VIA MEDICA

ORIGINAL ARTICLE

Joanna Siódmiak[1], Tomasz Siódmiak[2], Michał Falkowski[3], Adam Sikora[2], Michał Piotr Marszałł[2]

[1]Department of Laboratory Medicine, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland
[2]Department of Medicinal Chemistry, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Poland
[3]Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Poznan, Poland

Application of green chemistry in decreasing adverse effect of (*R*,*S*)-ibuprofen

Corresponding author:

Joanna Siódmiak
Department of Laboratory Medicine,
Collegium Medicum,
Nicolaus Copernicus University
Collegium Medicum
Sklodowskiej-Curie Street No. 9,
85–094 Bydgoszcz, Poland
Phone: +48 52 585 44 90
Fax: +48 52 585 36 03
E-mail: asiapollak@wp.pl

Folia Medica Copernicana 2015;
Volume 3, Number 2, 55–61

ISSN 2300–5432

ABSTRACT

Lipases from *Candida rugosa* (OF and MY) were tested for their application in the enzymatic kinetic resolution of (*R*,*S*)-ibuprofen by enantioselective esterification. In this study, screening of enzymes was performed, and lipase MY was selected as an optimal catalyst, which allows to obtain products with high enantiopurity. Additionally, the influence of reaction time on the enantiomeric ratio and conversion was tested. High values of enantiomeric ratio (E in the range of 40.1–71.3) of the esterification of (*R*,*S*)-ibuprofen were obtained using lipase MY, which has a great significance in the field of pharmaceutical synthesis of drugs. The chiral compounds (substrates and products) were analysed with the use of chiral stationary phases. As a result of the optimization, the reaction performed with the application of lipase MY allowed to achieve less toxic for human health (*S*)-enantiomer of ibuprofen with the high enantiomeric excess of product ee_p = 95%. Conversion of the reaction was c = 30.6% and enantioselectivity E = 58.9 after 126 h of incubation.

Key words: *Candida rugosa* lipase, green chemistry, (*R*,*S*)-ibuprofen, kinetic resolution

Folia Medica Copernicana 2015; 3 (2): 55–61

Introduction

Nowadays, the development of new methods of chiral drugs obtaining represents a major challenge for the pharmaceutical sciences. The REACH regulations (Registration, Evaluation, Authorisation and Restriction of Chemicals) and environmental legislation approved in the last decade by the United States, Japan and the European Union contributed to the discussion about the future direction of the chemical and pharmaceutical industries. Green Chemistry philosophy of promoting the use of environmentally safer chemicals and production conditions has a large impact on the ecological trend in the pharmaceutical synthesis [1].

Recently, biotechnological methods for the preparation of enantiomers using enzymes as catalysts have become an intensive study area in the pharmaceutical sciences. The use of these methods is advantageous, from an environmental and economic point of view, because enzymatically catalysed reactions do not require drastic conditions, which significantly reduces their toxicity. They also allow to simplify the procedures by omitting a number of steps in the enantioselective synthesis.

The latest reports of the American Food and Drug Administration (FDA) indicate that currently more than a half of the total investigational new drugs (INDs) are the optically pure compounds. This significant percentage of tested enantiomers translates into wide commercial availability. In the United States, eight out of top ten best selling drugs are chiral compounds in the form of a single enantiomer [2].

2-arylpropionic acids (profens) are known as the major nonsteroidal anti-inflammatory drugs (NSAID) widely used in the treatment of headache, rheumatoid arthritis, cephalgia or muscular strain. All those profen drugs have the chiral carbon atom within the propionic acid moiety. The kinetic resolution of profens is important from the pharmacological point of view because enantiomers of these drugs demonstrate different therapeutic activities. One of the most frequently used drugs within this therapeutic group is (*R*,*S*)-ibuprofen. The (*S*)-enantiomer of this medicine is about 160 times more active than its (*R*)-enantiomer in *in vitro* inhibition

