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Improvement of Lipase Catalytic Properties by Immobilization in Hybrid Matrices

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***Abstract*—**Lipases are enzymes particularly amenable for immobilization by entrapment methods, as they can work equally well in aqueous or non-conventional media and long-time stability of enzyme activity and enantioselectivity is needed to elaborate more efficient bioprocesses. The improvement of Pseudomonas fluorescens (Amano AK) lipase characteristics was investigated by optimizing the immobilization procedure in hybrid organic-inorganic matrices using ionic liquids as additives. Ionic liquids containing a more hydrophobic alkyl group in the cationic moiety are beneficial for the activity of immobilized lipase. Silanes with alkyl- or aryl nonhydrolizable groups used as precursors in combination with tetramethoxysilane could generate composites with higher enantioselectivity compared to the native enzyme in acylation reactions of secondary alcohols. The optimal effect on both activity and enantioselectivity was achieved for the composite made from octyltrimethoxysilane and tetramethoxysilane at 1:1 molar ratio (60% increase of total activity following immobilization and enantiomeric ratio of 30). Ionic liquids also demonstrated valuable properties as reaction media for the studied reactions, comparable with the usual organic solvent, hexane.

***Keywords*—**Ionic liquids, lipase, enantioselectivity, sol-gel immobilization

I. Introduction

INDUSTRIAL or "white" biotechnology can be defined as the application of biotechnology for the processing and production of chemicals, materials and energy. Based on a very impressive development in the last decade, it is able now to ensure new opportunities to chemical and life-science industries by using enzymes and microorganisms to produce valuable building blocks and materials that previously have been accessible only through complicated synthetic routes [1].

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Optically active intermediates: alcohols, amines, and carboxylic acids are important building blocks for the synthesis of pharmaceutical and agrochemical products. Around 80% of the active compounds manufactured by pharmaceutical companies are chiral, and this percentage is estimated to increase in the next period [2]. Using a pure enantiomer instead of racemic mixture allows lower dosage and improved efficacy of the product, but the availability of these compounds at large scale was limited. New technologies were needed and biocatalytic processes started to play an emerging role in this context. The excellent specificity and selectivity (including enantioselectivity) properties of enzymes have been employed to carry out processes of high complexity in less harmful experimental and environmental conditions [3]. There are several tools, as protein engineering, available to improve the enzyme characteristics, but some drawbacks associated with use of soluble enzymes like high price, product contamination, difficulty of separation from the reaction mixture, and insufficient operational stability may reduce the industrial application possibilities. In these circumstances, is not astonishing to see a revival of immobilization techniques, as they could enhance the stability, activity, specificity or selectivity of enzymes, if properly designed. Enzyme engineering by immobilization techniques was demonstrated to be perfectly compatible with other chemical or biological approaches to improve enzyme function [4]. However, the immobilization protocols should be tailored to every enzyme and reaction and is difficult to develop generic methods [5].

Among the immobilization methods described in the literature, bioencapsulation looks particularly valuable while direct linkage of the enzyme and support, frequently associated with activity loss, is avoided. A large diversity of natural or synthetic organic polymers, possessing a broad variety of available functional features, has been already studied for entrapment of biomolecules. They can fulfill several requirements needed for a highly efficient biocatalyst, but mechanical strength and chemical stability are often not appropriate [6]. Inorganic materials could be a solution for support matrices of improved quality for biomolecule entrapments, and silica gels are particularly suitable for this purpose. Silica has been widely used as an inert and stable matrix for enzyme immobilization owing to its high specific surface area and controllable pore diameters, which can be tailored (as microporous, mesoporous or macroporous silica) to the dimension of a specific enzyme. Since most enzymes

are of 3 to 6 nm in diameter, mesoporous materials (2–50 nm pore size) were most commonly employed [7]. Among the immobilization techniques based on silica gels, sol-gel encapsulation has proved to be a versatile alternative for a large variety of biomolecules. Sol-gel matrices are formed by hydrolytic polymerization of silane precursors, principally alcoxides. The first step is the hydrolysis to silicic acid, followed by condensation reactions to yield silica. Silica particles grow progressively as condensation proceeds, leading to the formation of colloidal solutions (sols) and gels. These gels can be partially dried at room temperature, giving a porous network of hydrated amorphous silica (called xerogel) with pores ranging typically between 1 and 10 nm in diameter [8].

