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Microfluidic separation of (*S*)-ibuprofen using enzymatic reaction

Yun Suk Huh [a], Young-Si Jun [a], Yeon Ki Hong [b], Won Hi Hong [a,*], Do Hyun Kim [a]

[a] *Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea*
[b] *Department of Chemical and Biological Engineering Chungju National University, 123 Geomdan-ri, Iryu-myeon, Chungju, Chungbuk 380-702, Republic of Korea*

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Abstract

This study was investigated for the enantioselective separation of (*S*)-ibuprofen using the ionic liquid in the microfluidic device. A stable and thin ionic liquid flow (ILF) was made by controlling the flow rate of the ILF in the microfluidic channel. In addition, coupling lipase as a biocatalyst with the ILF based on the microfluidic device showed the facilitative and selective transport of (*S*)-ibuprofen across the ILF, indicating successful optical resolution of a racemic mixture. Subsequently, the enantioselectivity was evaluated in the transport ratio (η) of (*R*)- and (*S*)-ibuprofen, the optical resolution ratio (α) and enantiomeric excess of (*S*)-ibuprofen (ee_S).

Keywords: Microfluidic device; Ionic liquid; lipase; Selective separation; (*S*)-Ibuprofen

1. Introduction

In recent years, there have been major research efforts to miniaturize biomedical diagnostic devices. Microfluidic technology allows the design and operation of analytical devices for high-throughput applications such as analysis of biomolecules and chemicals [1–3]. Micro-bioseparations and analytical applications for the biological component of interest will contribute to this rapidly developing microfluidic technology. In particular, the selective separation of organic compounds is a critical issue in the analysis of numerous biologically functional molecules [4–10]. The separation and analysis of optically active compounds is important field in the specialty chemicals. Despite growing interest and need to screen a large number of enantiomeric compounds, few rapid and selective separation method that is suitable for high-throughput screening (HTS) in drug discovery is available. This study takes advantage of the effective and high separation of the microchip that separates enantiomer based on ionic liquid and enzymatic catalyst for characteristic assay and HTS.

In the case of ibuprofen, only (*S*)-enantiomer is biologically active [4,5]. It is well known that racemic drugs often exhibit different pharmaceutical and/or toxicological effects according to the optical purity [5,6]. Thus, effective and selective separation technologies are essential to screen and characterize the optically pure enantiomer from a racemic mixture for avoiding the intake of unwanted enantiomer which is toxic to human health [8–10]. The optical resolution of racemic ibuprofen has been reported by several groups. A supported liquid membrane (SLM) employing the enantioselective enzyme has been widely studied as selective separation of (*S*)-ibuprofen [11–13]. Enzyme which has high substrate specificity and enantioselectivity has a potential for the selective and fast transport of (*S*)-ibuprofen. In particular, lipases have been studied as a biocatalyst for enantiospecific or substrate specific reactions [14–17]. Rethwisch et al. have reported the enzyme-facilitated transport of organic acid through a bulk liquid membrane [18]. Miyako et al. recently reported that a lipase-catalyzed reaction drove the transport of organic acids through a lipase-facilitated SLM [19]. In addition, Bhatia et al. has investigated that the high chemical and optical yields of (*S*)-ibuprofen acid is to be achieved in the enzymatic membrane reactor [20]. In the majority of bulk system, currently used separation and analytical procedures of (*S*)-Ibuprofen required expensive equipment in laboratory settings, highly trained personnel with extensive expertise and time [4,6,9]. In this respect, the microfluidic-based miniaturized tools have the advantages of smaller volumes of reagents and raw sample, speed of response, and cost effectiveness [21–23]. In detail, the short molecular diffusion distance in a miniaturized device promotes chemical reactions, the large specific interface benefits interfacial reactions

* Corresponding author. Tel.: +82 42 869 3919; fax: +82 42 869 3910.
E-mail address: whhong@kaist.ac.kr (W.H. Hong).

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such as solvent extraction allows the facile and fast control of reaction temperature [24–26]. Based on these improvements of operational conditions, a simple and rapid separation method was investigated using the microfluidic device for the enantioselective separation and analysis of (*S*)-ibuprofen from the racemic mixture. Thus, we tried combining lipase-catalyzed reactions with ionic liquid flow for the fast and selective transport of (*S*)-ibuprofen in the microfluidic device. It will be applicable to faster HTS assays for analysis of the quality and toxicity of enantiomer.

