NOVEL ASPECTS OF THE APPLICATION OF WATER-MISCIBLE IONIC LIQUIDS IN CATALYSIS BY PLANT PEROXIDASES: FORMATION OF THE REACTION MEDIA AND SUPPORTING MATERIAL FOR THE ENZYME IMMOBILIZATION

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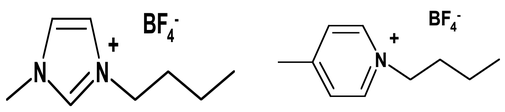
Abstract. Use of hydrophilic ILs (1-butyl-2-methylimidazolium ([**BMIm**]) and N-butyl-3-methylpyridinium **([BMPy]**) tetrafluoroborates instead of polar organic solvents (acetonitril, DMSO, and *etc.*) and the optimization of the reaction medium composition provided the oxidation of phenolic compounds catalyzed by plant peroxidases isolated from horseradish roots (**HRP**) and soybean hulls (**SBP**) in the presence of 60 – 80 vol% of IL. As a result, the procedures for the determination of 3 μM – 3 mM of the indicated substrates in samples with low water content (pharmaceuticals, for example) were developed. The catalytic activity of the considered plant peroxidases controlled by spectrophotometric method, and their substrate specificity were found to depend significantly on the nature of the enzyme, IL cation, and buffer solution used as a co-solvent for IL. Thus, SBP had the greatest catalytic activity and substrate specificity towards guaiacol, whereas HRP was more preferable for the transformation of *o*-chlorophenol in [BMIm][BF_4]-water mixture (70:30 vol%). The conditions for the formation, storage and applicability of the novel composite {cellulose-[BMIm][Cl]-peroxidase} in the reactions of aryldiamines (*o*-phenylendiamine, *o*-dianisidine, 3,3',5,5'-tetramethylbensidine, **TMB**) and catecholamines (dopamine, adrenaline, α-methyldopa, dobutamine) oxidation by H_2O_2 were optimized.

Application of ILs as a reaction media for the transformation of the model phenolic substrates of peroxidases (guaiacol and *o*-chlorophenol)[1]

Polarity of the diverse solvents, *logP*
(a logarithm of octanol-water partition coefficient)

CH_3CN (-0.33)	DMSO (-1.30)	[BMIm][BF_4] (-2.44)	[BMPy][BF_4] (-2.64)

ILs under investigation



Optimal conditions for the determination of the model substrates

Guaiacol	*o*-Chlorophenol
470 nm	415 nm
[BMIm][BF_4] – Im*, pH 7.0 (80:20 vol%)	[BMIm][BF_4] – MES**, pH 5.5 (70:30 vol%)
SBP (45 nM)	**HRP** (200 nM)
t-BuOOH 170 mM	50 mM

* – Imidazol-HCl; ** – Morpholine ethane sulfonate

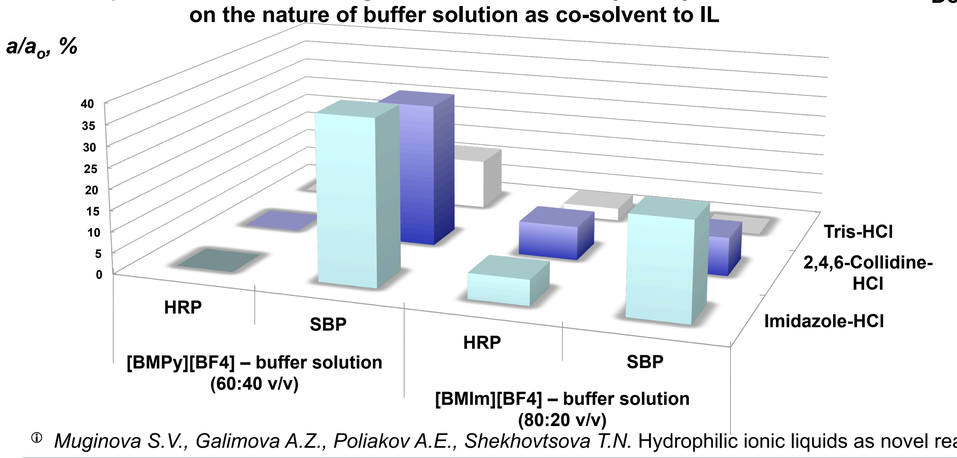
Analytical characteristics of the developed enzymatic procedures for the determination of guaiacol and *o*-chlorophenol (*n*=3, *P*=0.95)