The sol-gel process is particularly important because allows the low-temperature synthesis of silica and simultaneous entrapment of the enzyme throughout the gelation phase. Furthermore, silanes holding a non-hydrolyzable alkyl- or aryl group were used together with tetraalkoxysilanes, leading to more hydrophobic sol-gel matrices that have been proved particularly efficient in the case of lipases, facilitating their interfacial activation [9], [10]. Several other materials can be also incorporated during the sol-gel process as additives or templates, to obtain organic-inorganic hybrid materials with better characteristics. Organic molecules can be simply embedded or chemically linked within the silica matrix. Many additives such as polyethyleneglycol, glycerol, polyvinylalcohol, sugars, cyclodextrins, etc. have been tested [11]. A new and promising possibility could be the utilization of ionic liquids as template compounds for the sol-gel entrapment of enzymes [12].

The aim of this study was to investigate the feasibility of ionic liquids as additives for sol-gel entrapment of microbial lipases and the influence of precursor and ionic liquid nature on activity and enantioselectivity, examined for acylation reactions of aliphatic secondary alcohols.

II. Materials and Methods

Lipase AK "Amano" 20 (from *Pseudomonas fluorescens*) was donated by Amano Enzyme Inc. (Japan). Silane precursors propyl- (PrTMOS), octyl- (OcTMOS), and phenyltrimethoxysilane (PhTMOS), sodium fluoride, and vinyl acetate were purchased from Fluka. Methyl-trimethoxysilane (MeTMOS), tetramethoxysilane (TMOS), n-hexane (98%), isopropyl alcohol (99.7%), and racemic secondary alcohols: 2-butanol, 2-pentanol, 2-hexanol, 2-heptanol, and 2-octanol were from Fluka, 2-nonanol was supplied by Merck. All these reagents were of analytical grade and have been use without further purification. Hexadecane (99%, Merck) and decane (>99%, Aldrich) were used as internal standards for the chromatographic analysis.

Ionic liquids 1-ethyl-3-methyl-immidazolium tetrafluoroborate [Emim]BF_4, 1-butyl-3-methyl-immidazolium tetrafluoroborate [Bmim]BF_4, 1-hexyl-3-methyl-immidazolium tetrafluoroborate [Hmim]BF_4, and 1-butyl-3-methyl-immidazolium hexafluorophosphate [Bmim]PF_6, 1-ethyl-3-methyl-immidazolium trifluoroacetate [Emim]$OOCCF_3$, were purchased from Merck, 1-octyl-3-methyl-immidazolium tetrafluoroborate [Omim]BF_4 from Fluka. 1-Propyl-3-methyl-immidazolium tetrafluoroborate [Pmim]BF_4, 1-ethyl-3-methyl-immidazolium acetate [Emim]$OOCCH_3$, 1-ethyl-3-methyl-immidazolium octylsulphate [Emim]$OcSO_4$, were provided by the University of Bremen (Germany).

A. Immobilization Method 1

A previously described [13] procedure has been used, modified by replacing polyethyleneglycol as additive with ionic liquid. Lipase AK (100 mg/mL) was suspended in TRIS/HCl 0.1 M, pH 8.0 buffer, stirred at room temperature for 30 min, centrifuged, and the supernatant used for immobilization. In a 4 mL glass vial, Lipase AK filtrate (780 μL), ionic liquid (200 μL), 1M NaF (100 μL), and isopropyl alcohol (200 μL) were mixed. By continuous stirring, a mixture of silane precursors (total 6 mmoles) was added. The resulting mixture was magnetically stirred at room temperature until the gelation started. The gel was kept for 24 h at room temperature to complete polymerization. The bulk gel was washed with isopropyl alcohol (10 mL), TRIS/HCl buffer solution 0.1M, pH 8.0 (10 mL), isopropyl alcohol again (10 mL) and hexane (10 mL), and dried at room temperature for 48 hrs. Finally, it was crushed in a mortar and kept in the refrigerator.