This research focused on the enantioselective separation of (*S*)-enantiomer from racemic ibuprofen with a lipase-facilitated ILF in the microfluidic device. By varying the flow rate of ILF, the thickness of ILF could be controlled and thus the stable ILF was achieved. Following this, (*S*)-ibuprofen after the microfluidic separation was represented by the transport ratio (η) of (*R*)- and (*S*)-ibuprofen, the optical resolution ratio (α) and the enantiomeric excess (ee).

2. Materials and methods

2.1. Enzymes and chemicals

The lipases from *Candida rugosa* (CRL) and *porcine pancreas* (PPL) were obtained from Sigma–Aldrich (USA). (*R*, *S*)-Ibuprofen acid and (*S*)-ibuprofen acid were purchased from Acros (Belgium). Hexafluorophosphate (1-*n*-hexyl-3-methylimidazolium) ionic liquid was supplied from Fluka (Japan). A Si (1 0 0) wafer was obtained from LG Siltron Inc. (Korea); a negative photoresist, SU-8, was obtained from MicroChem. Corp.; and polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning (USA). Other reagents were used, and they were of analytical grade.

2.2. Preparation of microfluidic device

Microfluidic device was fabricated using the soft-lithography and replica molding methods, and the material for the replica molding was PDMS. PDMS is highly transparent so that it is suitable for optical detection. Fabrication procedure was as follows. First, a negative photoresist (SU-8) master was formed on a silicon wafer. PDMS prepolymer was then cast on the master and cured. After curing, the PDMS replica was peeled off from the master and then bonded with a slide glass plate (38 mm × 70 mm) after plasma treatment. The cross sectional shape was rectangular. And this device could be easily and rapidly fabricated [27].

2.3. Separation of (S)-ibuprofen in the microfluidic device

Fig. 1 shows the layout and dimensions of the microchannel with a three-phase flow. The three-channel microfluidic device used in this study for the selective separation of (*S*)-ibuprofen from racemic mixture, is about 17 cm long, has a 500 μm wide, and is 50 μm deep. The feeding phase consisted of 65 vol% ethanol, 10 mM racemic ibuprofen and 35 vol% McIlvaine buffer (pH 6.3) containing 30 mg/mL CRL. The receiving phase consisted of McIlvaine buffer (pH 6.3) containing 20 mg/mL PPL. The flow rate of both the feed phase and the receiving phase was kept at 1.5 mL/h, and that of ILF phase was varied from 0.15 to 0.60 mL/h by using a syringe pump (KDS200, KD Scientific Inc.). Flow rates of each stream phase were controlled independently. Formation of the three-phase flow was observed with an optical microscope (Olympus SZX12, Japan). Fig. 1 depicts the transport of (*R*, *S*)-ibuprofen through the ionic liquid flow (ILF) in the microchannel. Although lipases are usually known as ester-hydrolysis catalysts, some of them

