Ultrasound-Assisted Enzymatic Synthesis of Poly-\(\varepsilon\)-Caprolactone: Kinetic Behavior and Reactor Design

Abstract: Lipase-mediated, ultrasound-assisted synthesis of poly-\(\varepsilon\)-caprolactone was investigated. It was found that ultrasound irradiation helped to improve the rate constant of poly-\(\varepsilon\)-caprolactone chain propagation (\(k_p\)) at high initial monomer (\(\varepsilon\)-caprolactone) concentration. The enhancement of \(k_p\) ranged from 34% to 46% at 22.5–18.0 M initial monomer concentration, respectively. In a system prone to time-dependent mass-transfer limitation due to polymer chain extension, the acoustic effects could have also allowed the reaction to continue longer compared to non-sonicated process until it became impossible at highly elevated reaction mixture viscosity (>2,000 times increase from initial viscosity). Consequently, it also helped to improve monomer conversion. In a continuous flow polymerization system, a plug flow reactor system is recommended due to its lowest volume for maximum conversion compared to a continuously stirred tank reactor system.

Keywords: biodegradable polymer, lipase, poly-\(\varepsilon\)-caprolactone, reactor design, polymerization, ultrasound

1 Introduction

Biodegradable polymers are increasingly being used in diverse applications. They are especially important in various industrial applications including food packaging [1, 2], drug delivery and tissue engineering applications [3, 4]. Polyhydroxyalkanoates (PHAs) can be synthesized chemically by polycondensation of carboxylic acids and alcohols [5]. They can be made also by anionic [6], cationic [7] or enzyme-mediated [8, 9] ring-opening polymerization (ROP). Of specific importance are poly-\(\varepsilon\)-caprolactone, a PHA, that is reported to be a useful semicrystalline polymer [10] and can be synthesized via benign enzymatic ROP of \(\varepsilon\)-caprolactone monomer [11].

Unfortunately, enzyme-catalyzed ROP has certain limitations such as progressive increase in the medium’s viscosity with time due to polymer synthesis. The increased viscosity results in low molecular weight polymer, since continued elongation of the polymer chain may not sustain due to curtailed mass transfer [8]. In addition, reaction solvents commonly used in such polymerization system are said to adversely affect the enzyme stability [12]. Attempts have been made to improve lipase-mediated polymerization through various methods [13–15]. In this respect, high dissipating energy generated by cavitational bubbles collapse in the course of ultrasound irradiation is hypothesized to enhance mass transfer within the reaction medium as compared to non-sonicated reaction [16–18]. During the rarefaction phase of sonication, microbubbles of gas and vapor are produced by cavitation. The collapse of these microbubbles during the compression phase dissipates enormous amount of energy and temperature into the liquid medium [19–21]. The induction of these short-lived high temperatures and pressures, combined with extraordinarily rapid cooling, has been used to influence derivation of chemical reactions for production of useful molecules [22]. In every (bio)chemical conversion process, converting reactant(s) to valuable product(s) is the core objective. Thus, reactor design should take into consideration the type, size and interconnections of reactors involved for optimal productivity and conversion efficiency of the process. As such, rational reactor design could have a positive impact on the overall process economics.
of reactant concentration on kinetic constant \(k_p\) of \(\varepsilon\)-caprolactone polymerization were investigated and compared for non-sonicated and sonicated systems. The polymerization was carried out in ionic liquid (1-ethyl-3-methylimidazolium tetrafluoroborate) as reaction media. Ionic liquids have been promoted as an environmentally-benign solvents. Subsequently, continuously stirred tank reactor (CSTR) and plug flow reactor (PFR) were modeled based on experimental rate of non-sonicated and sonicated systems to determine the most productive reactor design. The most efficient reactor design is expected to be retro-fitted with ultrasonication contrivance.