B. Immobilization Method 2

This method was employed only for preparates made from MeTMOS and TMOS silane precursors, as those prepared according to *Method 1* exhibited very low activities, probably as a result of a too dense structure of the sol-gel. In a 4 mL vial, the silane precursors (total 6 mmoles) and ethanol (0.5 mL) were mixed for 15 minutes to form a homogeneous sol. In a second 4 mL vial lipase AK (75 mg) was suspended in TRIS/HCl 0.1 M, pH 8.0 buffer (1.1 mL), then 200 μL ionic liquid was added and mixed for 15 min. Subsequently, the two previous solutions were mixted, and magnetically stirred until gelation occurred. The resulting gel was kept at room temperature for 24 hrs to complete polymerization. The bulk gel was washed and dried as described in Method 1..

C. Transesterification Procedure

For the acylation studies, 4 mL capacity glass vials were charged with a mixture of secondary alcohol (1 mmole), vinyl acetate (3 mmole), reaction medium (hexane or ionic liquid, 2 mL) and free (5 mg) or sol-gel immobilized (25 mg) Lipase AK. The mixture was shaked at 300 strokes/min and 40ºC (MIR-S100 orbital shaker, Sanyo E&E, Japan). Samples taken at different time intervals were analyzed for conversion and enantiomeric excess by a Dani 86.10 gas chromatograph (Dani Instruments S.p.A., Italy) equipped with flame ionization detector, using a 30 m x 0,32 mm CYDEX-B chiral column (SGE, Australia). The analysis conditions were set as follows: oven temperature: 50 to 120ºC with 10ºC/min heating

rate, injector temperature 240ºC, detector temperature 280ºC, carrier gas (helium) flow 0.6 mL/min. Decane and hexadecane have been used as internal standards for quantitative determination of alcohol conversion. Only the enantiomers of the ester products have been separated on this column. All assays have been run at least in duplicate.

Transesterification activities were calculated based on the alcohol conversion at 24 hrs and expressed as the average amount of the forming 2-acetoxy-alcohol (in micromole) per hour by 1 mg of free or immobilized enzyme. Based on previous data [13] it was assumed that Lipase AK is (*R*)-specific in the acylation reaction of the tested secondary alcohols, therefore the main product is the (*R*)-ester. Enantiomeric excess (ee_p) of the resulted ester product was determined based on the enantiomers peak area. The enantiomeric ratio (*E*) has been calculated using the relation [14]:

$$E = \frac{\ln\left[1 - C\left(1 + ee_P\right)\right]}{\ln\left[1 - C\left(1 - ee_P\right)\right]},$$ where *C* represent the conversion at 24 hrs.

III. Results and Discussion

Ionic liquids attracted interest in the last period especially as "green" reaction media, because of their lack of vapour pressure and high thermal stability. In biocatalysis, ionic liquids with 1,3-dialkylimmidazolium or N-alkylpyridinium cation and a non-coordinating anion have been most frequently used, as their polarity and hydrophobicity properties were found appropriate to ensure selectivity and operational stability even higher than in traditional media [15]. This study was focused on the utilization of ionic liquids as nonstructural template additives for sol-gel immobilized lipases. The beneficial influence of ionic liquids as additives to protect the inactivation of lipase by the released alcohol and shrinking effect throughout the formation and drying of gel was recently described [16]. We studied the influence of ionic liquid structure, immobilization parameters and substrate structure on the activity and enantioselectivity of *Pseudomonas fluorescens* (AK "Amano" 20) lipase immobilized by sol-gel entrapment. The examined reaction was the irreversible transesterification of aliphatic secondary alcohols using vinyl acetate as acyl donor. Generation of acetaldehyde by tautomerization of the vinyl alcohol side product makes the reaction irreversible.

Transformations of racemic substrates catalyzed by enzymes are frequently enantioselective, and in optimal conditions only one enantiomer is converted in reaction product, leaving the unreacted enantiomer in optically active form. Acylation of a secondary alcohol is such a reaction, but usually the discrimination between the two isomers is not absolute, yielding enriched optical isomers instead of pure enantiomers (Fig. 1).