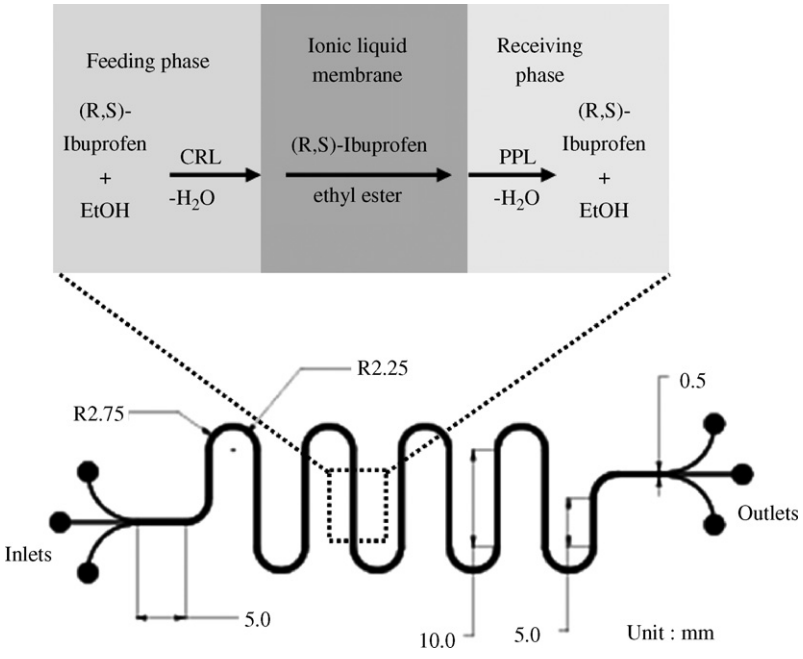

Fig. 1. Schematic drawing of the microfluidic channel and the enantioselective transport of (*S*)-ibuprofen with the ILF in the microfluidic device.

such as CRL are able to catalyze ester synthesis [13,18,28,29]. CRL selectively catalyses the reaction of esterification for (*S*)-ibuprofen rather than that for (*R*)-ibuprofen in the feeding phase, and the resulting ester dissolves into the ILF of the middle phase and diffuses across the ILF. In the receiving phase, PPL catalyzes the ester hydrolysis to produce the native ibuprofen and ethanol, which are water soluble. Employing ionic liquid as a liquid flow phase resulted in the stabilization of the liquid membrane due to the negligible vapor pressure and water-immiscible. Thus, as an organic phase, ILF phase has an important role in dissolving the resulting ester into the ILF and dividing two aqueous phase of the feeding phase and the receiving phase [12,13].

2.4. Analytical method

The concentration of the optically pure (*R*)- and (*S*)-ibuprofen was determined by HPLC (Waters 2487) analysis using a KR100-5CHI-TBB chiral column (Kromasil, USA) with a UV detector at 220 nm. The mobile phase used was hexane/methyl *tert*-butyl ether/acetic acid (55:45:1, v/v/v). Every result was averaged and the reproducibility was checked by triplicate experiments.

3. Results and discussion

3.1. Formation of stable ionic liquid flow in the microfluidic device

Fig. 2 shows the three-phase (feeding aqueous phase/ILF/receiving aqueous phase) flow in the microchannel. The three-phase flow and the clear liquid–liquid interface were formed over the entire microchannel. Neither the ILF nor the aqueous flow invaded the other flow at the arc junction of the microchannel and each phase was collected from each outlet port. The three-phase was formed as larminar flow, where the Reynolds number of the ILF ranged from 1.6×10^{-3} to 3.3×10^{-2} with the change of flow rate in the ILF (0.3–0.6 mL/h) and that of both aqueous phases were about 4.1×10^{-1}. It is related to the fact that mass transfer in the microfluidic device, where the turbulence flow cannot be occurred, was under the control of a diffusion, not a convection force. The flow of ILF was broken into droplet below the flow rate of 0.15 mL/h. This behavior can be attributed to the fact that the aqueous flow invaded the ILF because of the shear stress of the interface between aqueous flow and ILF below the flow rate of 0.15 mL/h. Fig. 2 exhibits the thickness of ILF was smaller than that of the aqueous phase as it flowed from inlet port to outlet port due to the high viscosity of ionic liquid. In the microfluidic device system, stable ILF was made at the various flow rates (0.3–0.6 mL/h). One of the attractive features of a microfluidic device is the ability to precisely control the thickness of ILF with the flow rate. In this microfluidic separation system, the interfacial contact area and the thickness of ILF play a significant role for the more rapid and selective transportation of (*S*)-ibuprofen in the microchannel. Therefore, it can be said that, for reducing the transportation resistance of (*S*)-ibuprofen in the ILF, the thickness of ILF should be decreased and thus the flow rate should be controlled. The thickness of ILF became thinner as the flow rate was decreased, but the feeding and receiving flow invaded the central channel of the ILF at a low flow rate.

