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Synthesis of polyesters containing disiloxane subunits: Structural characterization, kinetics, and an examination of the thermal tolerance of Novozym-435

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ABSTRACT

This paper reports the Novozym-435 mediated polymerization of disiloxane-containing polyester monomers under solvent-free conditions. The thermal tolerance of the immobilized enzyme was examined by conducting polymerization cycles over a temperature range of 35–150 °C. Increasing the temperature up to 100 °C afforded an increase in the apparent second order rate constant. Residual activity was measured using the production of octyl palmitate. The enzyme was shown to retain on average greater than 90% of its residual activity regardless of the polymerization temperature. This prompted a study of the long term thermal tolerance of the biocatalyst in which it was determined that over ten reaction cycles there was a significant decrease in the initial polymerization rate, but no change in the degree of monomer conversion after 24 h. The disiloxane containing polyesters were characterized using nuclear magnetic resonance spectroscopy and Fourier-transform infrared spectroscopy. Differential scanning calorimetry was used to determine the thermal properties of the disiloxane-containing polyesters.

1. Introduction

Silicones and other siloxane-derived materials are of great industrial and economic importance [1]. Low molecular weight cyclic silicones are commonly found in the health care and cosmetics industries where they are typically incorporated into topical applications, the food and beverage industry where they are used as antifoaming agents, and in sealants and coatings where their inherent hydrophobicity is the desired characteristic.

The popularity of siloxanes is a result of their physical and chemical properties, which makes them well suited for a range of applications. Siloxanes, and in particular silicones, are valued for their thermal stability (silicones typically do not degrade until temperatures in excess of 350 °C are obtained), low glass transition temperatures which result from the dynamic nature of the siloxane bond, resistance to oxidation, and low permittivity values [1,2]. The basis for these physical characteristics is derived from the Si—O—Si bond. The angle of the siloxane linkage is rather larger, typically 145°, compared to 109.5° for typical tetrahedral organic systems. It has been postulated that this angle may be flexible enough to actually range from 90° to 180°. The siloxane linkage is highly polarisable as a result of the high ionic nature of the Si—O bond which has been determined to be approximately 51% ionic [3]. The Si—O bond possesses very low rotational bond energy as well as being one of the strongest chemical bonds known with a covalent bond energy of 452 kJ/mol (108 kcal/mol) and ionic bond energy of 1013 kJ/mol (242 kcal/mol) [2].

Many methods have been designed to produce linear, branched, or cross linked silicones. Most commonly used are catalysts based on the platinum complexes developed by Karstedt and Speier, and on alkoxytitanium complexes. Tin carboxylates are commonly used, particularly in the room temperature vulcanization of silicone elastomers. However, the potential toxicity of alkyl tin complexes [4] renders this class of catalyst undesirable especially in the synthesis of biomaterials. Other general cure methods include high temperature vulcanizations, and thermal- or copper-mediated [1,3]-dipolar additions [5,6]. More recent work on the Piers–Rubensztajn reaction has lead to the development of $B(C_6F_5)_3$ as a catalyst for the preparation of star-shaped siloxanes and functionalized silicones from hydrosilanes and alkoxysilanes [7–9].

Polyesters are commercially available in a variety of products such as fibres, fillings, coatings, and textiles and are typically produced under extremes of heat, sometimes in excess of 250 °C, and under reduced pressure [10]. A variety of catalytic methods have been developed for producing polyester materials, typically using

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strong acids over stoichiometric quantities of both diols and diacids. Alternatively, dibutyltin oxide or dibutyltin dilaurate have been employed, but given the aforementioned toxicity concerns, these catalysts may wish to be avoided. While strong acids are ideal for polyester synthesis, they are not always compatible with siloxane polymers due to the possibility for redistribution of the siloxane backbone, or cleavage of the siloxane network [1]. Additionally, the condensation of diols with acyl chlorides, and the ring opening polymerization of lactones have been explored as viable routes to polyester synthesis [11].