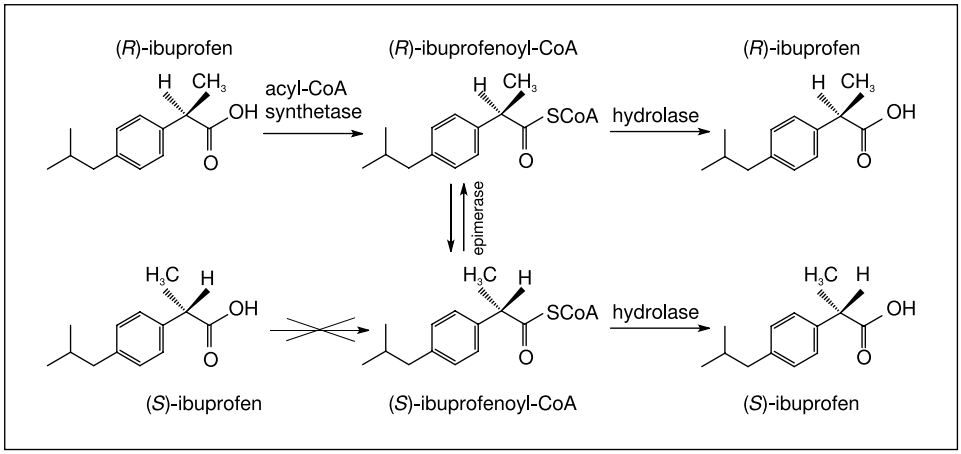

Figure 1. Metabolic chiral inversion of (*R*,S)-ibuprofen

of prostaglandin synthesis. (*S*)-ibuprofen inhibits equally both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), while (*R*)-ibuprofen is much less potent than (*S*)-enantiomer in inhibiting the activity of COX-1, and even less towards COX-2. It should be emphasized that (*R*)-ibuprofen contributes to increased side effects affecting the gastrointestinal tract, normal lipid metabolism and the membrane function [3–7].

It has been shown that in the presence of coenzyme A (CoA), adenosine 5'-triphosphate (ATP) and Mg^{2+}, the therapeutically inactive (*R*)-enantiomer is converted to the active (*S*)-isomer (Fig. 1). The reaction occurs in three steps: the synthesis of the (*R*)-ibuprofen thioester with coenzyme A, racemization of the resulting thioester and hydrolysis. The first step is based on the creation of thioester from (*R*)-ibuprofen and coenzyme A, catalysed by acyl-CoA synthetase. Next, due to the action of epimerase 2-arylpropionic-CoA, racemization (epimerization) occurs to form a mixture of (*R*)- and (*S*)-thioesters. The final step is the hydrolysis of the resulting thioester to (*R*)- and (*S*)-enantiomers of ibuprofen, catalysed by the hydrolase. It is estimated that the degree of chiral inversion of (*R*)-ibuprofen in humans ranges from 35% to 70% as a result of the physiological condition of the liver and the pharmacological treatment.

Received from the coenzyme A and ibuprofen thioesters, the compound may incorporate into triglycerides or phospholipids and form so-called 'hybrids'. The estimated elimination half-life ($t_{1/2}$) of the formed 'hybrids' in adipose tissue is about 7 days. In the performed studies, in which the pure enantiomers of ibuprofen were given to the patients, it was shown that (*R*)-enantiomer accumulated in adipose tissue, whereas this phenomenon was not observed in the case of the (*S*)-isomer. Currently, there are studies conducted in order to explain the effect of forming the 'hybrids' in the adipose tissue and their long $t_{1/2}$ in the human body. It is believed that the formation of 'hybrids' with phospholipids may change the structure and function of the cell membranes [8, 9].

It has been shown that oral administration of (*S*)-ibuprofen allows to obtain stronger analgesic effect in a shorter time comparing to the racemic mixture containing the same amount of the active enantiomer. Moreover, the results of the study on a group of 1,400 patients showed higher efficiency and significant reduction of therapy side effects when (*S*)–ibuprofen was administered [10, 11].

In the present study, the analysis of lipase from *Candida rugosa*, OF and MY, for the resolution of (*R*,*S*)-ibuprofen and its butyl ester has been conducted. The tested enzyme-reaction systems were assessed as the potential technique to obtain less toxic (*S*)-enantiomer of ibuprofen. The chiral compounds achieved as a result of the application of lipases were analysed with the use of chiral stationary phases. The optimization of chiral chromatographic conditions involved the selection of stationary phases, mobile phase composition, flow rate, volume of the injected analytes and the temperature of chromatographic process.

Materials and methods

Chemicals

Racemic (*R*,*S*)-ibuprofen and pure *S*(+)-enantiomer were purchased from Sigma-Aldrich Co. (Poland). Cyclohexane, *n*-butanol, *n*-hexane, acetic acid, molecular sieves 4Å and sulphuric acid were purchased from POCH S.A. (Poland). Lipases OF from *Candida rugosa* (activity 380 000 units/g solid) and lipase MY from *Candida rugosa* (activity 32 000 units/g solid) were a gift from Meito Sangyo Co., LTD. (Japan). The (*R*)- and

(*S*)-esters of ibuprofen were obtained as the products of a standard esterification reaction of (*R*,*S*)-ibuprofen and (*S*)-ibuprofen with *n*-butanol using sulphuric acid (H_2SO_4) as a catalyst [12]. Water used in the study was prepared with the Milli-QWater Purification System (Millipore, Bedford, MA, United States). All incubations were performed at 37°C and with a fixed number of rotations (600 rpm) in Thermomixer comfort (Eppendorf, Germany). Cyclohexane was dried over molecular sieves.

