

# Heterogeneous Catalysts

Subjects: Nanoscience & Nanotechnology

Contributor: Anna Chrobok

Nanomaterials are significant carriers for enzymes in heterogeneous biocatalysis. Large amounts of the active phase can be immobilized due to the large specific surface area. An interesting approach combines the stabilization of enzymes in ionic liquids and an immobilization of the active phase on a solid support, which allows to biocatalyst's recycling and its application in continuous flow and batch processes.

Keywords: supported ionic liquid phase ; supported ionic liquid-like phase ; biocatalysis ; enzyme ; heterogeneous catalysis ; immobilization ; nanomaterials

## 1. Introduction

Four different techniques (SILC, SCIL, SILP, and SILLP) of combining ionic liquids and the solid matrix, for enzyme stabilization, were applied in the biocatalysis. Due to the hydrophobic character, ionic liquids that are composed of an imidazolium cation with a long alkyl chain and less nucleophilicity of anion, are the most suitable for enzyme stabilization. The use of hydrophobic ionic liquids enables high specific activities of the enzymes to be obtained, for all types of techniques. It is associated with "essential" water, which is important to the enzymes action. Protein immobilization, via covalent bonding or physical adsorption on the support, ensures high stabilities and activities. The presented techniques also enable easy separation of the biocatalyst from the reaction mixture, and reusability. All these factors allow high conversions, yields, selectivity, and enantioselectivities to be achieved, and make SILC, SCIL, SILP, and SILLP methods attractive for reactions that are catalyzed by the enzymes.

## 2. Contribution of Nanomaterials to Effectiveness of Supported Ionic Liquid Phases

Nanomaterials are an attractive group of materials for supported ionic liquid phase techniques. Due to their small particle size, nanomaterials offer a larger specific surface area than conventional materials, which can improve the immobilization of the protein's particles on the support. Moreover, nanomaterials have unique mechanical, thermo-physical properties and surface morphology, which are important in immobilization methods <sup>[1][2]</sup>. The nanomaterials used in the supported ionic liquid phases, for the biocatalytic reactions described before, are summarized in **Table 2**.

**Table 2.** Nanomaterials used for supported ionic liquid phases in the biocatalysis.

Type	Nanomaterial	Ionic Liquid	Enzyme	Reaction	Ref.
SILLP	Chitosan-silica hybrid	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PLL (2482 U/g <sup>1</sup> , 132.1 mg/g <sup>2</sup> )	Triacetin hydrolysis 35 °C, 10 cycles	[3]
SILLP	Chitosan-Fe <sub>3</sub> O <sub>4</sub> hybrid	Imidazolium [PF <sub>6</sub> ] <sup>-</sup>	PPL (2879 U/g, 118 mg/g)	Triacetin hydrolysis 50 °C, 10 cycles	[4]
SILLP	Fe <sub>3</sub> O <sub>4</sub>	Imidazolium [PF <sub>6</sub> ] <sup>-</sup>	CRL (132.3 U/g, 639 mg/g)	Oleic acid esterification 30 °C, 5 cycles	[5]
SILLP	Fe <sub>3</sub> O <sub>4</sub>	Imidazolium [Cl] <sup>-</sup>	Penicillin G acylase (261 U/g, 209 mg/g)	Penicillin G potassium salts hydrolysis 37 °C, 10 cycles	[6]
SILLP	Fe <sub>3</sub> O <sub>4</sub> -silica hybrid	Imidazolium [Cl] <sup>-</sup>	CRL	Palm stearin interesterification 45 °C, 4 cycles	[7]

