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Deactivation of Novozym® 435 during the esterification of ibuprofen with ethanol: evidences of the detrimental effect of the alcohol

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Abstract The stability of Novozym® 435 in the esterification of ibuprofen using ethanol as reactant and solvent was investigated. Additionally, the surface interaction of the isomers of ibuprofen and ethanol with the biocatalyst was screened through conventional adsorption isotherms and temperature programmed surface reaction (TPSR). These investigations evidenced strong alcohol adsorption and dissolution of the biocatalyst, which explains its deactivation upon reuses in the esterification reaction.

Keywords Ethanol · Ibuprofen · Novozym® 435 · Biocatalyst

Introduction

The literature reports a lot of investigations about the chiral resolution of racemates through enantioselective esterification with a variety of lipases as biocatalysts [1]. Novozym® 435 produced by the Novozymes Co., is the most widely tested biocatalyst in the esterification of profens. This catalyst is composed of *Candida antarctica B* lipase physically immobilized within beads of a macroporous resin. Although this biocatalyst usually operates with the addition of a cosolvent such as isooctane, there are few investigations on solventless type of operation. In this context, Pepin et al. [2, 3] reported the enantioselective esterification of ibuprofen with 1-dodecanol to yield the R-ester of ibuprofen. The substrates are heated to 70 °C in order to melt the ibuprofen avoiding the use of a cosolvent for the dissolution of the reactants. More recently, our research group demonstrated that the esterification of ibuprofen using only ethanol as reactant and solvent yields 62% of ibuprofen conversion with an enantiomeric excess of 54% towards the S-isomer in 72 h of reaction [4].

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Following these previous findings, the present investigation shows preliminary results about the molecular interaction of ibuprofen and ethanol with Novozym® 435 in order to further understand the effect of the alcohol on the catalytic activity.

Experimental

Materials and methods

The esterification of racemic ibuprofen (0.500 g) with excess of ethanol (1.00 mL and 4.8% water) over Novozym® 435 (160 mg) was assayed in sealed flasks at 45 °C and 200 rpm for 48 h. These optimum conditions of reaction, the determination of ibuprofen conversion and the enantiomeric excess towards the S-isomer were previously published [4]. The ibuprofen conversion was calculated as the difference between the starting concentration of ibuprofen and the concentration remaining in the solution after 48 h of reaction relative to the starting concentration of that reactant,

$$X = \left[\left([\text{ibuprofen}]_o - [\text{ibuprofen}]_{48\,h}\right)/\left([\text{ibuprofen}]\right)_o\right] * 100 \tag{1}$$

Enantiomeric excess (ee) towards the form (S) of ibuprofen remaining after 48 h of reaction and the enantiomeric ratio (E) were calculated according to Eqs. 2 and 3 where [S] and [R] account for the concentrations of the S and R enantiomers respectively,

$$\text{ee} = [([S] - [R])/([S] + [R])] * 100 \tag{2}$$

$$E = \ln[(1 - X)(1 - \text{ee})]/\ln[(1 - X)(1 + \text{ee})] \tag{3}$$

The interaction of a solution of ibuprofen (Parafarm 99.23%) in ethanol (Carlo Erba, 99.8%) over Novozym® 435 (Novozymes, 71.59 m^2/g) was assayed at 298, 301 and 305 K by contacting the biocatalyst (80–100 mg) with 20.60 mL of a solution containing 60 ppm of racemic ibuprofen in ethanol for 40 min. The experiments were performed in sealed flasks placed in a shaker bath at 120 rpm and controlled temperature. The ibuprofen solution (200 μL) was withdrawn at 0, 5, 10, 15, 20, 25, 30 and 35 min during the experiment.

The analysis of both enantiomers of ibuprofen and the ethyl esters was conducted by chiral HPLC analysis using a Nucleodex beta-PM (Macherey–Nagel) with an UV detector operated at 230 nm. The mobile phase (methanol/0.1% TEAA pH 4.0 (60/40 v/v)) was operated at a flow rate of 0.700 mL/min.

The interaction of ethanol with the surface of Novozym® 435 was investigated through the adsorption of the alcohol at 305 K followed by in situ temperature programmed surface reaction (TPSR). Details of the equipment used in this investigation have been published before [5]. The sample (46.6 mg) was pretreated at 305 K for 40 min under a flow of pure helium (35 cm^3(NTP) min^{-1}) prior to adsorption and TPSR analysis. Then successive pulses of 0.5 μL of ethanol (Merck P.A., 100%) were dosed through a heated septum until the saturation was reached. The adsorption process was monitored in situ through a mass spectrometer that detects the non-adsorbed alcohol and/or the species desorbed from the sample. After the saturation, the sample was heated up to 473 K at 10 K/min for the temperature programmed surface reaction experiment.