Instrumentation

The Shimadzu HPLC system (Japan) used in the study was equipped with a pump, model LC-20AD; a UV-VIS detector, model SPD-20A; a degasser, model DGU-20A5; an autosampler, model SIL-20ACHT and a column oven, model CTO-10ASVP. A Lux Cellulose-1 (LC-1) (4.6 mm × 250 mm) column with cellulose tris(3,5-dimethylphenylcarbamate) as the chiral selector, a Lux Cellulose-2 (LC-2) (4.6 mm × 250 mm) column with cellulose tris(3-chloro-4-methylphenylcarbamate) as the chiral selector, a Lux Cellulose-3 (LC-3) (4.6 mm × 250 mm) column with cellulose tris(4-methylbenzoate) as the chiral selector and a Guard Cartridge System model KJO-4282 were purchased from Phenomenex (Torrance, United States). All columns had 5 μm particle sizes.

Chromatographic conditions

The effect of different compositions of mobile phase consisting of three compounds: *n*-hexane, 2-propanol and acetic acid on the separation selectivity of both substrates and products was investigated. Finally, the most appropriate chromatographic conditions were optimized with *n*-hexane/2-propanol/ acetic acid (99.6/0.4/0.15 v/v/v) mobile phase with the flow rate of 1 mL/min for *n*-butanol as the acyl acceptor. In order to obtain optimum HPLC conditions for separation of (*R*,*S)*-ibuprofen and its ester, three types of chiral chromatographic columns were investigated, including Lux Cellulose-1, Lux Cellulose-2 and Lux Cellulose-3. Lux Cellulose-2 and Lux Cellulose-3 did not prove to be suitable for this purpose because of the insufficient chromatographic resolution of enantiomers. The use of Lux Cellulose-1 (4.6 mm × 250 mm × 5 μm) HPLC column gave an enhanced resolution for the studied compounds. The chromatographic process was performed at 30°C, due to the better mass transfer and lower viscosity of the eluent. The detection UV wavelength was 254 nm.

The enantiomeric excesses of the substrate (ee_s) and the product (ee_p) as well as the conversion (c), enantiomeric ratio (also called enantioselectivity) (E) were calculated using the equations described in the literature [13–16].

The enantiomeric ratio (E) was calculated as follows:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \quad (1)$$

the ee_s and ee_p values were determined as:

$$ee_s = \frac{R-S}{R+S} \quad (2)$$

$$ee_p = \frac{R-S}{R+S} \quad (3)$$

For R > S

where S and R represent the chromatographic peak areas of the (S)- and (R)-enantiomers respectively. The quantities of ibuprofen and its ester were expressed by the value of the chromatographic peak areas.

The result values (ee_s and ee_p) were expressed in percentage using the following equations:

$$\%ee_s = \frac{R-S}{R+S} \times 100 \quad (4)$$

$$\%ee_p = \frac{R-S}{R+S} \times 100 \quad (5)$$

The conversion (c):

$$c = \frac{ee_s}{ee_s + ee_p} \quad (6)$$

Lipase-catalysed esterification of (R,S)-Ibuprofen

The reaction mixture was composed of cyclohexane (700 μL), racemic ibuprofen (0.02 mM) and *n*-butanol (0.06 mM) as the acyl acceptor (Fig. 2). The reaction was initiated by adding crude (free) lipase (8.75 mg) to the solution. The suspension was incubated at 37°C, shaken (600 rpm) in Thermomixer (Thermomixer comfort from Eppendorf Co.) for 126 h. Samples (50 μL) were withdrawn at several time intervals. The collected samples were dried by evaporation at room temperature and the residues redissolved in 0.7 mL mobile phase and, after filtration (0.45 μm), injected (25 μL) on the HPLC column.

Results

Influence of time reaction

Table 1 shows the enantiomeric excess of the substrate (ee_s), the enantiomeric excess of the product (ee_p), the conversion (c) and the enantioselectivity (E-value) as a function of time reaction. In this esterification reaction conducted at 37°C, lipases from *Candida rugosa,* OF and MY, were used as the catalysts in cyclohexane.

