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Enzyme kinetics of kinetic resolution of racemic ibuprofen ester using enzymatic membrane reactor

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Abstract

An ultrafiltration hollow fiber enzymatic membrane reactor was employed to study the kinetics of lipase-catalyzed kinetic resolution of racemic ibuprofen ester. Lipase from *Candida rugosa* was employed in the hydrolysis reaction both in free form in a batch system and in immobilized form in an enzymatic membrane reactor (EMR). The half life ($t_{1/2}$) of immobilized lipase on spongy layer was 105 h at reaction temperature of 40 °C and 62 h at 45 °C. This value was 94 h for lipase immobilized on the inner lumen and 45 h for free lipase in batch system at 40 °C . Excessive substrate was found to inhibit the reaction as an uncompetitive inhibitor. The by-product 2-ethoxyethanol was found to be non-competitive inhibitor to the reaction when it was present in an excess. Michaelis constant (K_m) and maximum reaction rate ($V_{\max}$) for immobilized lipase were 36.47 mmol L^{-1} and 3.27 mmol L^{-1} h^{-1}, respectively; and that for free lipase were 63.43 mmol L^{-1} and 2.83 mmol L^{-1} h^{-1}, respectively.

Keywords: Enzyme; Kinetics; Multiphase reactors; Selectivity; Convective transport; Diffusion; Membrane

1. Introduction

(S)-ibuprofen acid was reported 160 times more reactive in their analgesic effect than the (R)-acid (Adams et al., 1976). There is increasing demand for economical and highly efficient methods for commercial synthesis of pure enantiomers which mainly focuses on chiral drugs production. Most of the modern medicines comprise of only single pure enantiomer. The demand for these optically pure compounds is becoming more crucial due to its more target-specific therapeutic effect than that possessed by racemic mixtures. Many of the conventional technologies produce a mixture of both enantiomers either to be consumed as racemate (1:1 molar ratio) or at different molar ratios. Chiral resolutions can be initiated either by a prochiral intermediate, precursor or natural occurring racemate. The cost of substrates influences the choice of method as well as the possibility of recycling the catalyst or the resolving agent. Kinetic resolution utilizes natural occurring racemic compounds (racemate) as its starting material and hence is beneficial in terms of process economics. Being a subject of recent investigation, kinetic resolution is a method in which the residual substrate fraction can be obtained in high enantiomeric purity. Ideally one enantiomer reacts faster than the other with a chiral entity (Sheldon, 1993). A number of literature reported on enzymatic resolution of naproxen and ibuprofen acids and esters by lipases in which high enantioselectivity was observed for the (S)-form of the racemate (Battistel et al., 1991; Sheldon, 1993; Arroyo and Sinisterra, 1994; Roure et al., 1997; Lopez-Belmonte et al., 1997; Sanchez et al., 2000).

Of all the lipases available, lipase from *Candida rugosa* is given particular attention as an ideal biocatalyst for the resolution of racemic esters and alcohols (Kirchner et al., 1985; Holmberg et al., 1991; Parida and Dordick, 1991; Holmberg and Hult, 1992; Kim and Lee, 1996; Sanchez

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et al., 1996; Roure et al., 1997; Cardenas et al., 2001), as it acts enantioselectively and prefers to catalyze the synthesis of one of the enantiomers through hydrolysis with higher preference. Enzymes have started to be utilized by the fine chemicals manufacturers. For example, Gist-Brocades found that addition of the methyl ester of racemic ibuprofen to growing cultures of *Pseudomonas*, *Mucor*, *Arthrobacter* or *Bacillus* species produced (S)-ibuprofen at 74–98% *ee* (Stahly and Starrett, 1997). Gist-Brocades uses a growing microorganism, Sepracor uses isolated enzyme preparations and Rhône-Poulenc uses highly purified enzyme called isozymes (Stahly and Starrett, 1997). An advantage of many enzymatic kinetic resolutions employing lipases and esterases in organic media, is that they can be performed at high substrate concentration. These features possessed by enzymes have made kinetic resolutions of racemic mixtures practical for industrial applications. On the other hand, catalytic asymmetric syntheses often require high dilutions in order to achieve acceptable enantioselectivity (Sheldon, 1996). Furthermore, hydrolytic enzymes are sufficiently inexpensive.