Enzymatic methods are becoming increasingly popular in organic, bioorganic, and polymer chemistry. Lipase B from *Candida antarctica* immobilized on a macroporous acrylic resin and sold under the trade name Novozym-435, has been the work horse for synthesizing polymeric materials [12–15] and has been used to synthesize organosilicon amides and esters [16]. Furthermore, an Amberzyme-immobilized cutinase from *Humicola insolens* has garnered some recent attention for its polyester synthase ability [17,18]. This cutinase possessed stricter substrate specificity than N435 showing a preference for C10 and C13 diacids whereas those diacids with chain lengths shorter than C10 were not processed particularly well.

The synthesis of polyesters incorporating siloxanes has been demonstrated although, typically, the siloxane is only a minor component of the final polymer system [13,19–22]. The enzymatic synthesis of polyesters derived exclusively from siloxane-derived monomers has been described [23]. The number of siloxane units of the diol monomer did not affect the polymerization kinetics or the activation energy of the polymerization process [23].

In this paper disiloxane polyesters were synthesized employing N435 catalysis under solvent-free reaction conditions. The disiloxane polyesters were subsequently characterized by nuclear magnetic resonance (NMR) spectroscopy and Fourier-transform infrared (FT-IR) spectroscopy. Differential scanning calorimetry (DSC) was employed to determine the thermal transitions of the monomers and resulting siloxane containing polyesters.

In order for biocatalysis to be viable on an industrial scale cost must be minimized. One method to facilitate this would be to design a catalyst with a high turnover number, or a catalyst that would be amenable to multiple reaction cycles. The residual activity of N435 was examined, after each polymerization, by using a standard enzymatic assay in which the production of octyl palmitate from 1-octanol and palmitic acid was monitored. In conjunction with the residual activity assays, a single batch of N435 was used for multiple 24 h reaction cycles at 100 °C to gain some insight into the thermal tolerance and reusability of N435 as a polymerization catalyst.

2. Experimental

2.1. Materials

Lipase B from *C. antarctica* immobilized on acrylic resin (sold under the trade name Novozym-435, N435; EC.3.1.1.3, 10,000 U/g), activated carbon, 1,1,3,3-tetramethyldisiloxane (TMDS, 97%) and platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl disiloxane complex (Karstedt's catalyst, Pt^0(dvs)) in xylenes were obtained from Sigma–Aldrich (Oakville, Ontario, Canada). 1,3-Bis(3-carboxypropyl)-1,1,3,3-tetramethyldisiloxane (CPr-TMDS) was obtained from Gelest (Morristown, PA, USA). Allyl acetate (98%) was obtained from Alfa Aesar (Ward Hill, MA, USA). Isooctane (2,2,4-trimethylpentane, 99%) was obtained from Caledon Chemicals (Georgetown, Ontario, Canada). Chloroform-*d* ($CDCl_3$, 99.8% deuterated) was a product of Cambridge Isotope Laboratories, Inc. (Andover, MD, USA). Diethyl ether (99%) was acquired from Anachemia Science (Montréal, Québec, Canada). Distilled water was used when necessary. Chemicals were used as received without further modification or purification unless otherwise stated. All of the commercially available reagents were at a minimum pure by NMR (≥95%).

2.2. Methods

2.2.1. Nuclear magnetic resonance spectroscopy (NMR)

All NMR spectra were recorded in $CDCl_3$ on a Bruker Avance AV-300 spectrometer (^{1}H at 300 MHz, ^{13}C at 77 MHz, and ^{29}Si at 59.6 MHz) using the residual signal of $CHCl_3$ as an internal reference for ^{1}H spectra, and the three ^{13}C resonances of $CDCl_3$ as the internal reference for ^{13}C NMR spectra; tetramethylsilane (TMS) was used as an internal standard for ^{29}Si NMR spectra. NMR spectra were analysed using the Bruker Topspin v2.0 software interface.