Lipase OF from *C. rugosa* has shown the highest conversion degree (c = 68.4% after 126h), with enantiomeric excess of substrate (ee_s) amounting to 98.7%. It should be emphasised that despite the high conversion

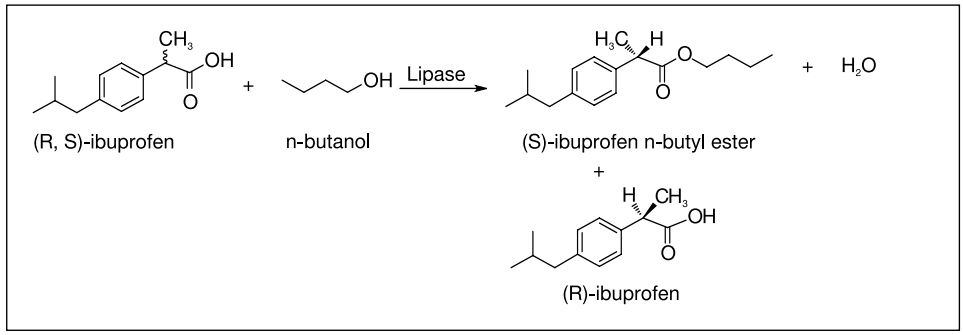

Figure 2. Biocatalyzed esterification of (*R*,*S*)-ibuprofen with *n*-butanol

Table 1. Influence of reaction time on the esterification reaction of (*R*,*S*)-ibuprofen catalyzed by *Candida rugosa* lipase

	Lipase MY				
Reaction time [h]	**ee_s (%)**	**ee_p (%)**	**c (%)**	**E**	**Alcohol**
30	7.3	95.1	7.2	42.9	*n*-butanol
54	14.2	96.8	12.8	71.3	
78	22.3	95.6	18.9	54.7	
102	30.9	93.5	24.8	40.1	
126	41.8	95.0	30.6	58.9	
	Lipase OF				
Reaction time [h]	**ee_s (%)**	**ee_p (%)**	**c (%)**	**E**	**Alcohol**
30	42.7	82.9	34.0	16.2	*n*-butanol
54	78.8	76.4	50.8	17.7	
78	94.1	67.7	58.1	17.8	
102	93.8	55.8	62.7	11.6	
126	98.7	45.6	68.4	11.8	

Reaction conditions: (*R*,*S*)-ibuprofen (0.02 mM), *n*-butanol (0.06 mM), lipase (8.75 mg), cyclohexane (0.7 mL), temp. 37°C; c-conversion, ee_s — enantiomeric excess of substrate, ee_p — enantiomeric excess of product, E — enantiomeric ratio

achieved by the use of this lipase, the E-value was not high. The obtained results indicate that lipase MY has the highest enantioselectivity (E = 58.9 after 126 h), with the enantiomeric excess of product ee_p = 95% and conversion c = 30.6%.

It was demonstrated that with the increasing time of the esterification reaction, it comes to an increase of conversion and enantiomeric excess of acid, both in various intensity depending on the applied lipase (Fig. 3, 4). It was also observed that the lipase with the highest conversion degree (OF) shows the lowest enantioselectivity (E = 11.6–17.8) in comparison to the lipase MY.

Effect of alcohol moiety

A suitable selection of alcohol moiety has a significant influence on the conversion degree and on the enantioselectivity of the esterification reaction. In this experiment, (*R*,*S)*-ibuprofen was esterified by the use of a primary alcohol. The study presented herein shows that *n*-butanol is a good substrate for the esterification of ibuprofen, due to the high conversion degree and enantiomeric ratio (Tab. 1). The application of a secondary alcohol as a substrate in the esterification reaction gives much lower values of conversion and enantiomeric ratio, in comparison to a primary alcohol [17]. Based on the results, it is essential to apply a primary alcohol in order to achieve high conversion degree and good enantioselectivity with the use of the lipase from *Candida rugosa* as a catalyst in the enantioselective esterification (Fig. 5).

Selection of lipases

Commercially available lipases from *Candida rugosa* were tested for their catalytic properties in the

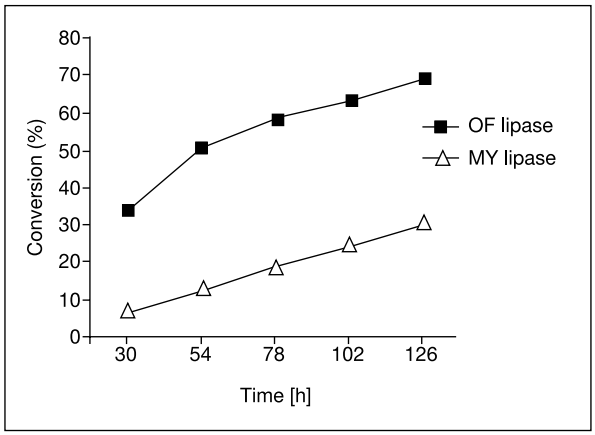

Figure 3. Influence of reaction time on the conversion of (*R*,*S*)-ibuprofen. Reaction conditions: (*R*,*S*)-ibuprofen (0.02 mM), *n*-butanol (0.06 mM), lipase (8.75 mg), cyclohexane (0.7 mL), temp. 37°C

