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REVIEW ARTICLE

Enzymatic kinetic resolution of racemic ibuprofen: past, present and future

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Abstract

This review is a journey concerning the investigations of the kinetic resolution of racemic ibuprofen for the last 20 years. The relevancy of the pharmacological uses of the S(+) enantiomer along with its higher cost compared with racemic profen are the driving forces of a variety of scientific research studies addressing the enzymatic resolution of ibuprofen through enantiomeric esterification using lipases as biocatalysts. Lipases of fungal sources such as *Candida rugosa, Rhizomucor miehei* and the lipase B of *Candida antarctica* have been extensively studied both in homogeneous and heterogeneous (immobilized on solid supports) processes. In this context, the various alcohols and organic co-solvents frequently used in the esterification of racemic ibuprofen are summarized and discussed in this review. Moreover, recent investigations using membranes as reactors coupled with the separation of the desired product and microfluidic devices are presented. Finally, some guidelines about future perspectives regarding the technology of the kinetic resolution of profens and research niches are given.

Keywords

Enantiomeric esterification, ibuprofen, kinetic resolution, lipases

History

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Introduction

Analgesics are the most widely used pharmaceuticals and more than 90% belong to the non-steroidal anti-inflammatory NSAIDs group. Ibuprofen is within this group of worldwide usage drugs. In this context, a 7 years survey (from 2003 to 2009) discusses an increase in ibuprofen consumption in Europe. In fact, the Czech Republic (30.2–31.7 describes daily doses/1000 inhabitans/day) and Finland (32.9–43.3 describes daily doses/1000 inhabitans/day) registered the highest consumption among the European countries (Hudec et al., 2012).

Racemic ibuprofen that contains equal quantities of the *R*(−) and *S*(+) isomers exhibited multiple pharmacological properties. Although it is regularly administered as an analgesic and anti-inflammatory, more recently it has been demonstrated that the long-term use of ibuprofen prevents the risk of Alzheimer's disease (Goldberg, 2008). The pharmacological effectiveness of ibuprofen is based on the ability of reducing the synthesis of prostaglandins by inhibiting cyclooxygenase (COX) that is exclusively exerted by the *S*(+)-enantiomer. The stereoisomers of ibuprofen present differences not only in their pharmacological properties but also in their metabolic pathways (Evans, 2001). In contrast to the *S*(+)-enantiomer, the *R*(−) is able to form a thioester with the coenzyme A which leads to a 50–60% inversion to the *S*(+)-enantiomer. In addition, the *R*(−)-enantiomer reacts with triglycerides interfering with the normal metabolism of lipids. Those hybrid triglycerides are deposited in adipose tissue from where are released slowly.

The carboxylic functional group of ibuprofen reacts with glucuronic acid forming an ester that also binds with endogenous macromolecules such as albumin. The formation of such adducts, primarily in the liver and kidney, is associated with the adverse side effects of NSAIDs. The administration of enantiomerically pure preparations of the *S*(+)-enantiomer possesses advantages compared to the racemic drugs such as: reduced metabolic load, diminished probability of the pharmacokinetic interactions with other drugs, no interference with lipid metabolism, lower doses, neither inversion nor interaction with COX that typically causes variability in the pharmacokinetic (Evans, 2001). In fact, clinical trials with over 1463 patients (with osteoarthritis of the knee and hip, chronic inflammatory and degenerative rheumatic diseases and dysmenorrhea) and post-marketing surveillance of over 7133 outpatients performed by Gebro Pharma (Austria) proved the higher efficiency and gastro-intestinal tolerability, and lower adverse side effects of the *S*(+)-ibuprofen versus racemic ibuprofen and diclofenac (Phleps, 2001).

The above-discussed observations provides evidence of the relevance of obtaining the *S*(+)-enantiomer. In this context, the racemic resolution and the asymmetric synthesis are ways

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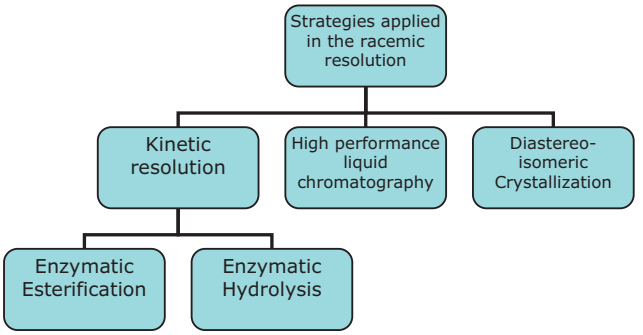

Scheme 1. Strategies applied in the racemic resolution of enantiomers.

of obtaining chiral substances. The former is based on applying a chiral substrate or a catalyst in order to differentiate the rate of reaction of the enantiomers that compose a racemate with a given substance. The asymmetric synthesis involves the creation of a chiral center in a starting non-optically active substrate (De Oliveira Carvalho et al., 2006a).

The racemic resolution might be performed through chromatographic based techniques, selective crystallization (physical method) or enzymatic kinetic resolution (Scheme 1). The most widely used method to resolve racemic mixtures is high-performance liquid chromatography (HPLC). This methodology involves either the direct resolution of the enantiomers with a chiral stationary phase (based on cellulose, amylose, cyclodextrins, amino acids and proteins) or the derivatization (modification of the molecule) of the enantiomers in order to generate diastereoisomers able to be separated.

Diastereoisomeric crystallization is used mostly at the industrial scale to resolve racemic ibuprofen. The amino acid L-lysine is regularly used to form D-ibuprofen-L-lysinate and L-ibuprofen-L-lysinate this latter one is more soluble in water than the former (De Oliveira Carvalho et al., 2006a).

The enzymatic kinetic resolution involves the use of a biocatalyst to discriminate between the enantiomers and improve the rate of hydrolysis or esterification of one instead of the other. The particularity of this process is that the highest desired conversion is 50% that is one enantiomer should react selectively in order to separate the other one. The scientific achievements of the enzymatic kinetic resolution of ibuprofen through esterification with alcohols are presented in this review.

Enzymes in the kinetic resolution of racemic ibuprofen

Enzymes are proteins designed by biological systems usually (but not always) to perform a catalytic action over reactions that take place inside the cells of living systems. The environment of the native enzymes in the cell is composed of lipids, proteins and ionic species usually in an aqueous medium. In this sense, an aqueous environment is the ''conventional medium'' for enzyme action. On the other hand, a ''non-conventional medium'' refers to a non-aqueous medium. In the past, the use of biocatalysts in organic synthesis becomes an attractive alternative to replace conventional methods due to the high chemo-, regio- and enantio-selectivity of enzymes along with mild conditions for the reactions to take place. In general, strategies and typical biocatalytic systems for the enzymatic kinetic resolution of profens are the enantioselective hydrolysis of a racemic ester (lipases, esterases, nitrilases, nitrile-hydratases and amidases), the enantioselective synthesis of an ester (lipases), stereo-selective biooxidation (oxidases), racemization of profenals (hidrolases and oxireductases), enzymatic decarboxylation of malonic acid esters (decarboxylases) and rearrangement of epoxides (styrene oxide isomerase) (Arroyo Sánchez, 1995; Kourist et al., 2011).