2.2.2. Fourier-transform infrared spectroscopy (FTIR)

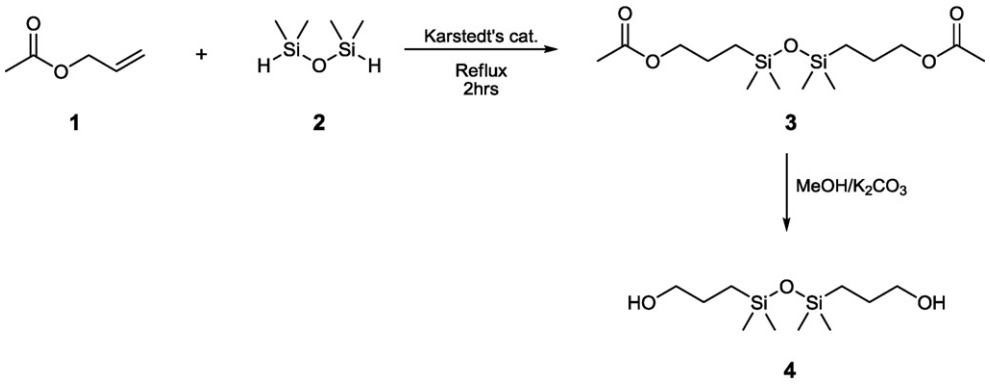
FTIR spectra were recorded on a Mattson research series infrared spectrometer operating in transmittance mode. Samples were prepared as neat, thin films on KBr plates. Each spectrum was comprised of 32–64 scans at 2 cm^{-1} resolution. Analysis of the FTIR data was performed using the Winfirst software platform. Peak assignments were made based on data previously reported in the literature [24].

2.2.3. Differential scanning calorimetry (DSC)

DSC thermograms were acquired on a Shimadzu DSC-60 differential scanning calorimeter. Polymer samples (approximately 10 mg) were transferred into aluminium pans and cooled to −150 °C at a rate of 10 °C/min. Samples were heated at 20 °C/min to 200 °C and subsequently cooled at 10 °C/min to −150 °C. A second heating scan was done at 20 °C/min to 200 °C. DSC data was analysed using the TA60 version 2.11 software platform.

Synthesis of 1,3-*bis*(3-*hydroxypropyl*)-1,1,3,3-*tetramethyldisiloxane* (**4**, 3HP-TMDS, Scheme 1). A round bottomed flask was charged with 1,1,3,3-tetramethyldisiloxane (**2**) and Karstedt's catalyst and stirred for 10 min. Allyl acetate (**1**) was added drop-wise through a septum over 30 min and the reaction mixture was allowed to reflux for 2 h to give 1,3-bis(3-acetoxypropyl)-1,1,3,3-tetramethyldisiloxane (**3**) in 83% isolated yield. ^{1}H NMR (300 MHz, $CDCl_3$, 7.26 ppm): 0.06, 0.11, 0.25 and 0.28 ppm ($\underline{\mathbf{Me_2}}$SiO—), 0.5 ppm (*m*, $CH_2CH_2\underline{\mathbf{CH_2}}$Si, 4H), 1.63 ppm (*m*, $CH_2\underline{\mathbf{CH_2}}CH_2$Si, 4H), 4.01 ppm (*m*, AcO$\underline{\mathbf{CH_2}}CH_2CH_2$Si, 4H), $\underline{\mathbf{CH_3}}$C=O (*s*, 3H); ^{13}C NMR (77.0 MHz, $CDCl_3$, 77.0 ppm): 0.22 ppm (($\underline{\mathbf{CH_3}}$)$_2$SiO—), 14.12 ppm ($CH_2CH_2\underline{\mathbf{CH_2}}$Si), 21.00 ppm ($CH_2\underline{\mathbf{CH_2}}CH_2$Si), 22.59 ppm ($\underline{\mathbf{CH_3}}CO_2R$), 66.94 ppm (AcO$\underline{\mathbf{CH_2}}CH_2CH_2$), 171.16 ppm ($\underline{\mathbf{C}}$=O). De-acylation of bis-acetate **3** was routinely carried out using an 8 fold excess of anhydrous MeOH and 20 mol% K_2CO_3 for 2 h at room temperature to yield diol **4** in nearly 87–95% yield as a clear to straw coloured liquid [25]. ^{1}H NMR (300 MHz, $CDCl_3$, 7.26 ppm): 0.05 ppm (*s*, 12H, $\mathbf{Me_2}$Si), 0.528 ppm (*m*, 4H, $CH_2CH_2\mathbf{CH_2}$Si), 1.646 ppm (*m*, 4H, $CH_2\mathbf{CH_2}CH_2$Si), 2.327 ppm (*t*, 4H, $\mathbf{CH_2}CH_2CH_2$Si, J = 7.5 Hz); ^{13}C NMR (77 MHz, $CDCl_3$, 77.01 ppm): 0.25 ppm ($\mathbf{Me_2}$Si), 18.03 ppm ($CH_2CH_2\mathbf{CH_2}$Si), 19.12 ppm ($CH_2\mathbf{CH_2}CH_2$Si), 37.48 ppm ($\mathbf{CH_2}CH_2CH_2$Si); ^{29}Si NMR (59.6 MHz, $CDCl_3$, TMS): 7.28 ppm; EI-MS: (M^+) 250 *m*/*z*.