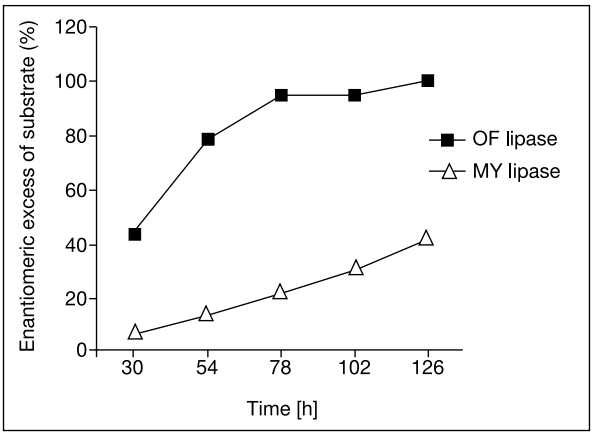

Figure 4. Influence of reaction time on the enantiomeric excess of (*R*,*S*)-ibuprofen. Reaction conditions: (*R*,*S*)-ibuprofen (0.02 mM), *n*-butanol (0.06 mM), lipase (8.75 mg), cyclohexane (0.7 mL), temp. 37°C

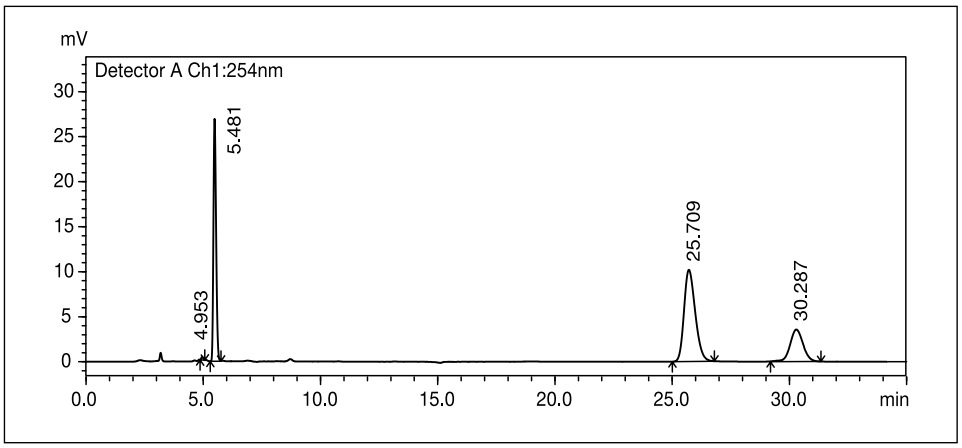

Figure 5. Representative HPLC chromatogram of ibuprofen and *n*-butyl esters of ibuprofen with retention time of *R*-enantiomer of ester (t_R = 4.953), *S*-enantiomer of ester (t_R = 5.481), *R*-ibuprofen (t_R = 25.709) and *S*-ibuprofen (t_R = 30.287); HPLC condition: Lux Cellulose-1 (4.6 mm × 250 mm × 5 μm) HPLC; mobile phase: *n*-hexane/ /2-propanol/acetic acid (99.6/0.4/0.15 v/v/v), F = 1mL/min, t = 30°C, UV = 254 nm, lipase MY, after 126 h

enantioselective esterification of racemic ibuprofen with *n*-butyl alcohol using cyclohexane as a solvent. The conducted study proved the ability of these lipases to perform the enantioselective catalysis of (*R*,*S*)-ibuprofen. All tested lipases preferentially catalysed the esterification of the (*S*)-enantiomer of ibuprofen, however, each of them demonstrated a different catalytic activity. The highest conversion degrees were obtained from lipase OF, the second one (lipase MY) exhibited lower performances in the same conditions of reaction (Tab. 1, Fig. 3). It should be noted, that values of enantiomeric ratio and enantiomeric excess of product were higher in reactions carried out with the use of lipase MY comparing to lipase OF (Fig. 6, 7). Therefore, the application of lipases from *Candida rugosa* in the enantioselective esterification of racemic ibuprofen requires a specific optimization of the reaction conditions, depending on the applied lipase. It should be emphasized that the reaction performed using lipase MY allowed to obtain less toxic for human health (*S*)-enantiomer of ibuprofen with the high enantiomeric excess of product (ee_p = 95%).