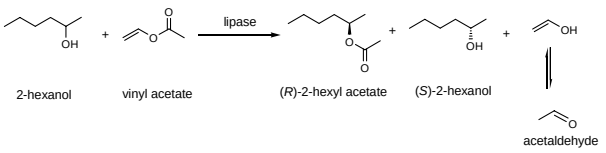

Fig. 1 Reaction scheme of 2-hexanol enantioselective acylation

Obtaining high enantiomeric excess depends on enzyme characteristics and reaction conditions, and consequently could be influenced by the immobilization conditions. A second target of every process is to reach high conversion. Obviously, the enantiomeric excess depends on conversion as well, being higher in the early stage of the reaction, based on the higher reaction rate of the faster reacting enantiomer. Therefore, the efficiency of an enantioselective enzymatic reaction is recommended to be expressed using the enantiomeric ratio E, a parameter that takes account of both enantiomeric excess and conversion [14].

A. Influence of the Nature of Ionic Liquids as Entrapment Additives

The role of additives should be the protection of the enzyme against diverse inactivation effects during immobilization and contribution to formation of a highly ordered silica matrix that could emerge in enhanced activity and stability. Several ionic liquids have been tested, replacing polyethyleneglycol in the original immobilization protocol, as follows:

- ionic liquids with increasing alkyl groups in the immidazolium cationic part and tetraflouroborate anion: 1-ethyl- ([Emim]BF_4), 1-propyl- ([Pmim]BF_4), 1-butyl- ([Bmim]BF_4) , 1-hexyl- ([Hmim]BF_4), and 1-octyl-3-methyl-immidazolium tetrafluoroborate ([Omim]BF_4);
- ionic liquids with the same cation and different anions: (1-ethyl-3-methyl-immidazolium)-tetrafluoroborate ([Emim]BF_4), -acetate ([Emim]$OOCCH_3$), -trifluoroacetate ([Emim]$OOCCF_3$), and -octylsulphate ([Emim]$OcSO_4$).

The results of acylation reactions of 2-hexanol by vinyl acetate are shown in Table I. The reactions were made at 40°C in hexane. The sol-gel matrix has been obtained with OcTMOS and TMOS precursors, at 1:1 molar ratio. Transesterification activities and activity recovery yields were calculated at 24 hrs reaction time. Relative total activity was determined as the ratio of total activity resulted following immobilization (effective activity · immobilized enzyme amount) and total activity of the native enzyme (effective activity · enzyme amount subjected to immobilization). Activity and enantioselectivity values of the native lipase were considered as reference.

TABLE I
INFLUENCE OF IONIC LIQUID ADDITIVE ON ACTIVITY AND ENANTIOSELECTIVITY OF SOL-GEL IMMOBILIZED LIPASE

Additive	Activity[1]	Relative total activity	e.e. (%)	E
- (native enzyme)	2.446	-	73	17
$[Emim]BF_4$	0.432	1.13	76	29
$[Pmim]BF_4$	0.440	1.23	70	22
$[Bmim]BF_4$	0.421	1.33	76	29
$[Hmim]BF_4$	0.481	1.55	67	19
$[Omim]BF_4$	0.507	1.61	74	30
$[Emim]COOCH_3$	0.440	1.40	72	34
$[Emim]COOCF_3$	0.425	1.17	76	19
$[Emim]OcSO_4$	0.265	0.60	51	4

[1]Effective activity, expressed in ($\mu mol \cdot h^{-1} \cdot mg^{-1}$)