Synthesis of 1,3-*bis*(3-*carboxypropyl*)-1,1,3,3-*tetramethyldisiloxane dimethyl ester* (**5**, **CPr-TMDS-DME**). Diester **5** was synthesized using previously published protocols [23]. Briefly, 5.01 g (16.34 mmol) of 1,3-bis(3-carboxypropyl)-1,1,3,3-tetramethyldisiloxane was refluxed in 15 mL of methanol in the presence of 5 mol% *p*-toluene sulfonic acid for 4 h to yield diester **5** as a clear and colourless liquid in 86% yield. ^{1}H NMR



Scheme 1. The synthesis of 1,3-bis(3-hydroxypropyl)-1,1,3,3-tetramethyldisiloxane.

(300 MHz, $CDCl_3$, 7.26 ppm): 0.05 ppm (*s*, 12H, **Me$_2$**Si), 0.528 ppm (*m*, 4H, CH_2CH_2**CH_2**Si), 1.646 ppm (*m*, 4H, CH_2**CH_2**CH_2Si), 2.327 ppm (*t*, 4H, **CH_2**CH_2CH_2Si, *J* = 7.5 Hz), and 3.663 ppm(*s*, 6H, **Me**OCO); ^{13}C NMR (77 MHz, $CDCl_3$, 77.01 ppm): 0.25 ppm (**Me$_2$**Si), 18.03 ppm (CH_2CH_2**CH_2**Si), 19.12 ppm (CH_2**CH_2**CH_2Si), 37.48 ppm (**CH_2**CH_2CH_2Si), 53.39 ppm (**Me**OCO), 174.09 ppm (MeO**C**=O); ^{29}Si NMR (59.6 MHz, $CDCl_3$, TMS): 7.28 ppm (**Si**—O—Si); EI-MS: ($M^+ - CH_3$) 319 *m*/*z*.

2.2.4. Preparation of disiloxane polyesters

Disiloxane polyesters were prepared under solvent-free conditions using 5 wt.% N435 (with respect to the mass of the combined monomers) following previously provided protocols with minor modifications [23]. Typically, diol **4** was combined in a 1:1 mole ratio with diester **5**, using approximately 0.5–0.75 g of the monomers. Individual reactions were incubated at temperatures between 35 °C and 150 °C with stirring for 24–72 h. The reactions were terminated with the addition of 5 mL of diethyl ether after cooling to RT. The enzyme beads were removed by filtration through a glass fritted Buchner filter of medium porosity and washed with two 10 mL volumes of diethyl ether. Solvents were removed *in vacuo* to yield siloxane-polyesters as clear and colourless, viscous liquids. ^{1}H NMR (300 MHz, $CDCl_3$, 7.26 ppm): 0.059 ppm (*s*, **Me$_2$**Si), 0.535 ppm (*m*, CH_2CH_2**CH_2**Si), 1.642 ppm (*m*, CH_2**CH_2**CH_2Si), 2.320 ppm (*t*, **CH_2**CH_2CH_2Si), and 3.667 ppm (*s*, **Me**OCO), 4.021 ppm (*t*, C(O)OC**H$_2$**—); ^{13}C NMR (77 MHz, $CDCl_3$, 77.01 ppm): 0.02 and 0.19 ppm (**Me$_2$**Si), 13.99 and 18.03 ppm (CH_2CH_2**CH_2**Si), 19.12 and 22.57 ppm (CH_2**CH_2**CH_2Si), 37.65 ppm (**CH_2**CH_2CH_2Si), 66.6 ppm (**CH_2**OCO), 173.58 ppm (MeO**C**=O); ^{29}Si NMR (59.6 MHz, $CDCl_3$, TMS): 7.30 and 7.72 ppm (**Si**—O—Si).