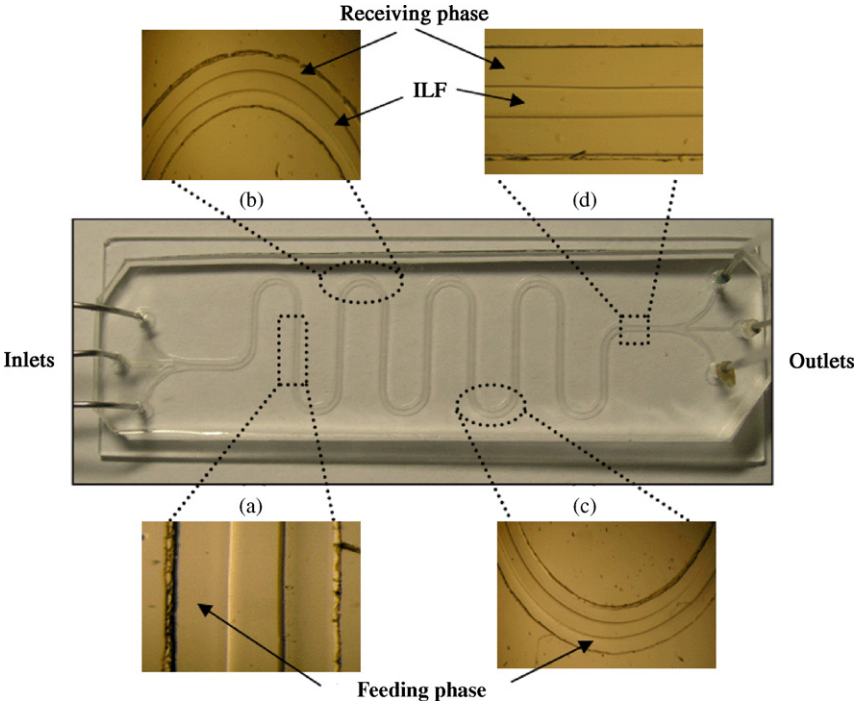

Fig. 2. Photographs of the three-phase flow in the microchannel: (a) center near the inlets of the microchannel; (b and c) arc of the microchannel; (d) center near the outlets of the microchannel. Flow rates of the aqueous phase and the ILF phase in (a–d) were 1.5 and 0.3 mL/h, respectively.

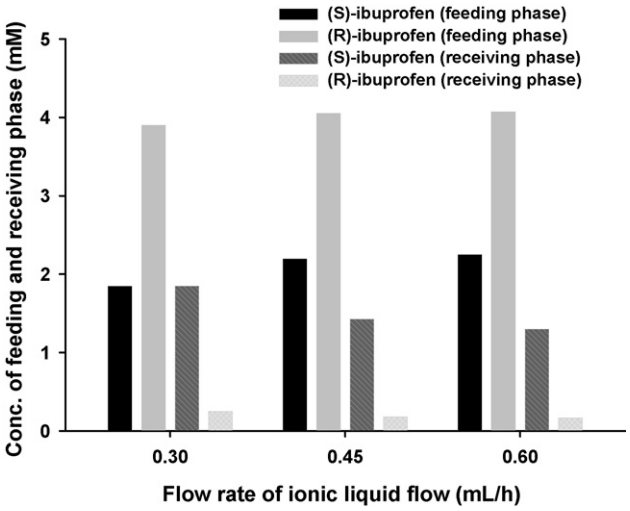

Fig. 3. Effect of flow rate of the ILF on the retentate concentration of (*R*, *S*)-ibuprofen in the feeding phase and the receiving phase.