2 Materials and methods

2.1 Materials

All materials used were of analytical grade. The ionic liquid 1-ethyl-3-methylimidazolium tetrafluoroborate [Emim][BF₄] (>99% pure) and \(\varepsilon\)-caprolactone monomer were purchased from Merck (www.merck-chemicals.com). Standard poly-\(\varepsilon\)-caprolactone of different molecular weights were purchased from Sigma–Aldrich (www.sigmaaldrich.com). Tetrahydrofuran (THF), chloroform and methanol were also sourced from Sigma–Aldrich. Immobilized \textit{Candida antarctica} lipase B 435 was obtained from Novozymes (www.novozymes.com).

2.2 Methods

2.2.1 Enzyme activity assay

The activities of the lipases were measured according to Teng and Xu [23]. In brief, Novozym® 435 (20 U mL⁻¹) was added to a vial containing 10 mL of a 10 mM 4-nitrophenyl palmitate solution in \(n\)-hexane. To this mixture, 60 µL of 1 M absolute ethanol was added. The resulting slurry was sonicated at 37 kHz, 30°C, 0.38 W cm⁻² power intensity or at 200 rpm, 30°C in conventional automatic shaking process. The reaction was allowed to proceed for a period of 60 min. Aliquots (30 µL each) of the reaction mixture were withdrawn at intervals and quenched by mixing with 1 mL of 0.1 M NaOH in a quartz cuvette. The 4-nitrophenol liberated by the reaction was measured at 412 nm (UV–Vis spectrophotometer V-630; Jasco, Japan) against a blank of distilled water. The enzyme activity was calculated as the slope of a plot of 4-nitrophenol released versus time.

2.2.2 Effects of \(\varepsilon\)-caprolactone concentration and ultrasound irradiation on polymerization constant \(k_p\)

In batch reaction, ionic liquid [Emim][BF₄] (2 mL) and different concentrations of \(\varepsilon\)-caprolactone (4.5–22.5 M) were mixed in a 20-mL capped reaction vial. Lipase B (1.5% w/v, g/100 mL) was added to initiate the reaction. Triplicate reaction vials were used in all experiments. A corresponding set of control vials that lack the enzyme were also added. A batch of the reaction vials were sonicated at 37 kHz (equivalent to 0.38 W cm⁻² power intensity) for 20 min by immersion in an ultrasonic bath (Elmasonic P30H; Elma, Germany) operated at \(2.5 \times 10^3\) W m⁻². This was followed by a further reaction period of up to 85 h without sonication. An identical batch of non-sonicated control vials was held in a shaker incubator (Daihan LabTech®, Korea) for 20 min and then under quiescent conditions. All reactions were performed at 50°C except stated otherwise. Lipase B activities at 50°C were studied previously [8, 17]. The progress of the reaction was monitored by removing vials (in triplicate) at specified intervals, adding 10 mL of chloroform to reduce the viscosity of the reaction mixture and immediate filtering (Büchner funnel with a sintered glass filter) to remove the enzyme beads and stop the reaction. The filtrate was further processed to recover the polymer as described in the next section.

2.2.3 Product extraction

The filtrate from the previous section was concentrated to about 3 mL using a rotary evaporator (LABOROTA C-311; www.heidolph-instruments.com) at 50°C under reduced pressure. The polymer was precipitated from the concentrate by adding cold methanol (10 mL, 4°C). The precipitate was recovered by centrifugation (Sorvall RC-5C centrifuge operated at 4°C, 1,912 g, 15 min). The white precipitate of poly-\(\varepsilon\)-caprolactone was redissolved in 10 mL of chloroform, and the aforementioned extraction process was repeated three times to further purify the product. The extracted product was dried to a constant weight under vacuum. A portion of the dried sample was subsequently subjected to further authentication analyses.