Ultrafiltration is an energy-efficient method used in product separations at a high degree of purity. Overall production costs can be reduced by the use of high-surface-to-volume catalytic hollow fiber membrane. The use of polyacrylonitrile (PAN) UF membrane for kinetic resolution of racemate has been reported by Matsumae et al. (1994) for the production of optically active 3-phenylglycidic acid ester. PAN hollow fiber membrane was also utilized for other non-enantiomeric resolution processes, such as hydrolysis of triacetin by immobilized lipase from *Candida rugosa* was reported by Guit et al. (1991). The use of enzymatic membrane reactor (EMR) allows easy replacement of enzyme (i.e., by removal of immobilized enzyme and repeated enzyme immobilization) and secondly EMR can also be used for biphasic reactions. Models that predict the performance of hollow fiber enzymatic membrane reactor on kinetic resolution of racemic ibuprofen ester had been reported in previous work (Long et al., 2003), where mathematical formulations were developed to simulate hydrolysis reaction in the membrane matrix support and product diffusion in the fiber lumen.

2. Process description

In the present study, the biphasic organic/aqueous membrane reactors are separated with the two liquid phases, the membrane is in contact with the organic phase on one side and with the aqueous phase on the other. As the organic/aqueous interface is located at the membrane level, the hollow fiber membrane provides high exchange surface per unit volume. The schematic diagram of the EMR system employed for the kinetic resolution is shown in Fig. 1. The racemic 2-ethoxyethyl-ibuprofen ester in organic solvent (isooctane) was circulated along the shell side and aqueous buffer in the fiber lumen, in a counter-current mode.

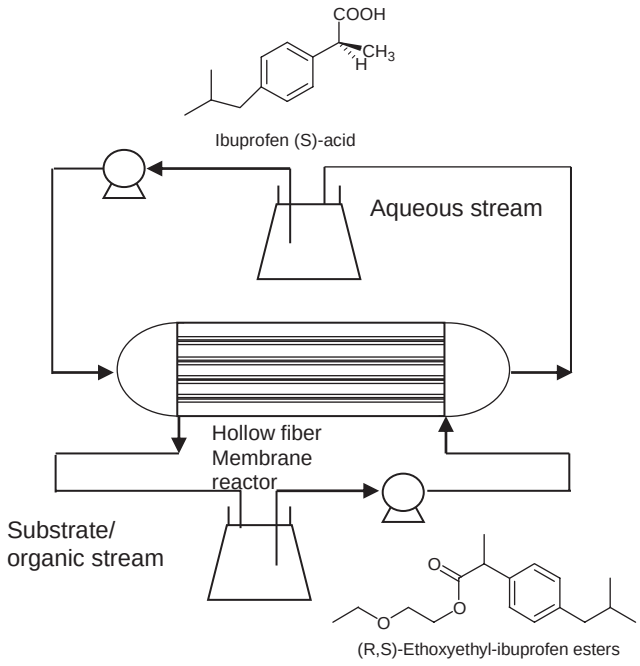

Fig. 1. Hollow fiber enzymatic membrane reactor for kinetic resolution of racemic 2-ethoxyethyl-ibuprofen ester.

In the hydrolysis reaction, the reaction layer is located at the aqueous–organic interface in the porous matrix. The use of circulating aqueous stream neglects the presence of water as a reactant; the *Candida rugosa* lipase catalyzes preferentially the (S)-ibuprofen ester, the (R)-ibuprofen ester is far less reactive, thus reducing the kinetics to one substrate-one product, as shown in Fig. 2. The substrate is in intimate contact with the immobilized enzyme at the membrane by means of a trans-membrane pressure (TMP) with excess pressure (40 kPa) from the shell side to avoid dispersion of aqueous phase through the hydrophilic membrane into the organic phase.

The enzyme reaction kinetics in this work was modeled on the basis of rapid equilibrium assumption. Rapid equilibrium condition (also known as quasi-equilibrium) assumes that only the early components of the reaction are at equilibrium (Segel, 1976). In rapid equilibrium condition, the E, S and ES equilibrate rapidly compared to the dissociation rate of ES into E and P. The product 2-ethoxyethanol was found to be a non-competitive inhibitor and (S)-ibuprofen showed uncompetitive inhibition. The proposed mechanism is given in Fig. 3. The overall enzyme reaction kinetics modified for uncompetitive substrate inhibitor and non-competitive product inhibitor was given in Eq. (1), which is in agreement with that obtained by Madhav and Ching (2001):

$$v = \frac{C_S V_{\max}}{(K_m + C_S)(1 + P/K_{IP}) + C_S^2/K_{IS}}, \quad (1)$$