2.2.5. Residual enzyme activity of recovered lipase

The recovered N435 was assayed for residual activity by measuring the production of octyl palmitate from 1-octanol and palmitic acid. A stock solution of 200 mM 1-octanol and 200 mM palmitic acid in isooctane was freshly prepared before each assay. For each reaction 5 mg of lipase was combined with 1 mL of the assay mixture and incubated at 35 °C for 1 h with magnetic stirring. A 20 μL aliquot of the reaction mixture was analysed by ^{1}H NMR. The ratio of the resonances associated with the *O*-methylene protons in 1-octanol and octyl palmitate was used to determine the degree of esterification. The methylene resonance in the ester is located at 4.02 ppm while the corresponding methylene resonance in the 1-octanol is located at 3.66 ppm. A fresh enzyme preparation, that is enzyme that had not been used to synthesize disiloxane polyester systems, was prepared for use as a control. The amount of esterification achieved from the fresh enzyme was set to a default value of 100% and all other assays were calibrated to this value. An enzyme-free control reaction was also performed to determine the background rate, if any, for the esterification of octyl palmitate.

2.2.6. Thermal tolerance and reuse of N435

A single batch of N435 was subjected to multiple 24 h reaction cycles at 100 °C to determine the number of times that the enzyme could be reused before it was determined to be unsuitable for catalysis. The apparent second order rate constant, which was derived from the slope of the line from a plot of the average degree of polymerization *versus* time, as well as the extent of monomer conversion were compared. Between each 24 h reaction cycle, the N435 beads were recovered by the addition of 5 mL of diethyl ether at room temperature for 10 min. After stirring, the beads were filtered through a glass filter of medium porosity and washed with three 10 mL aliquots of diethyl ether. The mass of the beads recovered was determined and the volumes of each reagent for subsequent reaction mixtures were adjusted accordingly.

3. Results and discussion

One of our research goals is the exploration of enzymatic methods to mediate traditional organosilicon transformations. To address these goals, we have employed enzymatic catalysis in the form of the immobilized lipase B from *C. antarctica* (N435) for the synthesis of disiloxane-containing materials. The reaction scheme for the enzyme-mediated polymerization under investigation is outlined briefly in Fig. 1.

3.1. Structural characterization of disiloxane polyesters

The structural environments of the disiloxane-derived polymers were probed primarily through NMR and FT-IR spectroscopies. A typical ^{1}H NMR spectrum for the disiloxane polyesters is presented in Fig. 2. There is a triplet positioned at 4.02 ppm which represents the methylene protons alpha to the oxygen atom in the newly formed ester linkage between the disiloxane-containing monomers. There is a smaller singlet located at 3.67 ppm that is assigned as unreacted methyl ester end groups [23]. The methylene protons alpha to the carbonyl in diester **2** experience a small shift up-field from 2.34 ppm in the monomer to 2.32 ppm in the final polymer, while the *O*-methylene protons of diol **4**, formally positioned at 3.60 ppm were only barely visible. The remaining multiplets, 0.54 ppm and 1.64 ppm, represent the methylene protons that are in the α- and β-positions with respect to silicon; the geminal methyl groups on silicon are positioned at 0.06 ppm and remained largely unchanged throughout the polymerization process.