2.3 Product authentication

2.3.1 FTIR spectroscopy

Non-destructive attenuated total reflectance FTIR spectra were recorded at room temperature over a range of
4,000–400 cm\(^{-1}\) on a Perkin-Elmer FTIR RX 1 spectrometer (Perkin-Elmer Inc., Wellesley, MA, USA) using NaCl crystal window. A sample of \(0.01\) g of the synthesized polymer was mixed with a dry dichloromethane forming a paste, which was applied on the NaCl crystal window and dried at room temperature before being analyzed in the spectrometer. A standard poly-\(\varepsilon\)-caprolactone authentic sample (CAS 24980-41-4, Sigma–Aldrich) was similarly treated and measured.

### 2.3.2 \(^1\)H-NMR analyses

Proton NMR spectra were recorded on a JEOL JNM-GSX 270 FT-NMR (JOEL Ltd, Tokyo, Japan) machine at 250 MHz. Measurements were made on the polymer (5 mg) dissolved in deuterated chloroform (2 mL) with tetramethylsilane (TMS) as the internal reference standard. About 1.5 mL of the dissolved mixture was withdrawn using borosilicate glass syringe into an NMR tube.

### 2.3.3 Gel permeation chromatography (GPC)

Waters 600 (Waters Corp, Milford, MA, USA) GPC system equipped with a Waters refractive index detector (model 2414) was used for molecular weight characterization. The instrument carried the following gel columns (7.8 mm internal diameter \(\times\) 300 mm) in series: HR1, HR2, HR5E and HR5E Waters Styrsgel HR-THF. Monodisperse polystyrene standards of different molecular weights (3.72 \(\times\) 10\(^3\), 2.63 \(\times\) 10\(^3\), 9.10 \(\times\) 10\(^3\), 3.79 \(\times\) 10\(^3\), 3.55 \(\times\) 10\(^3\), 7.06 \(\times\) 10\(^3\), 3.84 \(\times\) 10\(^4\) and 6.77 \(\times\) 10\(^4\) Da) were used to produce the calibration curve. The polymer samples were dissolved in THF at a concentration of 2.0 mg mL\(^{-1}\), filtered through a 0.22-µm polytetrafluoroethylene (PTFE) filter and injected (100 µL) at 40°C. THF was used as the mobile phase at a flow rate of 1.0 mL min\(^{-1}\).

### 2.3.4 Differential scanning calorimetry (DSC)

DSC analyses of the synthesized poly-\(\varepsilon\)-caprolactone were carried out using Perkin-Elmer differential scanning calorimeter (DSC 6; Perkin-Elmer Inc., Wellesley, MA, USA) running Pyris series 6 DSC software. Scans were made under a nitrogen flow rate of 50 mL min\(^{-1}\) at a temperature range from \(-60\) to \(125^\circ\)C. The heating/cooling rate was 20°C min\(^{-1}\). The melting temperature \((T_m)\) was taken at the peak of the DSC thermogram endotherm.

### 2.3.5 Thermogravimetric analysis (TGA)

TGA analysis was performed on a Perkin-Elmer TGA4000 instrument. The samples were heated from 50 to 900°C at a rate of 10°C min\(^{-1}\) under a nitrogen flow rate of 20 mL min\(^{-1}\).

### 2.3.6 Viscosity measurements

Viscosity of the reaction mixture was measured after removal of the enzyme beads, using a Sine-wave Vibro SV-10 viscometer (A and D Company, Japan).

### 2.3.7 Calculations

The DSC observed values of the endothermic melting enthalpy \((\Delta H_f)\) and the melting temperature \((T_m)\) were used to calculate the endothermic entropy of fusion \((\Delta S_f)\), as follows:

\[
\Delta S_f = \frac{\Delta H_f}{T_m} \quad (1)
\]

The degree of crystallinity \((X_c)\) of the polymer was calculated using the following equation:

\[
X_c = \frac{\Delta H_f}{\Delta H_f^0} \quad (2)
\]

where \(\Delta H_f^0\) is the endothermic melting enthalpy of the 100% crystalline poly-\(\varepsilon\)-caprolactone (142 J g\(^{-1}\)) as given in the literature [24].