Finally, Novozym® 435 was contacted with ethanol at 301 K and 200 rpm for 40 min in order to investigate the effect of the alcohol in the integrity of the biocatalyst. The biocatalyst was washed with ethanol in a 100 mg/mL ratio and 11 mg/mL ratio (weight of

sample per volume of ethanol). This last experiment was performed with a solution containing ethanol with 4.8% of water in order to mimic the conditions of the esterification reaction as described before. The solvent was separated and allowed to evaporate at room temperature. The resulting precipitate was analyzed through infrared spectroscopy with a Bruker Vertex 70 spectrophotometer.

Results and discussion

Previous investigation on the stereo-selective esterification of racemic ibuprofen using ethanol as reactant and solvent (without additional cosolvents) demonstrated that Novozym® 435 deactivates after reusing it for four cycles of 48 h of reaction [4]. The conversion of ibuprofen and the enantiomeric excess towards the S-isomer obtained in that investigation are recalled in Fig. 1 for a better understanding of the present study. Novozym® 435 loses 30% of its initial activity in the fourth cycle as is observed in the figure. This behavior was further investigated through the adsorption of ethanol and ibuprofen in order to obtain insights on the causes of deactivation.

In this sense, the interaction of ethanol with Novozym® 435 was investigated through the adsorption of the alcohol at 305 K followed by in situ temperature programmed surface reaction. The temperature programmed surface reaction shows a broad signal of ethanol and water desorption starting at 325 K (see Fig. 2). The observation that neither ethylene, CO nor CO_2 are produced during the experiment evidences that ethanol does not react over the surface of the biocatalyst. Furthermore, the profile of the signal indicates that ethanol desorbs even above 473 K which evidences a strong interaction of the alcohol with Novozym® 435. Temperature desorption experiments up to 673 K (that is without previous adsorption of alcohol) was carried on fresh Novozym® 435 and the bare support (Lewatit VP OC 1600). These experiments demonstrated that only water is desorbed out of the biocatalyst below 573 K and decomposes above that temperature (spectra not shown). This observation evidences that the desorbed species observed in the TPSR analysis do belong neither to the enzyme nor to the support decomposition.

Fig. 3 presents the adsorption of R-ibuprofen over Novozym® 435 as a function of time when the biocatalyst is in contact with a solution of the racemic ibuprofen in ethanol at

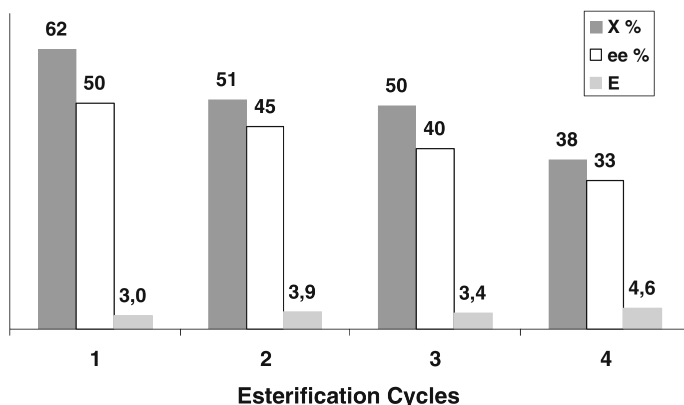

Fig. 1 Evolution of the conversion (*X*%), enantiomeric excess towards S-ibuprofen (ee%) and enantiomeric ratio (*E*) in the esterification of ibuprofen with ethanol catalyzed with Novozym® 435 in several reuses

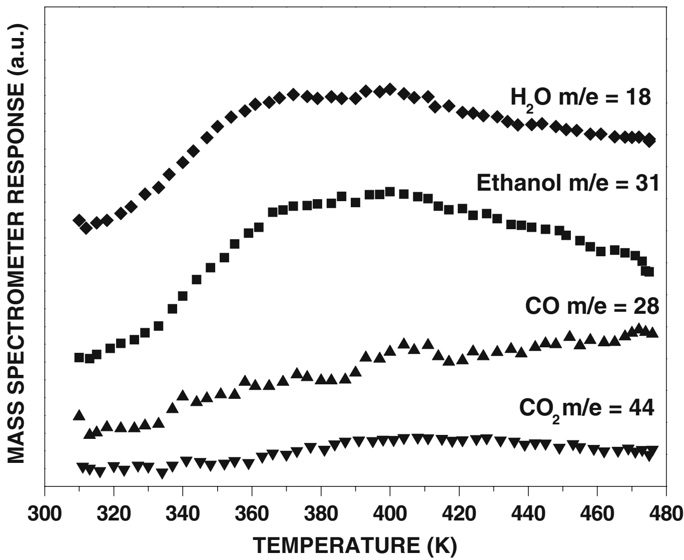

Fig. 2 Temperature programmed surface reaction of ethanol adsorbed on Novozym® 435