In particular, lipases are able to catalyze esterifications and transesterifications in non-conventional media with low water content. This property is fundamental in the kinetic resolution of racemic profens which are not soluble in aqueous media. It is worth emphasizing that the esterification of ibuprofen and other non-steroidal profens regardless of the enantios-electivity of the reaction, have been reported as an alternative way of producing pro-drugs to avoid the side effects associated with the free carboxylic group of the profens.

The most used lipases in the enantiomeric esterification of racemic ibuprofen are *Candida rugosa* and *Rizhomucor miehei* that catalyze preferentially the *S*(+)-ibuprofen and the lipase B of *Candida antarctica* that catalyzes the esterification of the *R*(−)-enantiomer. Table 1 shows the enzymes and the sources (commercial or not) that have been used in the kinetic resolution of racemic ibuprofen through enantiomeric esterification. Most of the research focuses on the use of the commercial biocatalyst Novozym®435 that is composed by the lipase B of *Candida antarctica* immobilized on polymethylmethacrylate. The lipase *Rizhomucor miehei* has also been used in the commercial Lipozyme® IM biocatalyst. In contrast, the catalytic performance of *Candida rugosa* lipase has been investigated as native enzyme and immobilized over agarose, SiO_2, Al_2O_3, cellulose acetate-titanium isopropoxide gel fiber, polypropylene and Accurel MP100 (hydrophobic polypropylene). However, none of these materials are commercially available. Other fungal lipases from *Candida sp* (Liu et al., 2009), *Aspergillus niger* (De Oliveira Carvalho et al., 2006b), *Pseudomona sp* (Ceynowa & Rauchfleisz, 2003), *Aspergillus terreus, Fusarium oxysporum*, *Mucor javanicus*, *Penicillium solitum*, *Rhizopus javanicus* (De Oliveira Carvalho et al., 2006c) and a thermophilic esterase from *Aeropyrum pernyx* K1 (Dan-tong et al., 2011) have been reported.

Alcohols as acyl acceptors in the kinetic resolution of racemic ibuprofen

The mechanism of the hydrolysis and esterification with lipases involved the formation of an acyl–enzyme complex between the carboxyl group C(O)OH of the substrate and the active site (catalytic triad) of the enzyme (Hokenson et al., 2000; Iqbal et al., 2009). Molecular modeling along with experimental studies demonstrated that the esterification of racemic ibuprofen with ethanol on the catalytic active triad of the lipase B of *Candida antarctica* proceeds through the formation of a tetrahedral intermediary between the profen and the lipase, followed by the transformation of the intermediary into the acyl–enzyme complex with

Table 1. Biocatalyst, acyl acceptors (alcohols), reaction conditions (molar ratio of substrates, temperature, others), co-solvents, conversion and enantiomeric excess in the kinetic resolution of racemic ibuprofen through enantiomeric esterification reported in the literature in the last 20 years.

Entry	Biocatalyst	Alcohol (profen:alcohol molar ratio)	Reaction conditions	Co-solvent	Activity and enantioselectivity	References
1	SP435A (lipase A of *Candida antarctica*)	1-Propanol (1:1)	24 °C, 100 rpm, 7 hs. non-controlled a_w Stereo-preference: *R*-ibuprofen	Isooctane	*X*% = 49.5 ee *S*-ibu % = 27	Arroyo & Sinisterra (1994)
2	Native *Candida rugosa* lipase and immobilized over SiO_2, Al_2O_3, agarose	1-Propanol (1:1)	30 °C, 500 rpm, Buffer Tris/HCl 0.1 M (pH 7). Stereo-preference: *S*-ibuprofen	Isooctane	Native CRL (192 h) *X*%:40, ee*R*-ibu%: 71.7 CRL/agarose (168 h) *X*%:29, ee*R*-ibu%: 37.2 CRL/SiO_2 (192 h) *X*%:40, ee*R*-ibu%: 75.3 CRL/Al_2O_3 (192 h) *X*%: 32, ee*R*-ibu%: 16	Arroyo et al. (1995)
3	Novozym® 435 (lipase B of *Candida antarctica*)	1-Dodecanol (1:1.3)	70 °C, 6 days, low water activity (not specified) Stereo-preference: *R*-ibuprofen	Cyclopentane	*X*%: 69 ee *S*-ibu% = 60%	Ducret et al. (1995)
4	Novozym® 435	1-Dodecanol (1:0.75 and 1:0.33)	70 °C, under vacuum. Two consecutive esterifications. Stereo-preference: *R*-ibuprofen	Solventless	1st reaction (24 h): *X*% = 71, ee *S*-ibu% = 86 2nd reaction (48 h): *X*% = 49, ee *S*-ibu % = 97	Trani et al. (1995)
5	Native *Candida rugosa* lipase	Methanol (1:2)	30 °C, 150 rpm, silica gel (desiccant), 200 h. Stereo = preference: *S*-ibuprofen	Various (carbon tetrachloride, xylene, cyclohexane, n-hexane, n-heptane, n-octane, etc.)	CCl_4: *X*%: 44.7; *E*: 492 Xylene: *X*%: 45.3; *E*: 510 Cyclohexane: *X*%: 44; *E*: 473 *n*-Hexane: *X*%: 42.2; *E*: 433 *n*-Heptane: *X*%: 46.5; *E*: 557 n-Octane: *X*%: 45.4; *E*: 514	Kim et al. (1996)
6	Lipozyme® IM (*Rhizomucor miehei* lipase)	1-butanol (1:1) 1-octanol (1:1)	(i) 1-Butanol, 37 °C, 72 h, non-controlled a_w Stereo-preference: *S*-ibuprofen (ii) Cyclohexane, 37 °C, 72 h, non-controlled a_w Stereo-preference: *S*-ibuprofen	Various (cyclohexane, isooctane, *n*-hexane, *n*-heptane, toluene, etc.)	(i) Alcohol = 1-butanol Cyclohexane. *X*%: 66; EF:0.92 Isooctane: *X*%: 75; EF: 0.78 *n*-Hexane *X*%: 73; EF:0.80 *n*-Heptane *X*%: 45; EF:0.90 Toluene *X*%: 55; EF: 0.70 (ii) Solvent = cyclohexane-1-butanol *X*%: 66; EF:0.99 eeR%: 99 -1-octanol *X*%: 34; EF: 0.98 eeR%:50	López-Belmonte et al. (1997)

(*continued*)