Type	Nanomaterial	Ionic Liquid	Enzyme	Reaction	Ref.
SILLP	MWCNTs	Imidazolium [PF <sub>6</sub> ] <sup>-</sup>	CALB (19,354 U/g, 96 mg/g)	Triacetin hydrolysis 60 °C, 4 cycles	[8]
SILLP	MWCNTs	Imidazolium [PF <sub>6</sub> ] <sup>-</sup>	CALB (25,350 U/g, 114 mg/g)	Triacetin hydrolysis 60 °C, 4 cycles	[9]
SILLP	MWCNTs	Imidazolium [PF <sub>6</sub> ] <sup>-</sup>	CALB (13,636 U/g, 66 mg/g)	Triacetin hydrolysis 60 °C, 4 cycles	[10]
SILP	MWCNTs	D-glucose based [NTf <sub>2</sub> ] <sup>-</sup>	CALB (42 mg/g)	Acrylic acid esterification 25 °C, 5 cycles, Y = 99% <sup>3</sup>	[11]
SILLP	MWCNTs	Imidazolium [Oc <sub>2</sub> PO <sub>4</sub> ] <sup>-</sup>	CALB (64 mg/g)	2-adamantanone oxidation 20 °C, 5 cycles, α = 91% <sup>4</sup>	[12]
SILP	MWCNTs	Imidazolium [NTf <sub>2</sub> ] <sup>-</sup>	CALB (22 mg/g)	2-adamantanone oxidation 20 °C, 4 cycles, α = 99%	[12]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PPL (975 U/mg)	Triacetin hydrolysis 36 °C, 5 cycles	[13]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PPL (975 U/mg)	Triacetin hydrolysis 35 °C, 5 cycles	[14]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	BCL (10205 U/g, 230 mg/g)	Triacetin hydrolysis 50 °C, 3 cycles	[15]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PPL (720 U/g, 227.5 mg/g)	Triacetin hydrolysis 35 °C, 4 cycles	[16]
SILLP	Silica	Imidazolium L-lysine	PPL (244 U/g, 197 mg/g)	Triacetin hydrolysis 50 °C, 5 cycles	[17]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PPL (392 U/g, 245 mg/g)	Triacetin hydrolysis 50 °C, 5 cycles	[18]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PPL (760 U/g, 117 mg/g)	Triacetin hydrolysis 45 °C, 5 cycles	[19]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	PPL (468 U/g, 186 mg/g)	Triacetin hydrolysis 45 °C, 5 cycles	[20]
SILLP	Silica	Imidazolium [Cl] <sup>-</sup>	Papain (0.8 U/mg, 261 mg/g)	L-tyrosine synthesis 50 °C	[21]
SILLP	Organosilica	Imidazolium [Cl] <sup>-</sup>	Amylase from <i>Bacillus amyloliquefaciens</i> (29.35 U/mg, 80 mg/g)	Starch hydrolysis 70 °C, 4 cycles	[22]
SILLP	Silica	Imidazolium [BF <sub>4</sub> ] <sup>-</sup>	CALB (5044.44 U/g)	Corn oil glycerolysis 50 °C, 5 cycles, α = 70.94%	[23]
SILP	Silica aerogel	Ammonium [C <sub>4</sub> H <sub>9</sub> COO] <sup>-</sup>	BCL (83% <sup>5</sup> )	Olive oil hydrolysis 37 °C, 23 cycles	[24]
SILP	Silica aerogel	Ammonium [C <sub>4</sub> H <sub>9</sub> COO] <sup>-</sup>	BCL (337 mg/g)	Coconut oil esterification 40 °C, α = 70%	[25]
SILP	Silica	Phosphonium [NTf <sub>2</sub> ] <sup>-</sup>	BCL (91.1%)	Olive oil hydrolysis 37 °C, 17 cycles	[26]

<sup>1</sup> Specific activity of enzyme. <sup>2</sup> Enzyme loading on the support. <sup>3</sup> Yield. <sup>4</sup> Conversion. <sup>5</sup> The total activity recovery yield.

As shown in **Table 2**, several groups of nanomaterials that used in the supported ionic liquid phase techniques can be distinguished. Magnetite  $\text{Fe}_3\text{O}_4$  nanoparticles are a very interesting group of nanomaterials, solving problems related to the separation of the nanobiocatalyst from the reaction mixture. The magnetite SILLP biocatalyst can be easily removed by a magnetic field. In the literature,  $\text{Fe}_3\text{O}_4$  nanoparticles were used as a supported ionic liquid biocatalyst, as well as nanocomposites hybrids with chitosan and silica, which prevent the aggregation of magnetite nanoparticles and improve their chemical stability. Additionally, a chemically modified magnetite nanomaterial surface, with hydrophobic ionic liquids, provides high enzyme stability and prevents leaching of the enzyme. Moreover, high ionic liquid loading on magnetite nanomaterials was reported, which can be easily explained by their large surface area [4][5][6][7]. The next significant group of nanomaterials that are used as matrices in SILP/SILLP, is carbon nanotube. Immobilized on carbon nanotubes, lipases exhibit high activity, due to the hydrophobicity of the MWCNTs outermost shells. There are reports where the ionic liquid is covalently grafted, or physically adsorbed, on the MWCNTs surface, which causes the increase in hydrophobicity of the SILP or SILLP matrixes. Therefore, lipases that are immobilized on SILP and SILLP, based on carbon nanotubes, showed great activity and enzyme loading. It is worth emphasizing the interesting approach with the D-glucose-based ionic liquid used for SILP synthesis. In consequence, bio-based SILP was obtained and successfully used in esterification reactions [8][9][10][11][12]. The most widely used in supported ionic liquid phases is silica. The surface of the silica nanomaterials can be easily functionalized. Moreover, the porosity of silica nanoparticles increases ionic liquid loading on the surface, and so on the efficiency of enzyme immobilization. The high activity and thermal stability of the enzymes were observed after immobilization on silica-based SILLP or SILLP [3][13][14][15][16][17][18][19][20][21][22][23][24][25][26].

For all the presented examples, nanomaterials improved the ionic liquid and enzyme loading caused by the large surface area:volume ratio. All of the described nanomaterials can be functionalized, which is key for the supported ionic liquid-like phase method. Nanomaterials based on supported ionic liquid phases, enabled the activity and stability of the employed enzymes to be increased. It was due to the imidazolium ionic liquids containing long alkyl chains and proper anions grafted to the nanomaterial, which increased the hydrophobicity of the support. CALB, PPL, and BCL are the most common lipases used in nanomaterial-based SILP and SILLP. Immobilized enzymes on the nanoparticles that are modified with ionic liquids, catalyzed in hydrolysis, esterification, transesterification and oxidation reactions, provide high yields and conversions. Moreover, biocatalysts could be easily separated from the reaction mixture, especially those with magnetic properties, and used in many cycles.

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