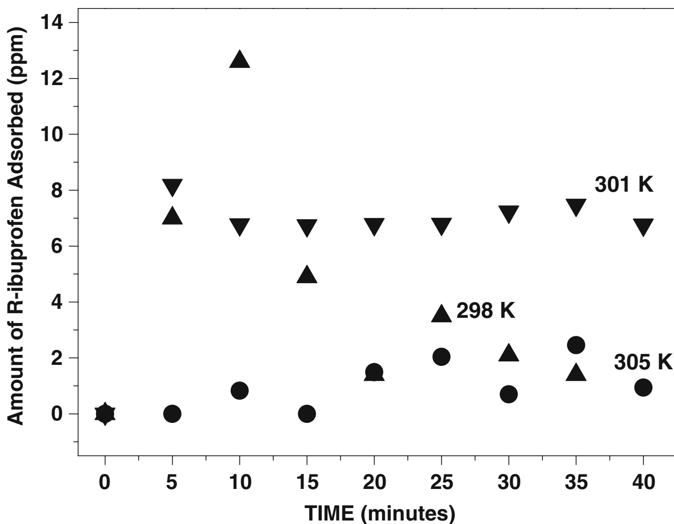

Fig. 3 Amount of R-ibuprofen adsorbed on Novozym® 435 in contact with racemic ibuprofen at different temperatures

different temperatures. The adsorption of the S-isomer possesses a similar behavior therefore is not shown for brevity. The adsorption of ibuprofen on Novozym® 435 at 298 K increases in the first 10 min of contact. However, it slowly desorbs afterwards indicating a weak physisorption (the experiment was performed three times with the same results). An irreversible adsorption of R-ibuprofen is detected at 301 K while at 305 K the amount of adsorbed ibuprofen greatly diminishes to about 2 ppm. This behavior cannot be attributed to the surface reaction of ibuprofen with ethanol since the HPLC analysis did not evidence

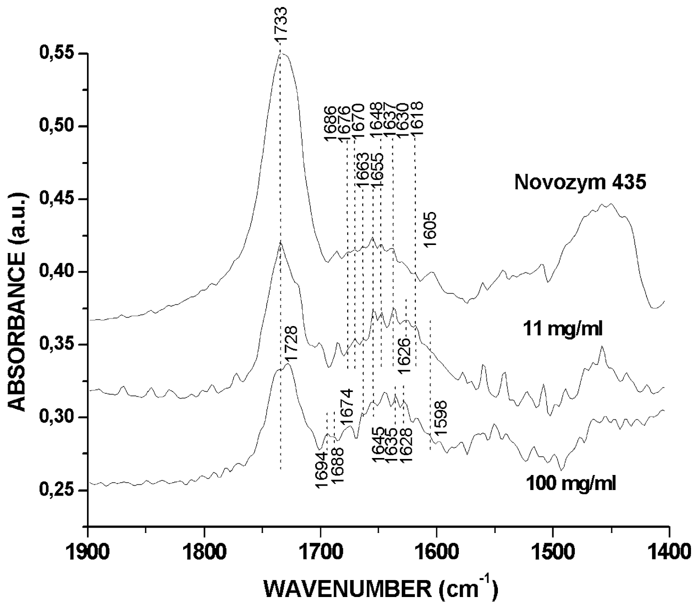

Fig. 4 Infrared spectra of the starting Novozym® 435 and the solids recovered from the liquid media after washing with pure ethanol and ethanol–4.8% water mixture

esterification products at the investigated temperatures. Nevertheless, further experiments contacting Novozym® 435 with pure ethanol evidenced the dissolution of the biocatalyst. Fig. 4 shows the infrared spectra of the starting Novozym® 435 and of the solids recovered from the solvent after treating the biocatalyst. The spectra show the characteristic signals of the Lewatit support at 1,733 cm^{-1} and the Amide I belonging to the *Candida antarctica B* lipase in the 1,598–1,694 cm^{-1} range [6]. This observation clearly demonstrates that the biocatalyst dissolves both in pure ethanol and an ethanol–water mixture.

Conclusion

The strong adsorption of ethanol at 305 K observed during the TPSR analysis allows suggesting that ethanol and ibuprofen exert a competitive adsorption over the same surface sites. This observation gives further evidence of the formation of “dead-end” species due to the irreversible adsorption of ethanol on the active sites of lipases producing its deactivation. Moreover, ethanol exerts a detrimental action on the integrity of Novozym® 435 causing its dissolution. This effect explains the progressive deactivation after several reuses of the biocatalyst during the enantioselective esterification of ibuprofen using ethanol as reactant.

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