The initial rate of monomer conversion \((r_p)\) determined by the initial gradient of the change in the number averaged molecular weight \([M_n]\) with time as shown in eq. (3).

\[
r_p = \frac{\delta [M_n]}{t} \quad (3)
\]

The polymer propagation rate constant \((k_p)\) is related to monomer concentration and initiator concentration [25], as follows:

\[
\ln \left( \frac{[M]_0 - [M]_{eq}}{[M]_{eq}} \right) = k_p [I]_0 t \quad (4)
\]

where \([I]_0\) is the concentration of the initiator, \([M]\) is the monomer concentration either at equilibrium (subscript eq) or at time \(t\) (subscript \(t\)). Eq. (4) assumes the presence of only one kind of active center in polymer propagation and a \(k_p\) value that is independent of the degree of polymerization. In this study, the initiator was the residual water present in the reaction mixture.
The sonic power intensity, \( I \) (W cm\(^{-2}\)) was calculated according to
\[
I = \frac{P}{A}
\]
where \( A \) is the reaction volumetric area (cm\(^2\)).

The sonication amplitude \( (P_A) \) and acoustic \( (P_a) \) pressures were calculated according to eqs (6) and (7), respectively [26].
\[
P_A = (2 \cdot \rho \cdot c \cdot I)^{1/2}
\]
(6)
\[
P_a = P_A \cdot \sin 2\pi f \cdot t
\]
(7)
where \( \rho \) is the bulk density (kg m\(^{-3}\)), \( c \) is the speed of sound (1,500 m s\(^{-1}\)) and \( f \) is the sonication frequency.

The particle’s maximum velocity \( (v_p) \) and displacement \( (\xi) \) are given by eqs (8) and (9), respectively.
\[
v_p = \frac{P_A}{\rho \cdot c}
\]
(8)
\[
\xi = \frac{v_p}{2\pi f}
\]
(9)
Particle acceleration \( (a) \) is given by eq. (10)
\[
a = 4 \cdot \pi^2 \cdot f^2 \cdot \xi
\]
(10)
The conversion \( (X_m) \) of the monomer (\( \epsilon \)-caprolactone) to poly-\( \epsilon \)-caprolactone was calculated as follows:
\[
X_m = \frac{\left[M_0 - |M_f|\right]}{M_0}
\]
(11)
In batch reactor, the time required to achieve a specified conversion is calculated using eq. (12)
\[
dt = M_0 \cdot \frac{X_m}{-r_p V}
\]
(12)
where \( V \) (L) is the reactor volume.

On the other hand, in comparison to batch process under homogenous mixing, the volume required to achieve a specified conversion in a CSTR and PFR were calculated by eqs (13) and (14), respectively,
\[
V = \frac{F_0}{-r_p \cdot (X_m)} \cdot X_m
\]
(13)
\[
V = F_0 \cdot \frac{X_m}{-r_p}
\]
(14)
where \( F_0 \) (mol s\(^{-1}\)) is the feeding molar flow rate given as the product of initial feeding concentration \( (M_0; \text{mol L}^{-1}) \) and the initial volumetric flow rate \( (v_0; \text{L s}^{-1}) \). Hence, a Levenspiel plot with ratio of molar flowrate to reaction rate against conversion was constructed. For CSTR sizing, a rectangle with a specified height \( (X_m) \) and base \( (X_m) \) was diagrammed. The rectangle area matched the volume required to achieve the desired conversion \( (X_m) \).