Table 1. Continued

Entry	Biocatalyst	Alcohol (profen:alcohol molar ratio)	Reaction conditions	Co-solvent	Activity and enantioselectivity	References
7	Novozym® 435	1-Dodecanol (1:0.75)	22 °C, controlled water activity $a_w = 0.04$–0.8. Stereo-preference: *R*-ibuprofen	Various	$V_R/V_S > 4$, $a_w = 0.04$ $-1 \leq \log P \leq 3$	Ducret et al. (1998)
8	Native *Candida rugosa* lipase	1-Butanol (1:1)	40 °C, water, celite (desiccant), 180 rpm. Stereo-preference: *S*-ibuprofen	Isooctane	$X\% = 50$ eeR%: 78.9 ee% product (*S*-ester): 95.3	Xie et al. (1998)
9	Novozym® 435	Decanol 1-Dodecanol 1-Tetradecanol 1-Hexadecanol (1: 1.33)	55 °C, controlled water activity $a_w = 0.05$–0.74. Profen dissolved in the alcohols at 70 °C	Solventless	$a_w = 0.10$, $V_R/V_S = 3.3$ $a_w = 0.03$, $V_R/V_S = 9.2$	Pepin et al. (1999)
10	*Candida rugosa* over Accurel MY lipase	N-Morpholinoethanol (1:1–1:7)	25–60 °C non-controlled a_w Stereopreference: *R*-ibuprofen	Cyclohexane	Specific activity $= 8 \times 10^{-3}$ μmol/h mg enzyme $E = 60$ $X\% = 40$, ee% = 60	Chen et al. (2000)
11	Lipozyme® IM20	1-Butanol (1:1.9)	Packed bed reactor, 35–45 °C, 120 h Stereo-preference: *S*-ibuprofen	water saturated isooctane	$X\%$: 45.5 ee%: 93.9 E: 113	Sánchez et al. (2000)
12	Lyophilized enzyme (lipase of various micro-organisms)	1-Propanol (1:1)	30 °C, non-controlled a_w Vigorous magnetic stirring Stereo-preference: depends on the lipase source	Isooctane	Lipase of *O. sulphureo-ochraceum* Stereo = preference: *S*-ibuprofen $X\% = 70.4\%$ (336 h) ee substrate (*R*-ibu)% = 65% Lipase of *F. oxysporum* Stereopreference: *S*-ibuprofen $X\% = 84.2$ (264 h) ee (*R*-ibu)% = 100	Cárdenas et al. (2001)
13	Native *Candida rugosa* lipase and immobilized over cellulose acetate-titanium isopropoxide gel fiber	1-Propanol (0.5:1) (1:2)	30/40 °C, 80 stokes/min, non-controlled a_w. Stereo-preference: *S*-ibuprofen	Water saturated isooctane	Native CRL 30 °C, 63 h: $X\% = 42.6$, $E = 4.7$ 40 °C, 40 h: $X\% = 37.2$, $E = 3.5$ Immobilized CRL 30 °C, 110 h: $X\% = 35.3$, $E = 12.9$ 40 °C, 94 h: $X\% = 27.4$, $E = 8.8$	Ikeda & Kurokawa (2002)
14	*Candida rugosa* immobilized over polypropylene (EP100®)	1-Butanol (1:1)	37 °C, 250 rpm. non-controlled a_w Stereopreference: *S*-ibuprofen	Isooctane	$X\% = 51\%$ (47 h) ee product % (*S*-ester) = 93 $E = 59$	López et al. (2002)
15	Native *Pseudomona* sp. and immobilized over polyamide membranes	Ethanol/*n*-propanol (1:10)	pH 7.2 controlled with buffer phosphate 0.1 M, 37 °C Stereo-preference: *R*-ibuprofen	*n*-Hexane	Ethanol $X\% = 25$ (24 h), ee% (*R*-ester) = 95, $E = 53$ 1-propanol $X\% = 19$ (24 h), ee% (*R*-ester) = 87, $E = 17$ Combined process of esterification followed by hydrolysis: Product% ee (*R*-ester) and E200 >99%	Ceynowa et al. (2003)

16	*Candida rugosa*	1-Propanol (1:3)	35 °C, 190 rpm, 24 hs	Isooctane [BMIM]PF_6 [BMIM]BF_4, [BMIM]$MeSO_4$, $(C_6H_{13})_3C_{14}H_{29}PN_3(C_4H_9)_3CH_3PTOs$, [MMIM]$MeSO_4$, [BMIM]$OctylSO_4$	Best results obtained with [BMIM][PF_6]: $X\% = 30$, ee *S*-ibu $\% = 38$, $E = 24.1$	Hongwei et al. (2005)
17	Commercial Novozym®435, Lipozyme RM, Lipozyme TL and CRL®. Native lipases from *Aspergillus niger* AC-54, *Aspergillus terreus*	1-Propanol (1:3)	35 °C, 350 rpm, 48 hs Mixtures isooctane-IL (1:1 v/v)	Pure isooctane and mixed with ionic liquids such as, [BMIM][PF_6] and [BMIM][PF_4]	Best results obtained with [BMIM][PF_6]/isooctane mixture: *Aspergillus niger* AC-54 $X\% = 12$, ee *S*-ibu $\% = 8$, $E = 4.6$ Novozym®435 $X\% = 35$, ee *S*-ibu $\% = 24$, $E = 3.3$ CRL® $X\% = 48$, ee% (*S*-ibu) $= 60$, $E = 8.5\%$	Contesini et al. (2006)
18	Novozym®435, CRL®, various native lipases	1-propanol (1:1)	180 rpm, 35 °C. with and without silica gel (desiccant) Stereo-preference: (i) *R*-ibuprofen: Novozym®435, lipases of *Aspergillus niger y terreus*. (ii) *S*-ibuprofen: CRL®.	anhydrous isooctane	Novozym®435 $X\% = 64.0$ (54 h), ee% (*S*-ibu) $= 82.6$; $E = 6.7$ CRL® (5 mg): $X\% = 53.0$ (54 h) ee% (*R*-ibu) $= 77.8$; $E = 12.0$ Lipasa *Aspergillus niger* (5 mg): $X\% = 25$ (162 h) ee% sustr (*R*-ibu) $= 19.9$; $E = 4.8$ CRL® (15 mg), silica gel: $X\% = 37$ (24 h) ee% (*R*-ibu): 49; $E = 20$ Lipase of *Aspergillus niger* (30 mg), silica gel (0.5% w/v): $X\% = 36$ (24 h) ee% (*R*-ibu) $= 46$, $E = 15$	De Oliveira Carvalho et al. (2006a)
19	Native *Aspergillus niger* AC-54 lipase	1-Propanol (1:3–3:1)	35 °C, 168 h, 180 rpm non-controlled a_w Stereo-preference: *R*-ibuprofen	Various (isooctane, hexane, toluene, chloroform, dichlorometane, diethyl eter, acetone)	$X\% = 48.0$ ee% substrate (*S*-ibu) $= 79.1$, $E = 32$	De Oliveira Carvalho et al. (2006b)
20	Immobilized lipase A and B of *Candida antarctica* (commercial SP382)	Primary alcohols C10-C18. (from 1:0.3 to 1:1.5)	70 °C, reduce pressure (5 mmHg) for water removal	Solventless	No effect of the chain length. $X\% \sim 60$ ee $S(+) = 95\%$	Ergan et al. (2006)
21	Native *Candida rugosa* lipase	1-Octanol (1:1), (1:2), (1:4), (1:8) and (2:1) 1-hexanol, 1-decanol (1:2)	30/40 °C, Celite®545 (desiccant) 24 h, with and without pervaporation. Stereo-preference: *S*-ibuprofen	Isooctane	1-octanol (1:1) $E = 400$, $v_0 = 100$ with pervaporation $E = 350$, $v_0 = 50$ without pervaporation 1-octanol (1:8) $E = 90$, $v_0 = 20$; $E = 100$, $v_0 = 20$ 1-hexanol (1:2) $E = 280$, $v_0 = 25$; $E = 140$, $v_0 = 20$ 1-octanol(1:2) $E = 480$, $v_0 = 45$; $E = 380$, $v_0 = 40$ 1-decanol (1:2) $E = 405$, $v_0 = 45$; $E = 390$, $v_0 = 40$	Won et al. (2006)