Substrate (enzyme)	Applicable concentration range, μM	a*	Δa	b	Δb	*r*	*RSD*, %
		10^{-3} min^{-1}		μM min^{-1}			
Guaiacol (SBP)	100 – 3000	2.8	0.6	1	1	0.985	6
o-Chlorophenol (HRP)	1.5 – 300	12	0.8	1.3	0.2	0.992	5

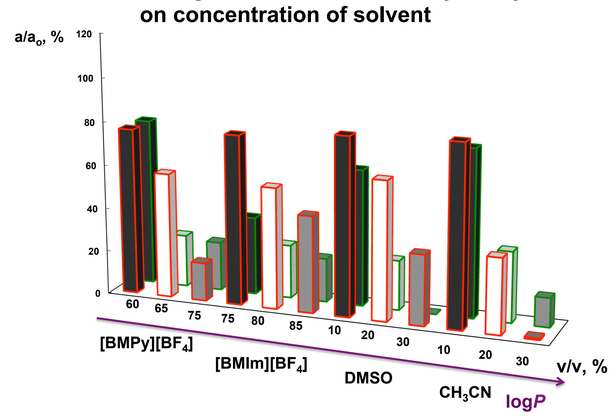
* – *V*= (a±Δa) *x* + (b±Δb)

Optimal order of adding the components of the indicator reaction: IL – *t*-BuOOH – buffer solution – phenolic substrate – peroxidase.

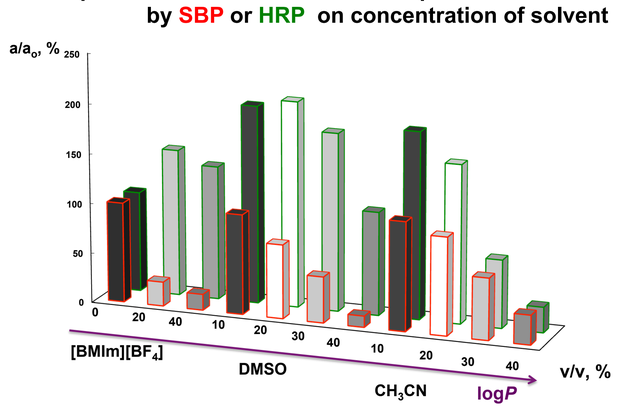
Dependence of the rate of guaiacol oxidation catalyzed by SBP or HRP on the nature of buffer solution as co-solvent to IL



Dependence of the rate of guaiacol oxidation catalyzed by SBP or HRP on concentration of solvent



Dependence of the rate of *o*-chlorophenol oxidation catalyzed by SBP or HRP on concentration of solvent



[1] *Muginova S.V., Galimova A.Z., Poliakov A.E., Shekhovtsova T.N.* Hydrophilic ionic liquids as novel reaction media for the determination of guaiacol using horseradish and soybean peroxidases. 2011, Mendeleev Comm. V. 21. p. 97-98.

Application of ILs for obtaining peroxidase-cellulose composites in the form of thin and transparent films as the sensitive element of the optical biosensors