The ^{13}C NMR spectrum confirms the synthesis of the disiloxane polyesters. The resonance for the carbonyl carbon resides

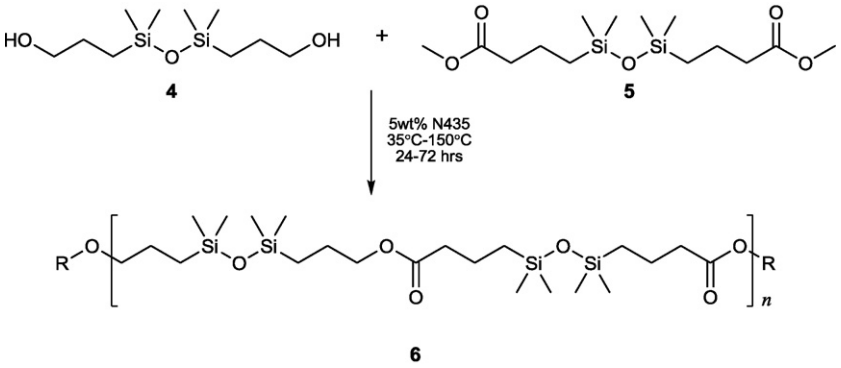


Fig. 1. The synthesis of disiloxane polyesters using N435 under solvent-free conditions.

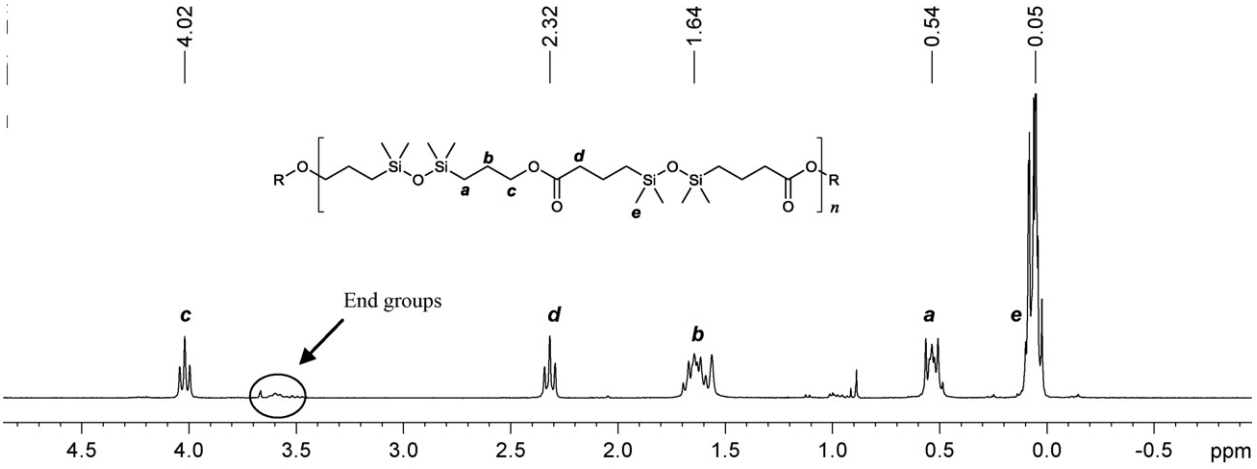


Fig. 2. A representative ^{1}H NMR spectrum of enzymatically synthesized disiloxane polyesters.

at 173.6 ppm while the *O*-methylene carbon can be found at 66.6 ppm; the carbon adjacent to the ester linkage was positioned at 37.7 ppm. The remaining resonances were located at 13.99 ppm and 17.96 ppm have been identified as $CH_2\mathbf{CH_2}Si$ (in monomer and polymer), and 19.06 ppm and 22.57 ppm has been identified as the $\mathbf{CH_2}CH_2Si$ environment in monomer. The ^{29}Si spectrum possessed two distinct resonances which were located at 7.30 ppm and 7.72 ppm. These signals were in the expected range for disiloxane linkages and do not represent a possible silanol or alkoxysilane.