(*continued*)

Table 1. Continued

Entry	Biocatalyst	Alcohol (profen:alcohol molar ratio)	Reaction conditions	Co-solvent	Activity and enantioselectivity	References
22	*Candida* sp. immobilized on pre-treated textile	Ethanol/*n*-propanol/*n*-butanol/2-methyl propan-1-ol (1:1)	180 h, 30 °C, Na_2SO_4/$Na_2SO_4 \cdot 10H_2O$ (500 mg) $a_w = 0.83$ Stereo-preference: *S*-ibuprofen	Various	Ethanol/hexane $v_0 = 111.7$ (μmol/h g), $E = 6.1$, t(eq) $= 333$ h 1-Propanol/hexane $v_0 = 173.9$, $E = 16.0$, t(eq) $= 120$ h, ee product $= 0.59$, $X\% = 60$ 1-Butanoll/hexane: $v_0 = 171.7$, $E = 17.1$, t(eq) $= 192$ h, ee products $= 0.55\%$, $X\% = 60$ 2-methylpropan-1-ol/hexane: $v_0 = 191.4$, $E = 19.4$, t(eq) $= 216$ h eeproducto $= 0.56\%$, $X\% = 60$ best system: 1-propanol/isooctane $E = 17.4$ and $K_{eq} = 0.016$ no enzyme activity in hydrophilic organic solvents	Liu et al. (2009)
23	Novozym® 435	Ethanol (1:7.08)	45 °C, 200 rpm, 48 h, non-controlled a_w Stereo-preference: *R*-ibuprofen	Solventless	$X\% = 62$ ee *S*-ibu% = 50	Foresti et al. (2009), José et al. (2010)
24	*Aeropyrum pernyx* K1 thermophilic esterase	Best results with 1-octanol (1:1)	Optimum temperature = 60 °C, optimum $a_w = 0.12$ Celite (desiccant) Stereo-preference: *R*-ibuprofen	Various	Activity: 216.5 μmol/h g $E = 38.1$ Equilibrium: 96 h $X\% = 57$, ee% *S*-ibu = 99	Dan-tong et al. (2011)
25	*Candida rugosa* lipase immobilized over magnetic beads	n-Propanol (1:3)	37°C, 600 rpm, 144 h. Controlled a_w with Na_2SO_4/$Na_2SO_4 \cdot 10H_2O$ and sieves molecular	Cyclohexane	Non-controlled a_w $X\% = 43.1$, $ee_p\ \% = 47.5$, $E = 4.0$ Controlled a_w $X\% = 42$, $ee_p = 83\%$, $E = 19$ Stable enzymatic activity for 5 cycles (30 days)	Marszałł & Siódmiak (2012)*
26	Novozym® 435	Ethanol (1:1), (1:1.4), (1:2), (1:4), (1:6), (1:7)	45 °C, 48 hs, with and without co-solvents added. 1-propanol and 2-propanol also tested	Acetonitrile, isooctane, *n*-hexane, carbon tetrachloride, ethyl acetate, tetrahydrofuran	(1:1) Profen: ethanol molar ratio with hexane, $X\% = 49$ and ee *S*-ibu% = 36 (1:7) Profen: ethanol molar ratio with isooctane, $X\% = 56$ and ee *S*-ibu% = 41	José et al. (2014)

X%: conversion; ee *S*-ibu %: enantiomeric excess towards the S-ibuprofen; ee *R*-ibu %: enantiomeric excess towards the *R*-ibuprofen; E: enantiomeric ratio; EF: enantiomeric factor, v_0: initial rate (μmol/h g of lipase).