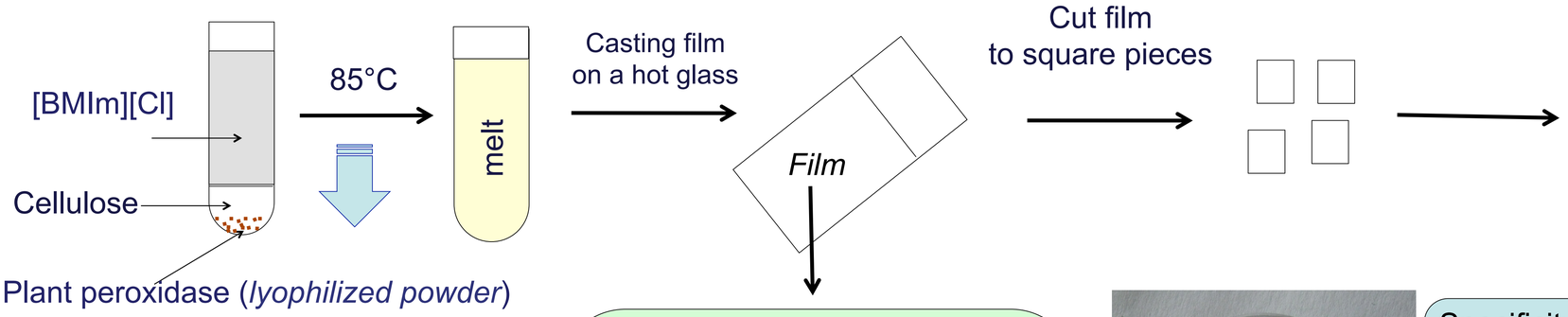
Optimization of the conditions for microcrystalline cellulose film preparation (for example HRP).

IL	[BMIm][Cl]	[BMIm][AcO] *	[BMIm][Br]
Solubility of cellulose (3.5 wt.%) in IL	**Soluble**	Soluble	Insoluble

** It is imposible to cast a film.*

Parameter	Content of the enzyme per test form, pmole	Ratio of cellulose/[BMIm][Cl], wt. %	Dissolution period, h	T, °C	Washing	Test form
Optimal value	40	3.5	6	85	2 portion of water (4 ml)	Square (0.5 cm^2)

Experimental details of the preparation of cellulose films with plant peroxidases and visual control of their catalytic activity.



Growing color into a cellulose film in the indicator system TMB-HRP-H_2O_2 in time

Time, min	ν(TMB), nmole					
	200	100	20	8	4	2
0.5						
1						
1.5						
2						

Enzyme or protein	*pI*	*Dissolution time, h*
HRP	*9.0*	*6*
Hemoglobine	*7.0*	*7*
SBP	*3.7*	*10*
Peanut peroxidase	*3.9*	*- (within 48 h)*

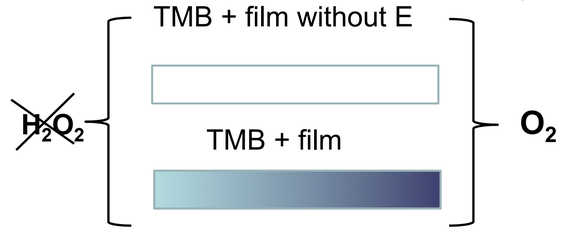
- Optically transparent (T,% = 94±3; *n* = 3, *P* =0.95);
- thin (thickness ≈ 0.3 mm);
- flexible;
- hard;
- has high catalytic activity (95%) and stability (at least 2 weeks).

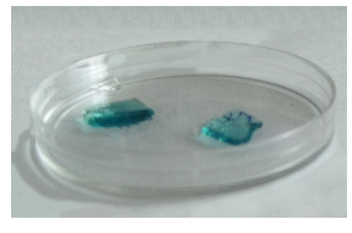
The obtained wet film was stored at a ambient temperature in closed container.

Specificity of HRP and SBP increases in the rows of substrates:

o-phenylenediamine – *o*-dianisidine ≈ TMB;
adrenaline - pyrocatechol – dopamine ≈ α-methyldopa - dobutamine.

Immobilized HRP and SBP have oxidase activity in the reaction of TMB oxidation.





Conclusions:

1. [BMIm][BF_4] is a suitable reaction medium for the enzymatic determination of phenolic substrates of plant peroxidases in samples with low water content.
2. The catalytic activity of native plant peroxidases and their substrate specificity depend significantly on the nature of peroxidase, IL cation, and buffer solution.
3. The use of [BMIm][Cl] for the development of optical biosensors based on cellulose films for the determination of peroxidase substrates is promising.

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