0.1 M phosphate buffer, pH 6.0 (20:80)	180 (HRP) 180 (SBP)	290 220	15 9
MeCN–0.1 M phosphate buffer, pH 6.0 (25:75)	180 (HRP) 180 (SBP)	145 220	9 9

The results demonstrate the applicability of [bmim][BF_4] and [bmpy][BF_4] to the determination of guaiacol using SBP. In our opinion, the application of hydrophilic IL expands the possibilities of enzymatic methods for the analysis of samples sparingly soluble and insoluble in water.

This work was supported by the Russian Foundation for Basic Research (grant no. 09-03-00823-a) and the RF Ministry of Education and Science (contract no. P991, 2010).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2011.03.013.

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Received: 21st September 2010; Com. 10/3594