Discussion

In the literature, the investigation of *Candida rugosa* lipase-catalyzed kinetic resolution of (*R*,*S*)-ibuprofen by

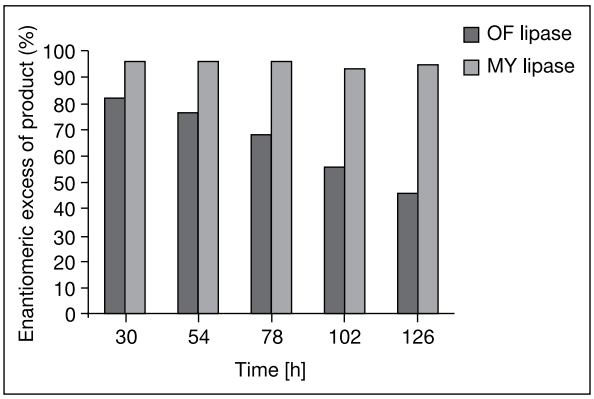

Figure 6. Enantiomeric excess of product depending on the used lipases. Reaction conditions: (*R*,*S*)-ibuprofen (0.02 mM), *n*-butanol (0.06 mM), lipase (8.75 mg), cyclohexane (0.7 mL), temp. 37°C

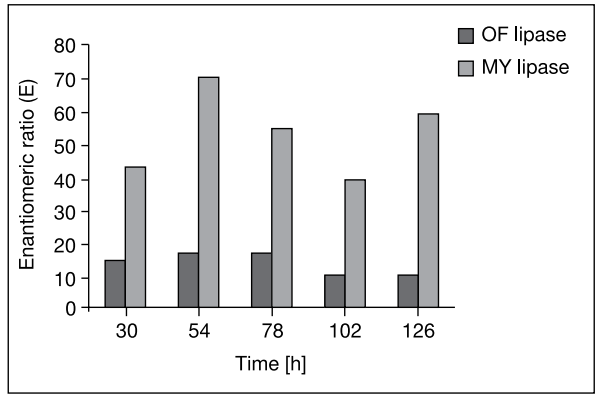

Figure 7. Enantiomeric ratio (E) depending on the used lipases. Reaction conditions: (*R*,*S*)-ibuprofen (0.02 mM), *n*-butanol (0.06 mM), lipase (8.75 mg), cyclohexane (0.7 mL), temp. 37°C

the enantioselective esterification has been described. For example, Contesini and de O. Carvalho studied kinetic resolution of (*R*,*S)*-ibuprofen using the lipase from *Candida rugosa* and *n*-propanol as the acyl acceptor in isooctane (without the addition of ionic liquids) as a reaction medium. After 48 h of incubation, the reaction product was obtained with the enantiomeric excess of product ee_p = 35% and conversion c = 42%. The enantioselectivity was E = 4.1 [18]. The enantioselective esterification of racemic ibuprofen with the use of four primary alcohols such as methanol, ethanol, *n*-propanol, and *n*-butanol in cyclohexane as a reaction medium was carried out by Siódmiak et al. *Candida rugosa* lipase was used as a biocatalyst immobilized on superparamagnetic nanoparticles and an enantiomerically pure product (methyl ester of (*S*)-ibuprofen) with the high value of ee_p = 93.5% and c = 36.7% was obtained. The use of *n*-butanol allowed to obtain product with ee_p = 76.5% and c = 50.8%, whereas the use of ethanol and *n*-propanol resulted in the products with ee_p = 61.7% and c = 39.3% / ee_p = 68.3% and c = 44.2% (respectively for ethanol/*n*-propanol). The enantioselectivities of the above reactions were: E = 50.6/E = 6.2/E = 9.1/E = 18.0 respectively for methanol/ethanol/*n*-propanol/*n*-butanol [5]. Yousefi et al. tested the ability of lipases from *Candida rugosa* immobilized on octyl-sepharose to catalyse the enantioselective esterification of (*R*,S*)*-ibuprofen with *n*-propanol in isooctane and two ionic liquids $[BMIM]BF_4$, $[BMIM]PF_6$. After 120 h of incubation, the following values of conversion in the applied reaction medium: isooctane, $[BMIM]BF_4$, $[BMIM]PF_6$ were achieved: c = 15.5%, c = 27.1%, and c = 31.0% respectively. Enantioselectivities of these reactions were E = 1.13, E = 1.24, E = 1.21 in isooctane, $[BMIM]BF_4$, $[BMIM]PF_6$ respectively [19]. Hong et al. carried out the immobilization of lipase from *Candia rugosa* in polyaniline nanofibers (PANFs) via a three-step process: enzyme adsorption, precipitation and cross-linking, called 'EAPC' [20]. The enantioselective esterification of (*R*,*S*)-ibuprofen with *n*-propanol, 1% (v/v) water and addition of dioctyl sulfosuccinate (AOT) was performed. After 102 h of incubation, the conversion c = 42% and the enantiomeric excess of product (*S*-ibuprofen) ee_p > 98% were obtained [20]. Ren et al. demonstrated results of kinetic resolution of (*R*,*S*)-ibuprofen with the use of *Candida rugosa* lipase in native and immobilized form. The lipase was lyophilized and next used as a catalyst of the enantioselective esterification of (*R*,*S*)-ibuprofen with *n*-propanol in isooctane containing water as a reaction medium. The hollow silica microspheres were used as the support for enzyme immobilization, and for cross-linking reaction aldehyde-containing dextrans were applied. The reaction with the native form allowed to obtain, after 72 h of incubation, the enantiomeric excess of substrate ee_s = 35 ± 1.5% and the conversion c = 30 ± 3.5%. The enantioselectivity was higher than 40. The authors were able to improve the activity of biocatalyst by its immobilization on support in two ways: by adsorption and cross-linking of the adsorbed lipase. The values of enantiomeric excess of substrate ee_s = 88 ± 1.5% and ee_s = 93 ± 0.5%, conversion c = 49.0 ± 1.5%, c = 50 ± 1.5%, and enantioselectivity E > 100 and E > 140 were reached for adsorbed and cross-linked adsorbed lipase forms respectively. The enantiomeric excess of the produced ester was ee_p = 95% ± 1.0% under all three conditions [21].