In PFR sizing, the volume required to achieve maximum conversion from the data was calculated using five point quadrature formula [27] for \( N + 1 \) data points, where \( N \) is an even number as shown in eq. (15).
\[
\int_{X_{m0}}^{X_{m1}} f(X) \cdot dX = \frac{h}{3} \left[F_0 \left(-r_p \cdot X_m \right)^{1} + 4 \cdot \frac{F_0}{-r_p} \left(-r_p \cdot X_m \right)^{2} + 2 \cdot \frac{F_0}{-r_p} \left(-r_p \cdot X_m \right)^{3} \right]
\]
(15)
where
\[
h = \frac{X_mN - X_{m0}}{N}.
\]

3 Results and discussion

3.1 Product characterization

The synthesized polymer from lipase-catalyzed, non-sonicated and sonicated processes was structurally characterized using FTIR (Figure 1) and \(^1\)H-NMR (Figure 2a and b).

![Figure 1 FTIR spectra of both synthesized and standard poly-\( \epsilon \)-hydroxyhexanoate](image-url)
In the FTIR spectra (Figure 1), the observed 3,438.19 cm\(^{-1}\) absorption band was reported to indicate the presence of hydroxyl group confirming the formation of a linear polymer chain [28]. Absorption bands at 2,945.12 and 2,866.16 cm\(^{-1}\) were allocated to asymmetric and symmetric methylene (CH\(_2\)) stretching vibrations, respectively. In both spectra, the absorption band of 1,724.15 cm\(^{-1}\) was assigned to carbonyl (C=O) stretching vibration. Absorption at 1,369.27 cm\(^{-1}\) was assigned to asymmetric COC stretching vibration. A series of absorption bands at 1,169 and 1,195 cm\(^{-1}\) were due to carboxyl (OC−O) stretching vibration. A series of absorption bands at 1,240.77 cm\(^{-1}\) was attributed to asymmetric COC stretching vibration. Similar observation was reported by Elzein et al. [24].

The absence of absorption at band 870 cm\(^{-1}\) showed that this polymer is not syndiotactic, while the general absorption at band 840, 842 and 1,047 cm\(^{-1}\) confirmed the synthesized polymer to be isotactic. Similar observation was reported [29]. The synthesized polymer showed a crystalline characteristics due to absorption band on wavelength 1,296–1,294 cm\(^{-1}\). This was found to agree with the observed bands of the standard poly-\(\varepsilon\)-caprolactone of similar \(M_n \cong 10,000\) (Figure 1) and was also found to be in good agreement with reported literatures [28, 30].

The proton NMR spectra of both synthesized and standard poly-\(\varepsilon\)-caprolactone were characterized against the internal standard reference of TMS; letter “a” representing triplet chemical shift (\(\delta = 2.39\) ppm) was assigned to methylene protons located at \(\alpha\)-position of the ester carbonyl carbon (Figure 2a and b). The broad quartet chemical shift at 1.66 ppm is represented by letters “b” and “d” that were assigned to methylene protons located at both \(\beta\) and \(\delta\)-position of the ester group, respectively. Letter “c” represents the chemical shift at 1.37 ppm and was assigned to protons of methylene at \(\gamma\)-position of the carbonyl ester. The triplet at chemical shift 4.17 ppm represented by letter “e” is assigned to methylene protons located at \(\epsilon\)-position of the ester group. Protons attached to carbinol carbon atom were assigned to narrow triplet chemical shift at 3.66 ppm represented by letter “f”. However, further triplets were observed down the field at chemical shift 4.20 ppm, these were reported to represent methylene protons in the dimer [28].

The molecular weight distribution of the synthesized poly-\(\varepsilon\)-caprolactone from both processes was also investigated. Sonicated synthesized polymer was observed to yield a polymer of an average molecular weight (\(M_n\)) as high as 20.6 kDa, compared to 9.8 kDa in non-sonicated synthesis. Stassin et al. [31] reported a similar polymer molecular weight (21 kDa), when observing PCL synthesis in supercritical CO\(_2\). Similarly, a PCL of 22 kDa was synthesized by means of supercritical CO\(_2\) in a variable-volume view reactor [32]. In general, all structural and molecular weight data of the synthesized polymer (either from sonicated or from non-sonicated process) agreed very well with the findings from our previous studies [17]. This indicated that the enzyme-mediated processes, regardless whether they were sonicated or not, are highly reproducible.