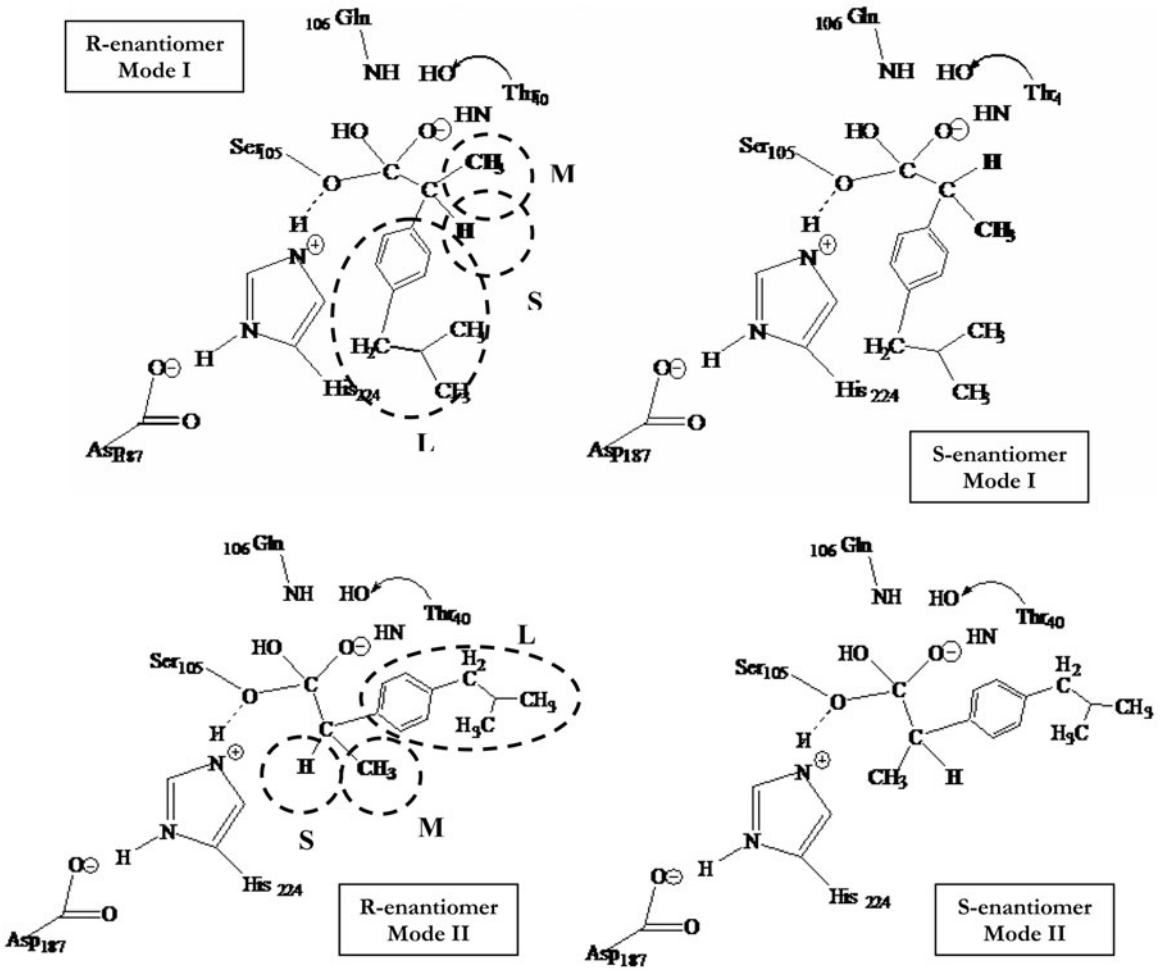

Figure 1. Binding modes of the (*R*) and (*S*) enantiomers of ibuprofen at the active site of CALB in order to for the acyl–enzyme complex. Taken form reference Foresti et al. (2009).

release of water. Figure 1 shows the different bindings of the two enantiomers in the active site of the lipase suggested by Foresti et al. (2009). The alcohol then interacts with the acyl–enzyme intermediate to produce a ternary complex that gives rise to the ethyl-ibuprofen and the free lipase. However, the calculations evidenced that the alcohol and water might irreversible interact with the histidine moiety of the catalytic triad prior to ibuprofen coordination, as shown in Figure 2. The irreversible adsorption of various alcohols was also experimentally proved through temperature programmed desorption which further enlightened the formation of non-reactive dead ends (spectator species; José et al., 2011). The authors also performed molecular modeling of the interaction of 2-propanol with *Candida antarctica* in order to understand the low yield observed in the esterification of racemic ibuprofen and ketoprofen with 2-propanol catalyzed with Novozym® 435 (Toledo et al., 2012). They found that the transfer of hydrogen from the 2-propanol to the His 224 (that forms the catalytic triad) may be sterically and electronically hindered if the methyl group of the adsorbed alcohol is located near the His. This situation requires a change of conformation in the alcohol to produce the reaction (Figure 3). On the other hand, the low activity observed was attributed to the higher adsorption stability of 2-propanol near the acyl enzyme in the Ping Pong Bi Bi mechanism for the *R*(−)-enantiomer. In other words, the adsorption of 2-propanol is less stable in the case of the acyl enzyme of the *S*(+)-enantiomer than the acyl enzyme of *R*(−)-enantiomer.

These investigations allow an understanding of the interaction of both the profens and the alcohols with the enzyme at a molecular level. The investigations summarized in the Table 1 indicate that the best enzymatic performance is obtained when linear primary alcohols are used as acyl acceptors. Tertiary alcohols and polyols do not show activity. However, the investigations concerning the catalytic activity with secondary alcohols are somewhat contradictory (Table 1) (Arroyo Sánchez, 1995). Various investigations demonstrated the influence of the chain length of linear primary alcohol in the catalytic performance of lipases in the enantiomeric esterification of racemic ibuprofen. In general, the authors observed that the lower the number of carbons of the alcohols the higher the catalytic activity, being 1-propanol the optimum acyl acceptor (Table 1). An early report by Pepin & Lortie (1999) determined that only the rate of esterification of the *R*(−)-enantiomer was influenced by the length of the alcohol chain and the water activity when Novozym® 435 was used. However, this dependency was not observed when a

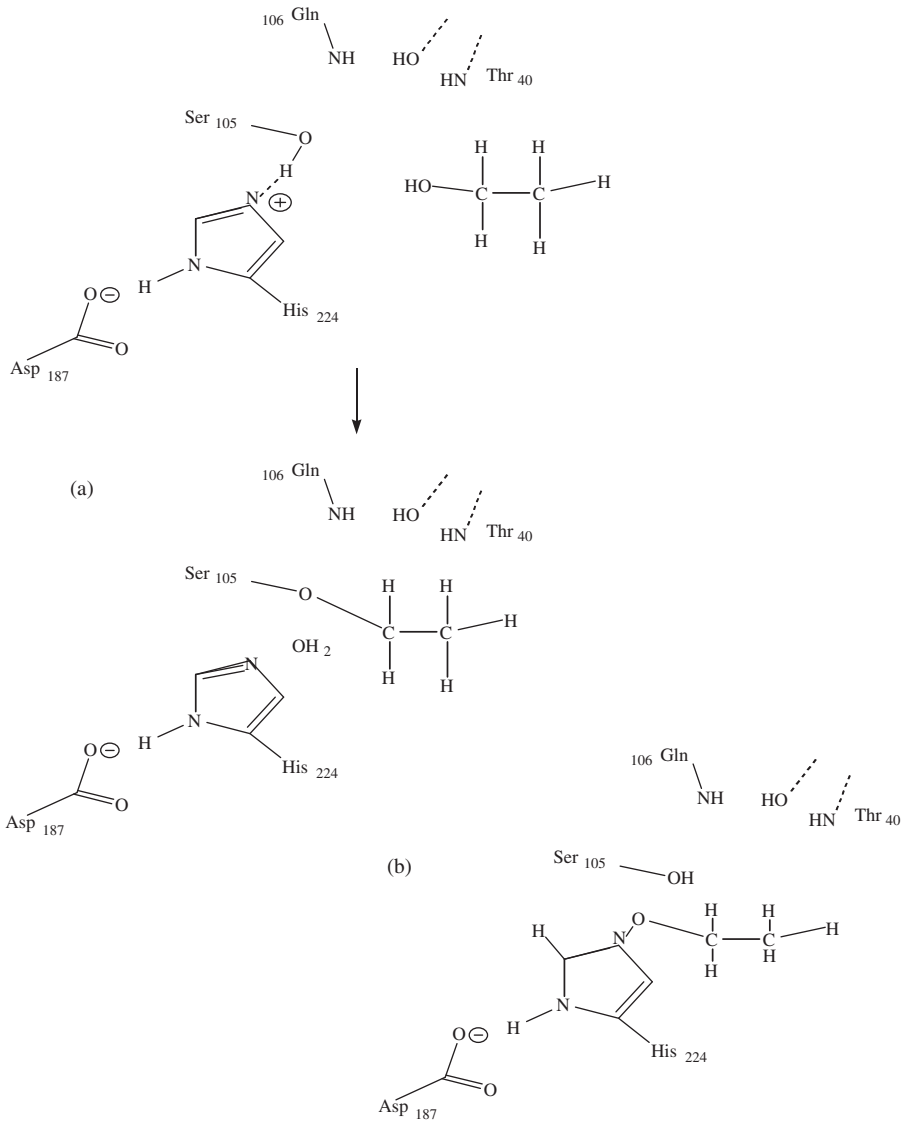

Figure 2. Inhibition by ethanol prior to ibuprofen coordination. Formation of dead-end complexes due to reaction of ethanol with amino acids of the catalytic triad. (a) Reaction with serine and (b) reaction with histidine. From reference Foresti et al. (2009).

mixture of the lipases A and B from *Candida antarctica* was investigated in the solventless esterification of ibuprofen with primary alcohols with 10–18 carbon atoms.