3.2. Selective separation of (S)-ibuprofen from racemic mixture

Fig. 3 shows the concentration of (*R*, *S*)-ibuprofen in the feeding phase and receiving phase. CRL was used as the biocatalyst for the enzymatic esterification reaction at the feeding phase. As the flow rate of the ILF was decreased, the concentration of (*R*, *S*)-ibuprofen at outlet port of the feeding phase was decreased, but that at the outlet port of receiving phase was increased. As the flow rate of ILF was decreased, the diffusion distance from feeding phase to receiving phase for (*S*)-ibuprofen became shorter because the thickness of the ILF became thinner and the retention time of (*R*, *S*)-ibuprofen ethyl ester in the middle phase became longer. In addition, it was shown that the amount of (*S*)-ibuprofen transported from feeding phase to receiving phase was more than that of (*R*)-ibuprofen. This is because CRL has enantioselectivity of (*S*)-ibuprofen in the esterification reaction. It was supposed that the (*S*)-ibuprofen in the feeding phase was selectively transferred into the ILF. And the (*R*, *S*)-ibuprofen ethyl ester in the ILF was diffused into the receiving phase because the hydrolysis reaction by the biocatalyst of PPL mainly took place at the interface between the ILF and receiving phase. The enantioselectivity was evaluated based on the transport ratio (%) of (*R*)- and (*S*)-ibuprofen (η_R and η_S, respectively), the optical resolution ratio (α was defined as η_S/η_R) and the enantiomeric excess (ee_S) which is expressed in terms of optical purity of the (*S*)-ibuprofen [4]. Transport ratio was calculated from the molar fraction of each enantiomer at the inlet and outlet of the each phase in the microfluidic device.

$$\eta_{f,i} = \frac{C_{f_{in},i} - C_{f_{out},i}}{C_{f_{in},i}} \times 100 \quad (1)$$

$$\eta_{r,i} = \frac{C_{r_{out},i}}{C_{f_{in},i} - C_{f_{out},i}} \times 100 \quad (2)$$

$$\eta_{t,i} = \frac{C_{r_{out},i}}{C_{f_{in},i}} \times 100 = \frac{\eta_{f,i} \times \eta_{r,i}}{100} \quad (3)$$

$$ee_S = \frac{C_S - C_R}{C_R + C_S} \times 100 \quad (4)$$

where $C_{f_{in},i}$ and $C_{f_{out},i}$ are the concentrations of ibuprofen at the inlet and outlet in the feeding phase. $C_{r_{out},i}$ is the concentration of ibuprofen at the outlet port of the receiving phase and i means the (*R*)- and (*S*)-ibuprofen, respectively. The transport ratios between feeding phase and the ILF and between the ILF and the receiving phase are expressed by $\eta_{f,i}$ and $\eta_{r,i}$, and the overall transport ratio from the feeding phase to the receiving phase is represented by $\eta_{t,i}$.

Table 1 summarizes the transport ratio of (*S*)- and (*R*)-ibuprofen, the optical resolution ratio and enantiomeric excess. As shown in Table 1, the amount of (*R*, *S*)-ibuprofen transported from the ILF into the receiving phase was smaller than that from the feeding phase into the ILF. In particular, as the flow rate of the ILF increased, the transport of (*R*, *S*)-ibuprofen from the ILF to the receiving phase decreased. It is plausible to say that this behavior is mainly attributed to the magnitude of concentration gradient in the ILF. Mass transfer in a microchannel is governed by molecular diffusion because the Reynolds number in this system is much less than 1. As shown in Table 1, in both the feeding and the receiving phases, the transport ratio of (*S*)-ibuprofen was two times higher than that of (*R*)-ibuprofen in the different flow rate of the ILF, meaning that the catalytic reaction by lipase drove the selective transport of (*S*)-ibuprofen through the ILF. In addition, the value of the optical resolution ratio in the feeding phase was higher than that of the receiving phase at the all flow rate of the ILF. This result indicates that CRL esterified selectively (*S*)-ibuprofen and then transported preferentially (*S*)-ibuprofen ethyl ester from the feeding phase into the ILF. The transport ratio of (*S*)-ibuprofen was larger than (*R*)-ibuprofen in the receiving phase, although the enzyme of PPL is not able to selectively produce (*S*)-ibuprofen in the interface between the ILF and the receiving phase. It has been well known that the PPL is appropriate for the fast ester hydrolysis catalyst, but it does not transport (*S*)-ibuprofen selectively [15,16,19]. The selectivity

Table 1
The transport ratio of (*R*)- and (*S*)-ibuprofen, optical resolution ratio and purity with the different flow rate of the ILF in the feeding and the receiving phase