### 3.2 Thermal analysis

In comparison to the conventional agitation process, the polymer made under ultrasound-assisted synthesis
showed a melting temperature ($T_m$) of 58.46°C. This observation was found to be in agreement with other reported literatures [33, 34]. The endothermic melting entropy ($\Delta S_f$) and enthalpy of fusion ($\Delta H_f$) [eq. (1)] were found to be 0.26 J g$^{-1}$ K$^{-1}$ and 86.38 J g$^{-1}$, respectively, which was higher than the reported $\Delta H_f$ of 68.2 J g$^{-1}$ for the same polymer synthesized conventionally [35]. Higher $\Delta H_f$ for the sample made via sonication was attributed to its higher degree of crystallinity and average molecular weight as compared to the conventionally synthesized product.

The polymer made via ultrasound-aided synthesis was found to have a higher degree of crystallinity [eq. (2)] of 0.61 (i.e. 61% crystalline) as compared to reported values of 51–55% [34, 35] for the conventionally produced polymer. This high degree of crystallinity could be due to sonication-associated microstreaming which may have promoted a better alignment of the polymer molecules. This in turn influences their closer packing in the polymer matrix resulting in the observed higher degree of crystallinity. Thermogravimetric measurements showed that the synthesized both polymer samples have shown an appreciable resistance to thermal degradation. For example, the onset of phase transition temperature ($T_i$) of the polymer synthesized under ultrasound-aided process was found to be 380°C with a final thermal decomposition temperature ($T_d$) of about 590°C. On the other hand, the $T_i$ of the polymer produced in conventional agitation process was about 330°C with a final $T_d$ of 464°C. The significant difference in the polymer degradation temperatures was attributed to the relatively higher molecular weight and degree of crystallinity of the polymer from an ultrasonic-aided synthesis.

### 3.3 Effects of ε-caprolactone concentration and ultrasound irradiation on $k_p$

The rate of monomer conversion ($r_p$) and polymer propagation rate constant ($k_p$) were determined according to eqs (3) and (4), respectively. In this study, for a fixed concentration of an initiator, a plot of $\ln\left(\frac{[M_0]-[M_{eq}]}{[M_{eq}]-[M_{eq}]}\right)$ versus the reaction time is expected to be a straight line with a slope of $k_p[I_0]$. Such plots are shown in Figures 1a and b for both the sonicated reaction systems and the non-sonicated ones.

It was observed that at lower monomer concentration (4.5–13.5 M), sonication has less pronounced effect on the polymerization rate constant ($k_p$) $3.31 \times 10^{-4}$ to $3.36 \times 10^{-4}$ s$^{-1}$ (Figure 3a and Table 1) compared to the non-sonicated synthesis with $k_p$ value spanning from 2.98 $\times 10^{-4}$ to 3.35 $\times 10^{-4}$ s$^{-1}$ (Figure 3b). However, increasing the initial monomer concentration to 18.0 M and beyond under sonication seems to influence the polymerization process. Under ultrasound irradiation, the rate constant $k_p$ increases from 3.68 $\times 10^{-4}$ to 5.64 $\times 10^{-4}$ s$^{-1}$ at monomer loading of 18.0 M and 4.17 $\times 10^{-4}$ to 5.58 $\times 10^{-4}$ s$^{-1}$ at monomer loading of 22.5 M. This observed increase in $k_p$ at higher concentration of ε-caprolactone monomer in comparison to the shaking process may be attributed to the reported effects of ultrasonic cavitation. This bubbles collapse was reported to generate high local pressures (up to 1,000 atm), temperatures (up to 5,000 K) and shear forces that increased the fluid velocity, which facilitates mass-transfer driving force.
improving the diffusion rate of the monomer from the bulk fluid to the active site of the enzyme [26, 36].