The investigations reported by Foresti et al. (2009) and José et al. (2014) concerning the kinetic resolution of racemic ibuprofen through esterification with ethanol as reactant and solvent are innovative. The authors performed the enantiomeric esterification at 45 °C in contrast with previous investigations in solventless media carried at 55–70 °C. The authors reported a 62% conversion of racemic ibuprofen with a 50% enantioselectivity towards the *S*(+)-enantiomer although some drawbacks in the physical integrity of Novozym® 435 were demonstrated (José & Briand, 2010; José et al., 2011).

Kinetic resolution of racemic ibuprofen in non-conventional media: co-solvents added

Although there is abundant literature regarding the effect of several solvents in enzymatic reaction systems to date, the clear or general trends have not yet been reported. In general, observations on the esterification catalyzed by lipases in organic media suggest that higher activities and selectivities are obtained with hydrophobic co-solvents. The hydrophobicity of a solvent is represented with the value of log *P* that is the logarithm of the distribution constant of the solvent in the two phase octanol–water system. In this context, the best values of conversion and enantioselectivity are reported with solvents with log *P* equals or higher than 2, and very low activity or no reaction for values of log *P* lower than 2 (Table 1) (Arroyo Sánchez, 1995). However, heterogeneous behaviors are reported for both regions of this parameter. In this sense, López-Belmonte et al. (1997) and Ceynowa & Rauchfleisz (2003) observed a linear dependence of the conversion with no trend in the enantioselectivity for log $P \geq 2$ (entries 5 and 15). Moreover, Liu et al. (2009) and Kim & Lee (1996) observed no trend for any of the reaction parameters with the nature of the solvent in the esterification of *R*/*S*-ibuprofen with various alcohols using *Candida rugosa* lipase and *Candida* sp., respectively (entries 5 and 22). The literature also reports exceptions in the presence of certain solvents with low log *P* values (entries 1 and 24; Arroyo Sánchez, 1995). In this regard, Dan-tong et al. (2011) demonstrated that the optimum conversion in the esterification of ibuprofen with 1-octanol catalyzed with APE1547

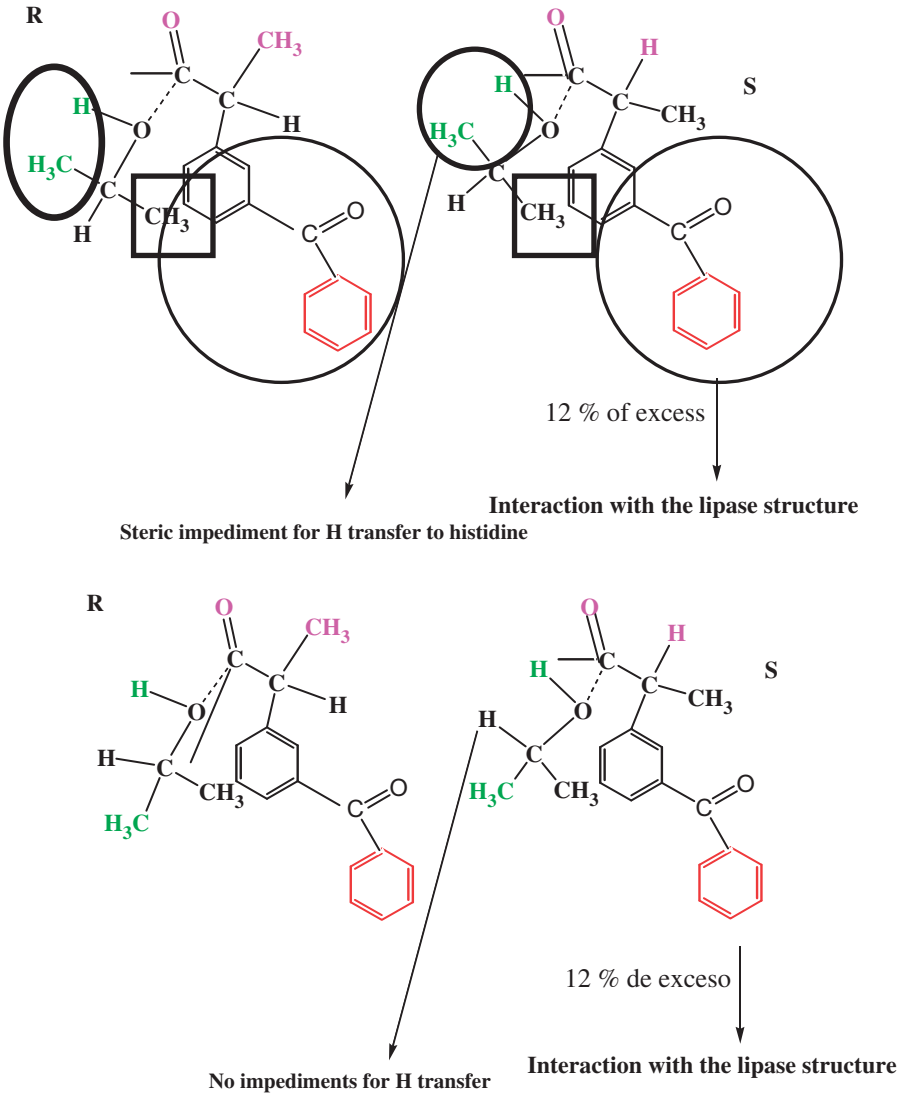

Figure 3. Representation of the interaction of the *S*(+)-ketoprofen, *R*(−)-ketoprofen and 2-propanol with the active catalytic triad of the lipase B of *Candida antarctica*. From reference Toledo et al. (2012).

(immobilized esterase) was reached when using solvents with intermediate values of log $P \sim 2.5$ (entry 24).

Persson et al. (2002) studied the esterification of *R*(−)-2-phenylpropionic and *S*(+)-2-phenylpropionic acids with 1-heptanol using catalysts based on lipases of various origins. The authors demonstrated that the enantioselectivity of Novozym® 435 was independent of the log *P* of the solvent. In contrast, immobilized *Candida rugosa* lipase showed an increase of enantioselectivity for increasing log *P* values of the co-solvent.

The numerous reports on this matter mostly exhibit experimental results without further explanation of the observed phenomena. At first, it was postulated that the more hydrophilic solvents have greater ability to remove water molecules essential to the enzyme molecules thus affecting its activity (Klibanov, 1997; Laane et al., 1987). However, Liu et al. (2009) demonstrated that *Candida* sp. was not active in the esterification of ibuprofen with 1-propanol in the presence of hydrophilic solvents even under controlled water activity. This observation means that somehow the hydrophilic solvents exert additional effects (along with water stripping) on the enzyme that inhibits its catalytic activity.