Ionic liquids with the same anion, tetrafluoroborate, enhanced the transesterification activity of the sol-gel immobilized lipase in accordance with the increasing chain length and hydrophobicity of the alkyl group from the cationic part. According to Zhou et al. [17], ionic liquids with tetrafluoroborate anion are templates for preparation of mesoporous nanostructures in which hydrogen bonds between the tetrafluoroborate anion and silanol group of silica gel and π-π interactions between neighboring immidazole groups have an important role in formation of the framework. Most likely, the ionic liquids are partially confined in the silica network and will influence the enzyme behavior. A well-ordered mesoporous structure should favor the enzymatic reaction reducing diffusion limitations, but the hydrophilic nature of the ionic liquids could inactivate lipases in non-aqueous media by taking off the water that is essential to maintain the active conformation. A more hydrophobic substituent in the cationic part of ionic liquid can equilibrate this tendency and induce higher activity. The enantioselectivity of the immobilized preparates was higher when ionic liquids with shorter alkyl chain have been used. This behavior could be explained by formation of a more compact framework favored by these additives that induce a better kinetic discrimination between the optical isomers.

As concerns ionic liquids with the same cation and different anions, large substituents in the anionic part decrease both activity and enantioselectivity of the sol-gel/enzyme composite. The explanation should be again the different influence for the formation of a well ordered mesoporous structure. For the studied reaction $[Emim]COOCH_3$ was the ionic liquid with the best results as immobilization additive, with a 40% increase of relative total activity compared to the native enzyme and enantiomeric ratio E value of 34.

B. Influence of Silane Precursor Structure

Tetramethoxysilane (TMOS) and an alkyl- or aryl-trimethoxy silane (at different molar ratios) have been used as precursors to investigate the influence of precursor nature on lipase catalytic efficiency. Tables II and III show the results obtained for acylation reactions of 2-hexanol with vinyl acetate catalyzed by sol-gel entrapped lipase obtained with $[Omim]BF_4$ and $[Bmim]PF_6$ as additives, respectively. All immobilizations have been carried out according *Method 1* (see Materials and Methods part), excepting those with MeTMOS where *Method 2* was used. Although the lower effective activities for immobilized lipases compared to the native enzyme, the total activities were higher for several preparates, up to 60% increase for the preparate made with OcTMOS and TMOS precursors at 1:1 molar ratio and $[Omim]BF_4$ as ionic liquid additive. Activity values for preparates obtained from the same precursors were slightly higher when the ionic liquid additive was $[Omim]BF_4$, than for $[Bmim]PF_6$. The nature of the second silane precursor (besides TMOS) was more influential on activities. Increasing the alkyl group density resulted in enhanced activities, but it seems to be a limit of this positive influence. Lipases are well known as enzymes being activated in hydrophobic environments and the hydrophobic groups from the immobilization matrix could act in this way. At 1:1 molar ratios of TMOS and the silane with nonhydrolizable alkyl group, the activities increased with the length of the alkyl chain, from methyl to octyl. Increasing the amount of alkyl-containing silane in the immobilization mixture (2:1 molar ratio), the highest activities were recorded for the preparates with propyl groups. The explanation is a possible negative effect of hydrophobic group excess.

TABLE II
INFLUENCE OF SILANE PRECURSOR NATURE AND MOLAR RATIO ON THE ACTIVITY AND ENANTIOSELECTIVITY OF SOL-GEL IMMOBILIZED LIPASE OBTAINED WITH $[OMIM]BF_4$ AS ADDITIVE

Silane precursor (molar ratio)	Activity[1]	Relative total activity	e.e. (%)	E
- (native lipase)	2.446	-	73	17
MeTMOS:TMOS (1:1)	0.113	0.21	> 99	> 100
MeTMOS:TMOS (2:1)	0.528	0.72	53	14
PrTMOS:TMOS (1:1)	0.483	1.09	77	24
PrTMOS:TMOS (2:1)	0.651	1.49	28	8
OcTMOS:TMOS (1:1)	0.507	1.61	74	30
OcTMOS:TMOS (2:1)	0.532	0.86	54	12
PhTMOS:TMOS (1:1)	0.354	1.06	82	18
PhTMOS:TMOS (2:1)	0.339	1.11	78	14

[1]Effective activity, expressed in ($\mu mol \cdot h^{-1} \cdot mg^{-1}$)

Pending alkyl groups cannot be part in the building of the gel network and their excess would avoid the formation of a well-ordered mesoporous silica matrix which is needed for a highly active composite.