For a given concentration of the monomer, the $k_0$ values in the non-sonicated reaction were consistently lower compared to the values for the sonicated reaction (Figure 3b and Table 1). This suggests that sonication enhanced the rate of polymer chain propagation possibly by enhancing the mass transfer of the monomer. This observation is in accordance with previously reported literatures [21, 37, 38]. The linearity of the plots in Figure 3 suggests that polymer continued to grow at a fixed rate for much of the reaction period for both sonicated and non-sonicated systems. A close inspection of the plots (Figure 3) showed that the slopes of the lines declined noticeably around 75 h, as the increasing viscosity (viz. $1.9 \times 10^{-3}$ cP at 0 h to 4.1 cP at 75 h) of the reaction mixture likely adversely affected the mass transfer of the monomer and, therefore, the continued growth of the polymer.

Concomitantly, in sonicated synthesis, the reactant conversion increased with initial monomer concentration fed to the system, i.e. from 46.2% (at 4.5 M) to 73.2% (at 18.0 M) (Table 1). Thurecht et al. [39] reported about 70% of monomer conversion in supercritical CO$_2$, and the authors reported the reaction to proceed via first-order kinetics. At 22.5 M initial monomer concentration, the conversion became almost constant at ~69.3%. Contrary to this observation, in conventional agitation process, despite initial increase of monomer conversion at 4.5 M (42.5%) to 9.0 M (50.1%) initial ε-caprolactone concentration, progressive decrease in conversion was observed for the subsequent monomer concentration fed to the system (Table 1). It is also noted that at 4.5 M initial monomer concentration, almost similar conversion in both sonicated (46.2%) and conventional agitation systems (42.5%) was observed. It is hypothesized that at the lowest monomer concentration tested, as the polymerization progresses, the residual monomer concentration dropped below the level of apparent affinity of the enzyme toward ε-caprolactone. This caused the efficiency of polymerization hence conversion to be reduced irrespective of application of acoustic assistance.

### 3.4 Effect of ultrasound irradiation on polymer molecular weight

As previously reported, in sonicated synthesis, both the number averaged molecular weight ($M_n$) and the weight averaged molecular weight ($M_w$) of the polymer were substantially higher at any instance of the reaction compared to the values in non-sonicated synthesis. For example, at initial ε-caprolactone concentration of 18.0 M, the polymer synthesized using ultrasound-assisted process showed $M_n = 1.1$ kDa after 5 h, and increased to ~10 kDa after 86 h. Similar trend was observed in $M_w$, i.e. from 1.2 kDa after 5 h to 20.6 kDa after 86 h. In contrast, the $M_n$ for polymer synthesized in conventional agitation process was found to increase within a limited extent only viz. 0.77 kDa after 5 h to 3.0 kDa after 86 h. Similarly, the $M_w$ was found to increase from 1.1 kDa after 5 h to 9.8 kDa after 86 h. This is in agreement with the findings of earlier investigation [17]. Thus, the sonication process assured a better supply of the monomer to the location of the polymer growth point due to improved mass transfer resulting in the synthesis of polymer molecules that are longer on average.

In this study, sonication power intensity ($I$) was applied at $2.5 \times 10^3$ W m$^{-2}$; amplitude pressure ($P_a$) and acoustic pressure ($P_r$) were calculated at 3,000 N m$^{-2}$ and 148,050 atm, respectively, a pressure that is ~99 times greater than the reported acoustic pressure of 1,500 atm required to cause cavitation in water [26]. This leads to calculated sonic particle velocity ($v_p$) of 1.7 m s$^{-1}$ with corresponding particle acceleration ($a$) of $3.9 \times 10^5$ m s$^{-2}$, an acceleration that is over 40,000 times greater than under normal gravitational influence.