More recently, we reported the effect of several organic co-solvents in the enantiomeric esterification of racemic ibuprofen with ethanol catalyzed with Novozym® 435. The results demonstrated that the best conversion (60%) and enantioselectivity towards *S*(+)-ibuprofen (51%) is achieved with an excess of ethanol instead of the addition of a co-solvent regardless of its nature.

The investigations of Hongwei et al. (2005) and Contesini & De Oliveira Carvalho (2006) discuss the use of non-conventional solvents such as, ionic liquids in the kinetic resolution of ibuprofen. Both investigations concluded that the catalytic performance was enhanced and even extended in ionic liquids (particularly, 1-butyl-3-methyl imidazolium hexafluorophosphate [BMIM]PF_6) compared with isooctane. In this regard, Contesini & De Oliveira Carvalho (2006) reported that a 48% conversion and 60% enantiomeric excess towards *S*(+)-ibuprofen in the esterification of ibuprofen with 1-propanol with *Candida rugosa* lipase in a mixture of isooctane-[BMIM]PF_6. Similarly, Hongwei et al. (2005) found that the enantiomeric excess towards *S*(+)-ibuprofen was 38% in [BMIM]PF_6 compared with 33% in isooctane at a similar conversion (30%) in the esterification of ibuprofen with 1-propanol in excess.

To the knowledge of the authors, no investigations regarding the enzymatic esterification of racemic ibuprofen using the so called green solvents have been reported. This observation will be further discussed in the following section.

Esterification of ibuprofen in membrane reactors: shifting thermodynamics towards the desired enantiomer

A membrane reactor is a flow reactor that is fed continuously with the substrates (with or without the enzyme) that react within the reactor with a continuous separation of the product through the membrane. The membrane mediates between the reaction system and a flow of solvent that carries the product after diffusing through the membrane. These systems are particularly useful when the substrate and the product possess different solubility therefore the solvent used in the feeding stream is different from the one carrying the product out of the reaction system.

Membranes are classified into microfiltration with a pore size in the range of 0.1–10 mm, ultrafiltration with a molecular weight cut off (MWCO) between 0.5 and 500 kDa, and reverse osmosis membrane suitable for low-molecular weight solute. Enzymes with sizes between 10 and 500 kDa are not retained by ultrafiltration membranes that are commercially available with a typical MWCO of 10 kDa (Long et al., 2002).

The enzyme molecules may be used in a freely circulating mode or immobilized onto the membrane surface or porous structure. In this last mode, the immobilized enzyme would increase its stability and resistance towards organic solvents (Ríos et al., 2004). This is the case of lipases (*Pseudomonas* sp., *Candida cylindracea*, *Porcine pancreas*) immobilized on an asymmetric polyamide capillary membrane that exhibited a stable activity in 500 hs of operation in the esterification (with various alcohols) and trans-esterification (with vinyl acetate) of 2-arylpropionic acids such as, (±)2-(2-fluoro-4-biphenyl)propanoic and ibuprofen (Ceynowa & Rauchfleisz, 2003). The esterification of ibuprofen was accomplished in the EMR with *Pseudomonas* sp. achieving 25% conversion with 95% enantiomeric excess towards the product (the *R*-enantiomer ester) when ethanol was used as the achiral substrate.

Another membrane based setup used in the esterification of racemic ibuprofen is a novel liquid type of membrane with ionic liquids (Lozano et al., 2011). These systems known as supported ionic liquid membranes (SILMs), are composed by a porous support (polymeric membrane) impregnated with ILs. In this particular application, an aqueous feeding mixture of racemic ibuprofen with ethanol and *Candida rugosa* produces the ethyl ester of the *S*(+)-enantiomer that flows towards the SILM. The membrane selectively allows only the ibuprofen ethyl esters to diffuse through it and rejects the *R*(−)-ibuprofen (Figure 4). The ethyl ester is then hydrolyzed with the lipase from porcine pancreas and the *S*(+)-ibuprofen is recovered (Miyako et al., 2003).

EMR inside microfluidic devices is the ultimate application of this technology in the kinetic resolution of ibuprofen and separation of the product in one step. In this context, El-Feky et al. (2013) developed a setup composed by a

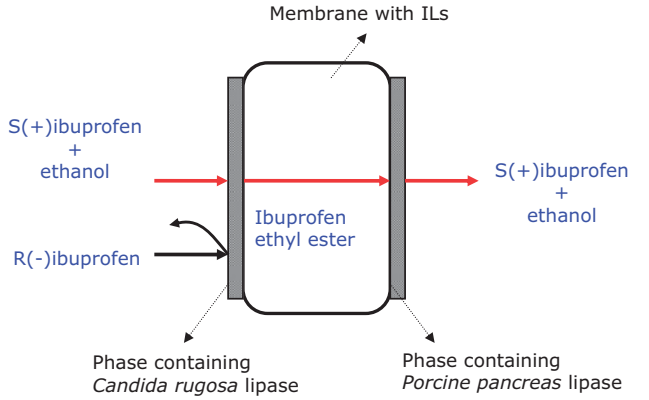

Figure 4. Schematic representation of a membrane containing ILs adapted from Miyako et al. (2003).

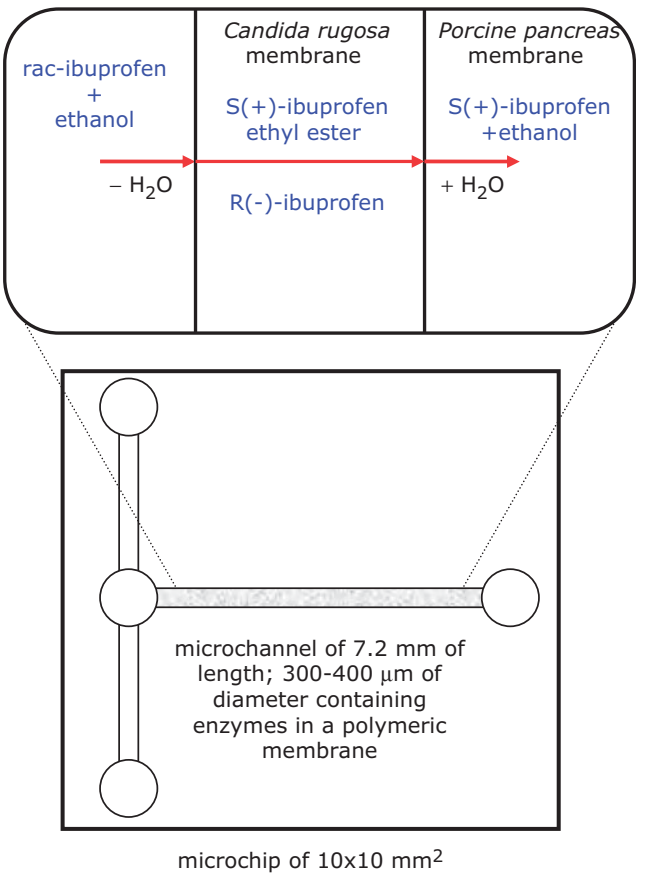

Figure 5. Schematic representation of a micro-fluidic device and the transport of ibuprofen within the polymeric membrane containing enzymes. Adapted from El-Feky et al. (2013).