The FTIR spectrum for a typical disiloxane polyester is presented in Fig. 3. The assignment of vibrational modes was made based on previously reported data [24]. The carbonyl bond shows a strong absorbance located at 1737 cm^{-1} which was formerly located at 1742 cm^{-1} in the methyl ester monomer. The characteristic vibrational modes of the siloxane fragments are present as the Si—O—Si stretches located at 1050 cm^{-1}. The FT-IR spectra support the hypothesis that the enzyme did not participate in Si—O—Si cleavage as the expected vibrational stretching mode for the free silanol (~3350 cm^{-1}) was not observed [26].

3.2. Thermal characteristics

The thermal characteristics of the disiloxane polyesters were probed using differential scanning calorimetry (DSC). DSC thermograms were acquired using a Shimadzu DC-60 differential scanning calorimeter under air. Disiloxane-derived monomers, as well as the enzymatically produced disiloxane polyesters, were cooled to −150 °C from room temperature at a rate of −10 °C/min. Each sample was then heated to 200 °C and subsequently cooled and reheated at 10 °C/min. The glass transition temperatures (T_g) were acquired from the second heating scan. DSC thermograms for a representative disiloxane polyester, along with diol **4** and diester **5**, are presented in Fig. 4. The T_g for diol **4** and diester **5** were determined to be −99 °C and −109 °C, respectively. These values are somewhat higher than that for a typical polydimethylsiloxane which shows a T_g near −125 °C [2]. The disiloxane polyester **6** exhibited intermediate T_g values at −104 °C. As a result of the dynamic nature of the disiloxane linkage, well defined melting temperatures and cold

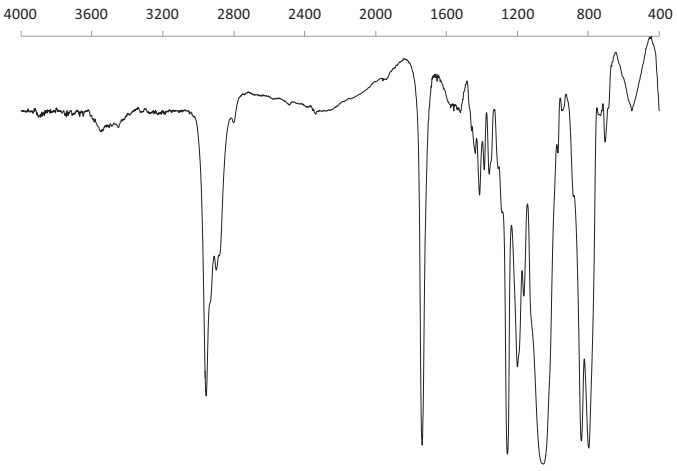


Fig. 3. Representative FTIR spectra of a disiloxane-containing polyester.

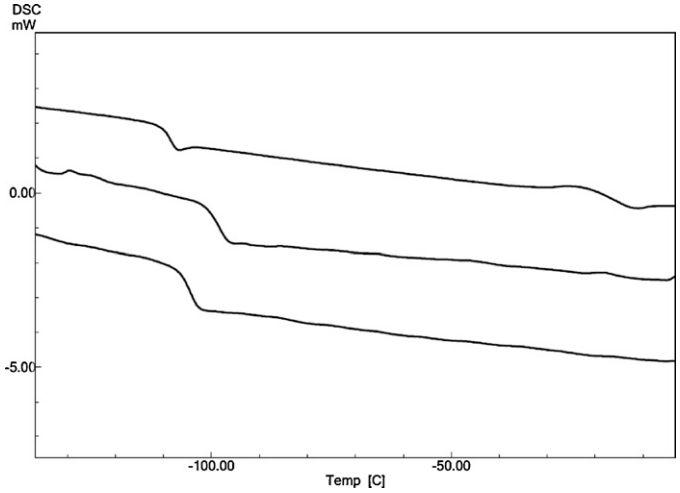


Fig. 4. Representative DSC thermograms of starting materials and the enzymatically produced disiloxane polyesters. Top thermogram: CPr-TMDS-DME, middle thermogram: 3HP-TMDS, and bottom thermogram: disiloxane polyester.