All esterification reaction studies published in the above mentioned literature were performed in different conditions. Comparing the described data with the results in our article, it can be seen that the received

parameters for tested lipase *Candida rugosa* MY are characterized by high value of enantioselectivity (enantiomeric ratio in the range of 40.1–58.9 (Tab. 1). (*S*)-ibuprofen butyl ester as a product of the enantioselective esterification was obtained with a high optical purity ee_p = 95.0% after 126h of incubation. The enantiomeric ratio of the reaction was E = 58.9, the conversion was c = 30.6%, and enantiomeric excess of the substrate ee_s = 41.8%. Despite existing reported literature data describing the enantioselective esterification of (*R*,S)-ibuprofen with the use of *Candida rugosa* lipase, the detailed optimization of the reaction conditions is still an important area of the pharmaceutical science, which allows to achieve high enzymatic activity and values of enantiomeric ratio of the reaction.

Conclusions

Based on the results, demonstrated in this paper and previously, there is a significant influence of the temperature, reaction time and type of alcohol on the conversion degree and the enantioselectivity of the studied product [17]. The performed study confirmed that the application of the primary alcohols gives good results of conversion and enantioselectivity.

The collected data indicate that there are many factors which affect the enantioselectivity and conversion of esterification reaction when applying *Candida rugosa* lipase. The obtained results demonstrate how important the optimization of reaction conditions is in relation to each lipase individually. The lipase OF in the applied reaction conditions is characterised by the highest conversion among the tested lipases, but the lowest values of enantiomeric excess of the product. Opposite to lipase OF, the application of lipase MY allowed to achieve good results of enantiomeric excess of the product, but, at the same time, lower conversion values. Is should be emphasized, that the reaction performed with the use of lipase MY allowed to obtain less toxic for human health (*S*)-enantiomer of ibuprofen with the high enantiomeric excess of product ee_p = 95%. Conversion of the reaction was 30.6%, after 126 h of incubation.

The comparison study of two commercially available *Candida rugosa* lipases showed their biocatalytic activity, indicating the necessity for a specific optimization of the reaction conditions, depending on the applied lipase. An appropriate optimization allows to conduct a simple and efficient enantioselective synthesis of ibuprofen enantiomers using biocatalysts with a potential application in the pharmaceutical industry. At the same time, a reliable liquid chromatographic system with the new commercially available cellulose-based stationary phase is proposed for reproducible determination of profens in the pharmaceutical analyses.

Acknowledgements

We would like to express our sincere gratitude to Meito Sangyo Co. (Nagoya, Japan) for the supply of lipase OF and MY. The project was supported by the research grant of the National Science Centre DEC-2013/09/N/NZ7/03557. The authors report no competing interests.