pre-membrane solution containing either hydroxy-methyl methacrylate, 2-aminoethylmethacrylate or 2-hydroxy-2-methylpropiophenone with the crosslinker ethylene dimethacrylate that is polymerized inside a microchannel of 7.2 mm long and 300–400 µm of diameter held by a microchip of $10 \times 10\,mm^2$ as can be observed in the Figure 5. The lipase *Candida rugosa* was adsorbed onto the polymeric membrane after the formation of the membrane. The microfluidic device feed with a mixture of 65% v/v of ethanol and 10 mM of racemic ibuprofen was able to preferentially esterify the *S*(+)-enantiomer. The *S*(+)-ibuprofen ethyl ester was further hydrolyzed with a receiving phase composed by the lipase

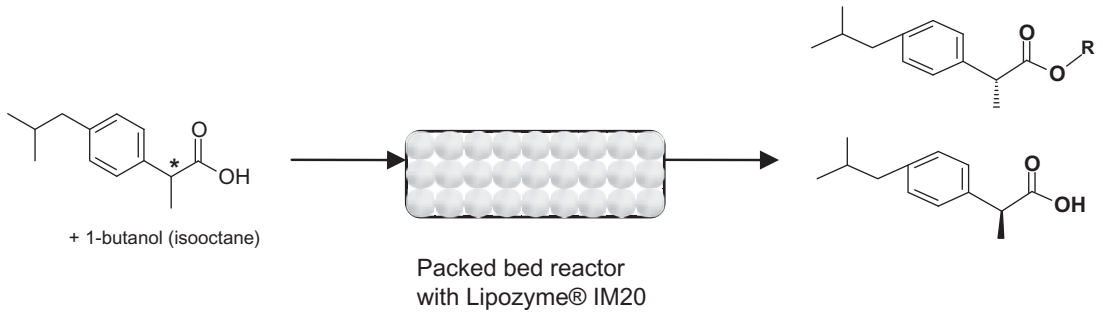

Figure 6. Schematic representation of the esterification of racemic ibuprofen with butanol in a packed bed reactor with Lipozyme® IM20 from reference Sanchez et al. (2000).

from porcine pancreas. This setup allowed obtaining a 40% enantiomeric excess towards the *S*(+)-ibuprofen.

Future perspectives

This review of the literature shows that the kinetic resolution of racemic ibuprofen through enantiomeric esterification with various alcohols and organic co-solvents has been investigated in detail. However, it is worth noticing that, to the knowledge of the authors, there is no report regarding the use of ''green solvents''. Green solvents such as 2-methyltetrahydrofuran, cyclopentyl methyl ether; *N,N′*-dimethylpropyleneurea; 1,3-propanodiol and 1,3-dioxolane are known as excellent substitutes of tetrahydrofuran, diethyl ether, dioxane, methyl tert-butyl ether; 1,2-dimethoxyethane, dimethylformamide, dichloromethane, dichloroethane and methyl ethyl ketone in biotransformations. In addition, deep eutectic solvents (known as the third generation of ILs) are attractive as replacement of conventional ILs and organic co-solvents due to their ease of preparation, biodegradability and low cost (Dominguez de María & Maugeri, 2011). These systems are composed of a quaternary ammonium salt, natural bases (choline chloride), amino acids and carboxylic acids. Recently, Maugeri et al. (2012) reported the separation of mixtures of an alcohol and its ester, alcohol–ketone and amine–ketone using a DES of choline chloride and glycerol. The authors proved a high recovery of the ester (83% in three extractions) that is dissolved preferentially in the upper phase formed using the DES. To the best of our knowledge, this novel idea has not been proved with the products of the esterification of racemic ibuprofen with alcohols.

Carbon dioxide at supercritical conditions (above the critical temperature and pressure) has also been used as an environmental benign solvent in bio-transformations since its low supercritical parameters (31 °C and 73.8 bar) are tolerated by enzymatic catalysts (Hoobs & Thomas, 2007). Moreover, supercritical CO_2 is a non-toxic, non-flammable, inexpensive and non-volatile organic compound. The esterification of racemic ibuprofen with ethanol catalyzed with Novozym® 435 under supercritical CO_2 reached 61% of enantiomeric excess at 45 °C (Hoobs & Thomas, 2007; Overmeyer et al., 1999). The esterification with *n*-propanol as acyl acceptor with supercritical CO_2 as solvent was also reported (Rantakylä & Aaltonen, 1994).

Another innovative strategy in continuing growth is the use of continuous-flow reactors in the kinetic resolution of racemic profens (Itabaiana et al., 2013). Typical continuous operation requires a tubular packed bed reactor such as a stainless steel HPLC column filled with the biocatalyst that works as a plug-flow reactor. The reactants are feed into the reactor in the liquid form with a pump at an accurate flow rate that in turn determines the residence time of the reactants and products with the biocatalyst. This is the case of the reactor reported by Sánchez et al. (2000) in the esterification of ibuprofen with butanol in isooctane (Table 1 and Figure 6). The authors compared the yield of a typical stirred (batch mode) versus the continuous plug-flow reactor filled with the commercial Lipozyme biocatalyst. They were able to obtain 93.9% of enantiomeric excess towards *S*(+)-ibuprofen with 45.5% of conversion in a continuous operation for 100 h. Chen & Tsai (2000) reported esterification with N-morpholinoethanol in a packed-bed reactor filled with the lipase MY (*Candida rugosa* type of lipase) immobilized on Accurel MP1000 (polypropylene hydrophobic support) (Table 1).

Conclusions

The investigation concerning the kinetic resolution of ibuprofen through enantiomeric esterification in the past 20 years has been extensively reviewed. There is no doubt that the esterification with various alcohols as acyl acceptors with the use of conventional organic co-solvents has been the focus of most of the investigations. However, there are no reports on the use of the so-called ''green solvents'' as co-solvents. Although, this observation might be related to operative difficulties such as the solubility of ibuprofen on those solvents there is no data on this matter. Nevertheless, the use of alcohols as reactants and solvents has recently been explored and is promising.

The confinement of immobilized enzymes in a packed bed reactor for continuous operation is an emerging technology. In this context, there is more work to be conducted in order to develop an integrated technology of esterification and separation of the desired enantiomer in the same operation.

Finally, broadening of the materials used as supports is another opportunity to find a sustainable technology to the opinion of the authors. Solid wastes from the agro-industry (between others) as an enzyme support might be cutting edge research that has not been explored in the kinetic resolution of ibuprofen.

Declaration of interest

The authors report no declaration of interest.

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