### Table 1 Comparison of $k_0$ between sonicated and non-sonicated reactions

<table>
<thead>
<tr>
<th>Monomer concentration [M]</th>
<th>Sonicated synthesis</th>
<th>Conversion $X_m$</th>
<th>Non-sonicated synthesis</th>
<th>Conversion $X_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_0 \times 10^{-4}$</td>
<td>$R^2$</td>
<td>$k_0 \times 10^{-4}$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>4.5</td>
<td>3.31</td>
<td>0.99</td>
<td>46.2</td>
<td>2.98</td>
</tr>
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<td>9.0</td>
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<td>0.99</td>
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</tr>
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<td>0.99</td>
<td>71.5</td>
<td>3.35</td>
</tr>
<tr>
<td>18.0</td>
<td>5.38</td>
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<td>3.68</td>
</tr>
<tr>
<td>22.5</td>
<td>5.58</td>
<td>0.98</td>
<td>69.3</td>
<td>4.17</td>
</tr>
</tbody>
</table>
The improvement in synthesized polymer molecular weight and \( k_p \) (at higher concentration of \( \varepsilon \)-caprolactone monomer) was obtained following 20 min exposure of reaction mixture to ultrasonic irradiation in the early period of biosynthesis. It seems that under earlier described acoustic conditions, this initial exposure proved beneficial towards reaction enhancement until late stage of polymerization where the effect of elevated viscosity in reaction mixture became untenable.

3.5 Reactor design for \( \varepsilon \)-caprolactone polymerization in a continuous flow system

The experimental data for \( r_p \) and \( X_m \) were simulated in a CSTR and PFR. In a CSTR, the volume required to achieve a maximum experimental monomer conversion of 74% was observed to increase with the increasing molar flowrate from 0.01 to 0.13 mol s\(^{-1}\) (Figure 4). When CSTR is operated at 0.13 mol s\(^{-1}\), the maximum reactor volume to achieve 74% \( \varepsilon \)-caprolactone conversion was found to be 11 L. On the other hand, reducing the molar flowrate to about 0.01 mol s\(^{-1}\) resulted in reduction of reactor volume by a factor of 10 (1.1 L). This increase in reactor volume for maximum conversion with increasing feed molar flowrate is due to the fact that in a CSTR conversion is a function of reactor volume that translates to the reaction species residence time [27]. Increase in molar flowrate results in a lower residence time between the enzyme and the reactant \( \varepsilon \)-caprolactone. Hence, for high conversion to take place at high molar flowrate, a longer residence time is required and this translates to a larger reactor volume. Similarly in PFR, the volume for maximum conversion was also observed to increase with increasing molar flowrate (Figure 4). However, in contrast to CSTR, simulating the \( \varepsilon \)-caprolactone polymerization reaction in PFR under the same feed conditions resulted in general reduction of the reactor volume for maximum conversion by a factor of 11 (Figure 5). For example, at a molar flowrate of 0.13 mol s\(^{-1}\), PFR volume for 74% conversion was found to be at 1 L as compared to 11 L in CSTR. This observation is in accord with the observation that in an isothermal reaction greater than zero order, the PFR volume for maximum conversion is lower than that of CSTR for the same conversion and reaction conditions [27].

4 Conclusions

Lipase-mediated, ultrasound-assisted \( \varepsilon \)-caprolactone polymerization was investigated in ionic solvent 1-ethyl-3-methylimidazolium tetrafluoroborate. Ultrasound irradiation helped to improve the rate constant of poly-\( \varepsilon \)-caprolactone chain propagation (\( k_p \)) at high initial monomer (\( \varepsilon \)-caprolactone) concentration. The acoustic effects could have also allowed the reaction to continue longer compared to non-sonicated process until it became impossible at highly elevated reaction mixture viscosity. Consequently, it also helped to improve monomer conversion. In a continuous flow polymerization system, PFR system is recommended due to its lowest volume for maximum conversion compared to CSTR.

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