TABLE III
INFLUENCE OF SILANE PRECURSOR NATURE AND MOLAR RATIO ON THE ACTIVITY AND ENANTIOSELECTIVITY OF SOL-GEL IMMOBILIZED LIPASE OBTAINED WITH $[BMIM]PF_6$ AS ADDITIVE

Silane precursor (molar ratio)	Activity[1]	Relative total activity	e.e. (%)	E
- (native lipase)	2.446	-	73	17
MeTMOS:TMOS (1:1)	0.125	0.27	> 99	> 100
MeTMOS:TMOS (2:1)	0.460	0.43	83	46
PrTMOS:TMOS (1:1)	0.474	1.02	70	22
PrTMOS:TMOS (2:1)	0.593	1.20	39	8
OcTMOS:TMOS (1:1)	0.530	0.48	52	12
OcTMOS:TMOS (2:1)	0.648	1.14	32	7
PhTMOS:TMOS (1:1)	0.245	0.65	61	5
PhTMOS:TMOS (2:1)	0.343	1.11	81	18

[1]Effective activity, expressed in ($\mu mol \cdot h^{-1} \cdot mg^{-1}$)

The enantioselectivity of the immobilized lipase preparates, as results from Tables II and III, could be increased by sol-gel entrapment using ionic liquids as additives. However, the highest enantioselectivity, observed for the preparate synthesized using MeTMOS and TMOS precursors at 1:1 molar ratio was associated with the lowest activity among all preparates. Most composites displayed enantiomeric ratio values close to the native lipase, but it must be noticed the enhanced values for both activity and enantioselectivity, obtained when OcTMOS and TMOS at 1:1 molar ratio have been used as precursors and $[Omim]BF_4$ as additive.

C. Influence of Substrate Structure

Six aliphatic secondary alcohols with increasing alkyl chains, from 2-butanol to 2-nonanol, have been investigated as substrates for transesterification reactions catalyzed by sol-gel immobilized Amano AK lipase composites. Hexane was used as reaction medium at 40°C, OcTMOS and TMOS as silane precursors (1:1 molar ratio), and $[Omim]BF_4$ as ionic liquid additive. The activity results are shown in Table IV, compared to the native lipase.

The substrate specificity of Amano AK lipase is not significantly influenced by the secondary alcohol alkyl chain length. The immobilization was demonstrated to be beneficial especially for secondary alcohols with longer alkyl chain, 2-octanol and 2-nonanol. It must be noticed the important activation effect, reflected in more than twofold increase of total activity following immobilization.

The enantioselectivity was enhanced for most of the immobilized sol-gel biocatalysts compared to the native lipase, as results from Fig. 2.

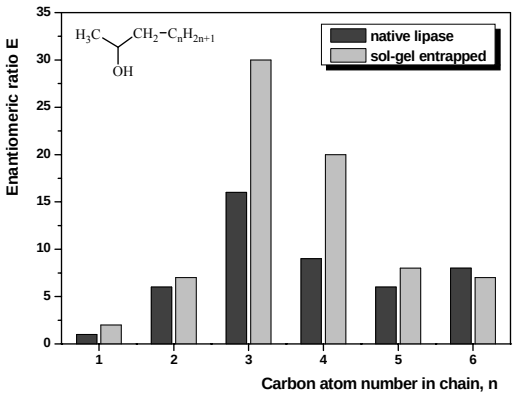

Fig. 2 Influence of secondary alcohol chain length on enantioselectivity of sol-gel immobilized lipase with ionic liquid additive

2-Hexanol and 2-heptanol are the highly enantiospecific substrates in the acylation reaction catalyzed by Amano AK lipase. The enantioselectivity on these substrates was considerably increased by immobilization. Such an effect is essential for technological applications, as optically active secondary alcohols are important chiral auxiliaries [18].