References

1. Muñoz Solano D, Hoyos P, Harnáiz MJ, Alcántara AR, Sánchez-Montero JM Industrial biotransformations in the synthesis of building blocks leading to enantiopure drugs. Bioresour Technol 2012; 115: 196–207.
2. Wu L, Vogt FG A review of recent advances in mass spectrometric methods for gas-phase chiral analysis of pharmaceutical and biological compounds. J Pharm Biomed Anal 2012; 69: 133–147.
3. Neupert W, Brugger R, Euchenhofer C, Brune K, Geisslinger G Effects of ibuprofen enantiomers and its coenzyme A thioesters on human prostaglandin endoperoxide synthases. Br J Pharmacol 1997; 122: 487–492.
4. Wsól V, Skálová L, Szotáková B Chiral inversion of drugs: coincidence or principle? Curr Drug Metab 2004; 5: 517–533.
5. Siódmiak T, Ziegler-Borowska M, Marszall MP Lipase-immobilized magnetic chitosan nanoparticles for kinetic resolution of (*R*,*S*)-ibuprofen. J Mol Catal B Enzym 2013; 94: 7–14.
6. Siódmiak T, Marszall MP, Proszowska A Ionic liquids: a new strategy in pharmaceutical synthesis. Mini Rev Org Chem 2012; 9: 203–208.
7. Sikora A, Siódmiak T, Marszall MP Kinetic Resolution of Profens by Enantioselective Esterification. Chirality 2014; 26: 663–669.
8. Tegeder I, Williams K, Geisslinger G Metabolic Chiral Inversion of 2-Arylpropionic Acids. Eichelbaum M, Testa B, Somogyi A Stereochemical Aspects of Drug Action and Disposition, Springer, Berlin–Heidelberg 2003; 341–354.
9. Rainsford KD Ibuprofen: A Critical Bibliography Review, Taylor & Francis, London 1999.
10. Bonabello A, Galmozzi MR, Canaparo R et al. Dexibuprofen (S+-isomer ibuprofen) reduces gastric damage and improves analgesic and antiinflammatory effects in rodents. Anesth Analg 2003; 97: 402–408.
11. Davies NM Clinical pharmacokinetics of ibuprofen. The first 30 years. Clin. Pharmacokinet 1998; 34: 101–154.
12. Ghanem A, Aboul-Enein MN, El-Azzouny A, El-Behairy MF Lipase--mediated enantioselective kinetic resolution of racemic acidic drugs in non-standard organic solvents: Direct chiral liquid chromatography monitoring and accurate determination of the enantiomeric excesses. J Chromatogr A 2010; 1217: 106–1074.
13. Chen CS, Fujimoto Y, Girdaukas G, Sih CJ Quantitative analyses of biochemical kinetic resolutions of enantiomers. J Am Chem Soc 1982; 104: 7294–7299.
14. Ghanem A, Aboul-Enein HY Application of lipases in kinetic resolution of racemates. Chirality 2005; 17: 1–15.
15. Marszall MP, Siódmiak T. Immobilization of Candida rugosa lipase onto magnetic beads for kinetic resolution of (*R*,*S*)-ibuprofen. Catal Commun 2012; 24: 80–84.
16. Siódmiak T, Mangelings D, Vander Heyden Y, Ziegler-Borowska M, Marszall MP High enantioselective Novozym 435-catalysed esterification of (*R*,*S*)-flurbiprofen monitored with a chiral stationary phase. Appl Biochem Biotechnol 2015; 175: 2769–85.
17. Siódmiak T, Ruminski JK, Marszall MP Application of lipases from Candida rugosa in the enantioselective esterification of (*R*,*S*)-ibuprofen. Curr Org Chem 2012; 16: 972–977.
18. Jares Contesini F, de Oliveira Carvalho P Esterification of (*R*,*S*)--Ibuprofen by native and commercial lipases in a two-phase system containing ionic liquids. Tetrahedron: Asymmetry 2006; 17: 2069–2073.
19. Yousefi M, Mohammadi M, Habibi Z Enantioselective resolution of racemic ibuprofen esters using different lipases immobilized on octyl sepharose. J Mol Catal B Enzym 2014; 104: 87–94.
20. Hong S-G, Sol Kim H, Jungbae K. Highly stabilized lipase in polyaniline nanofibers for surfactant-mediated esterification of ibuprofen. Langmuir 2014; 30: 911−915.
21. Ren L, Jia H, Yu M, Shen W, Zhou H, Wei P. Enhanced catalytic ability of Candida rugosa lipase immobilized on pore-enlarged hollow silica microspheres and cross-linked by modified dextran in both aqueous and non-aqueous phases. Biotechnol Bioprocess Eng 2013;18: 888–896.