Flow rate of ILM (mL/h)	CRL in the feed phase				PPL in the receiving phase				Overall transport ratio	
	$\eta_{f,R}$ (%)	$\eta_{f,S}$ (%)	α	ee (%)	$\eta_{r,R}$ (%)	$\eta_{r,S}$ (%)	α	ee (%)	$\eta_{t,R}$ (%)	$\eta_{t,S}$ (%)
0.30	22	63	3.13	48.34	23	59	2.62	77.67	5.1	37.2
0.45	19	56	2.84	47.89	19	51	2.69	76.83	3.6	28.6
0.60	19	55	2.82	47.71	18	47	2.68	76.71	3.4	25.9

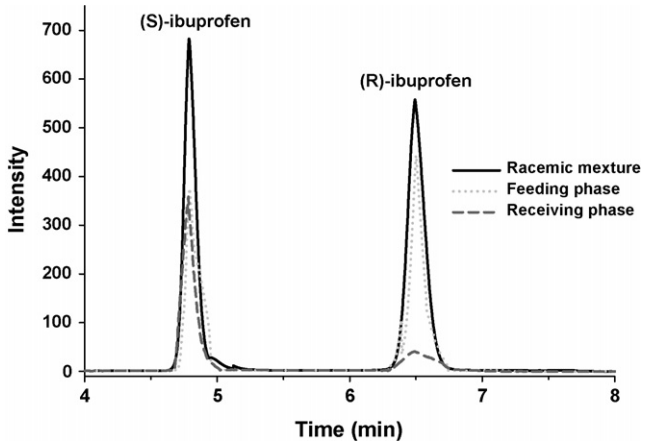

Fig. 4. HPLC chromatogram of the racemic mixture, the feeding phase sample at the outlet port and the receiving phase sample at the outlet port. The retention times of (*R*, *S*)-ibuprofen were 4.787 and 6.493 min, respectively.

of (*S*)-ibuprofen in the receiving phase was interpreted to be that of the diffusion with the difference of concentration from the ILF to the receiving phase. The larger the difference of concentration was, the more the transport of ibuprofen from the ILF to the receiving phase was. In the same manner to the above results, the value of ee was higher when the flow rate in the ILF phase was slower. Three samples were prepared. They were a recemic mixture sample, a feeding phase sample at the outlet port and a receiving phase sample at the outlet port. Each sample was analyzed by the HPLC. Fig. 4 shows the results of HPLC chromatogram of the initial racemic mixture sample, the feeding phase sample which has been remained at the outlet port of feeding phase and the receiving phase, (*R*, *S*)-ibuprofen transported from the feeding phase to the receiving phase through the ILF. It was shown that the (*S*)-ibuprofen was successfully separated from the racemic mixture using the microfluidic separation device coupled enzymatic reaction with the ILF.

In the comparison with microfluidic system and SLM bulk system, the SLM system had the more amount of (*R*, *S*)-ibuprofen transported from feed phase to receiving phase because of existence of the turbulence flow by stirring and the sufficient reaction time (about 20–40 h) in the both feeding phase and receiving phase, while the microfluidic system was able to separate (*S*)-ibuprofen efficiently for the short working time (about 30–60 s) although the value of overall transport ratio ($\eta_{t,i}$) was smaller than that of SLM system. It is supposed that the microfluidic channel, which is able to form a thin ILF with controlling the flow rate, could enlarge the contact area and reduce the ILF resistance between the aqueous phase and the ILF compared to the conventional bulk liquid membrane using the SLM with immobilization of enzyme as an extractant and stable separator between solute and extractant. Therefore, this microfluidic device is suitable system for the selective and rapid separation of (*S*)-ibuprofen required by following detection from the racemic mixture. And it will be effectively applied to analyze the biologically functional molecules and also lead to miniaturized portable devices that decrease reagent requirements and improve assay sensitivity.

4. Conclusions

Our investigation showed that the microfluidic channel was successfully achieved to form a stable and the thin ILF by controlling the flow rate of the ILF. This result demonstrates that the microfluidic device with the ILF can be applied for the enantioselective transport of (*S*)-ibuprofen. In addition, from an analytical point of view, the miniaturization of the ILF technique reduces the amount of sample required and enables the optically active compound to separate during the short time and transport selectively with the larger contact surface area to volume. Therefore, it is suggested that the microfluidic device is an appropriate analytical tool for the fast and selective separation of (*S*)-ibuprofen and the microfluidic channel is more efficient device to make a stable and thin the ILF.

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