TABLE IV
INFLUENCE OF SECONDARY ALCOHOL CHAIN LENGTH ON THE ACTIVITY OF SOL-GEL ENTRAPPED LIPASE WITH $[OMIM]BF_4$ AS ADDITIVE

Substrate	Lipase	Activity[1]	Relative total activity
2-butanol	Native	2.358	-
2-butanol	Immobilized	0.667	1.81
2-pentanol	Native	2.380	-
2-pentanol	Immobilized	0.611	1.71
2-hexanol	Native	2.446	-
2-hexanol	Immobilized	0.507	1.61
2-heptanol	Native	2.630	-
2-heptanol	Immobilized	0.508	1.29
2-octanol	Native	2.095	-
2-octanol	Immobilized	0.738	2.35
2-nonanol	Native	2.030	-
2-nonanol	Immobilized	0.668	2.19

[1]Effective activity, expressed in ($\mu mol \cdot h^{-1} \cdot mg^{-1}$)

D. Influence of Reaction Medium

The subject of biocatalytic reactions in non-conventional reaction media, including organic solvents and ionic liquids, is very well documented in the scientific literature [19]. It was not an objective of this work to investigate in detail the influence of reaction media in the studied transesterification reactions. However, a preliminary study was realized to show the influence of ionic liquids as both additives and reaction

media. The results are showed in TABLE 5, compared to hexane, the organic reaction medium used is the previous experiments. Two immobilized preparates have been evaluated, the first obtained from PrTMOS and TMOS precursors (at 1:1 molar ratio) with $[Bmim]PF_6$ ionic liquid additive, and the second from OcTMOS and TMOS at the same molar ratio with $[Omim]BF_4$ additive. The same ionic liquids, $[Omim]BF_4$ and $[Omim]BF_4$ were tested as reaction media of 2-hexanol transesterification by vinyl acetate at 40°C.

TABLE V
COMPARATIVE EVALUATION OF CATALYTIC EFFICIENCY AND ENANTIOSELECTIVITY OF FREE AND IMMOBILIZED LIPASE IN ORGANIC SOLVENT AND IONIC LIQUID REACTION MEDIA

Silane precursors (ionic liquid additive)	Reaction medium	Activity[1]	Relative total activity	e.e. (%)	E
-	n-hexane	2.446	-	73	17
(native enzyme)	$[Bmim]PF_6$	2.605	-	58	18
	$[Omim]BF_4$	2.247	-	80	40
PrTMOS:TMOS	n-hexane	0.474	1.02	70	22
($[Bmim]PF_6$)	$[Bmim]PF_6$	0.541	1.09	52	12
	$[Omim]BF_4$	0.492	1.15	72	34
OcTMOS:TMOS	n-hexane	0.507	1.61	74	30
($[Omim]BF_4$)	$[Bmim]PF_6$	0.700	1.63	20	5
	$[Omim]BF_4$	0.511	1.38	59	15

[1]Effective activity, expressed in ($\mu mol \cdot h^{-1} \cdot mg^{-1}$)

Compared to hexane, the most commonly used organic solvent, the ionic liquids demonstrated comparable or slighter higher activities. The enantioselectivity was higher when $[Omim]BF_4$ has been used as reaction medium and lower for the other ionic liquid. Thus, the appropriate ionic liquid must be selected for every application. The primary importance of ionic liquids as reaction media in biocatalytic reactions is related to their reduced vapour pressure and lower toxicity compared to volatile organic solvents, but at this moment their high price is a serious drawback for large-scale biotechnological applications.

IV. CONCLUSION

The sol-gel technique is an emerging procedure for immobilization of biomolecules. Improvement of enzyme characteristics as activity and enantioselectivity is the most important aim for every biocatalytic process. Selecting the appropriate precursors, additives and template compounds is fundamental to obtain solid-phase biocatalysts with higher efficiency and operational stability. Ionic liquids could have an important function in this issue, as they contribute to the formation of the sol-gel framework and can also facilitate the enzymatic reaction by their hydrophilic or hydrophobic nature. The results of this study provide that fine tuning of ionic liquid and silane precursor structure can result in entrapped lipase composites with higher activity and enantioselectivity than the native enzyme. However, several other aspects are yet to be improved, as avoiding sol-gel matrix shrinkage and enzyme aggregation during the immobilization process. More detailed studies on different substrates, also involving the relation between structure of additive and nature of the most effective reaction medium will be completed, to extend the application possibilities of this topic.

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