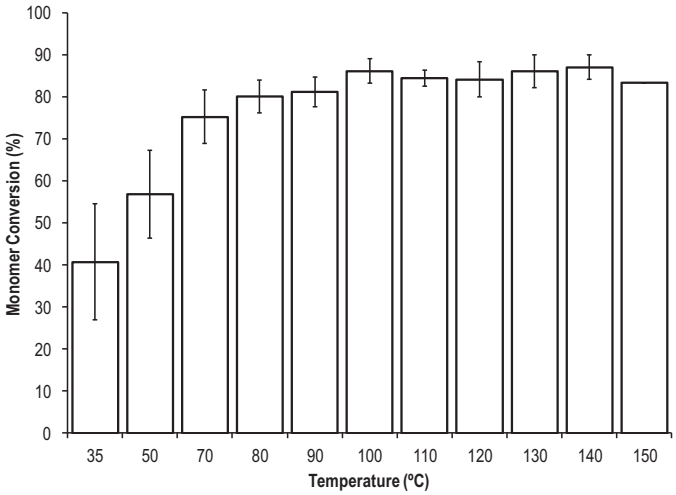


Fig. 6. Monomer conversion after 24 h of polymerization using N435 as catalyst. Each bar is the average of multiple replicate trials; error bars represent the standard deviation.

crystallization temperatures could not be determined for any of the polyester samples.

3.3. Disiloxane polymerization

The synthesis of polyesters from the polymerization of diols, with diesters, diacids or acid chlorides is generally thought to be a second order process [27]. The apparent rate constant for these reactions can be determined using a plot of the average degree of polymerization (DP) *versus* time, where $DP = 1/(1-p)$ in which p is the extent of monomer conversion during the first hour of the reactions. Enzyme-free control reactions were performed at all temperatures and no background rate of transesterification was observed. Our experiments showed that the reaction temperature at which each polymerization was conducted had a significant impact on the initial reaction rate (Fig. 5). At reaction temperatures equal to or less than 70 °C, the polymerization of monomers was slow while increasing the reaction temperature above 70 °C afforded higher polymerization rates. This increase can be partially attributed to the ease of removal of the methanol by-product

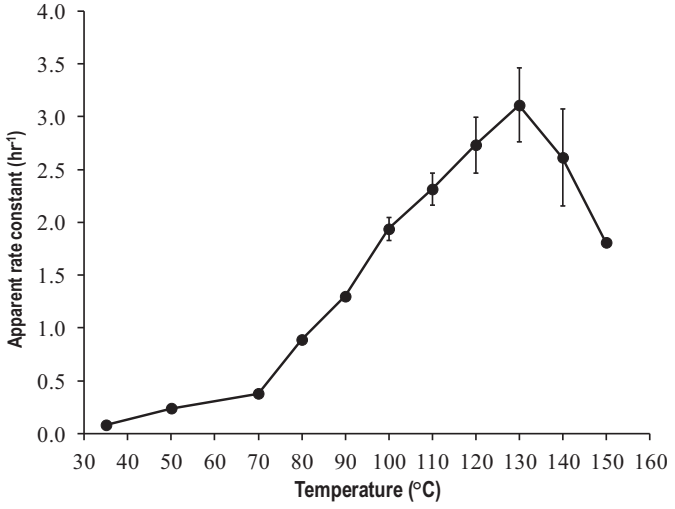


Fig. 5. The effect of temperature on the apparent second order rate constant for the N435-catalysed polymerization of diol **4** with diester **5**. Each data point is the average of three trials; error bars represent the standard error of the means. The line is included to guide the eye.

thus forcing the equilibrium to favour the products in accordance with Le Châtelier's principle. Further increases in temperature were hypothesized to be deleterious to the functioning of the enzyme as a result of thermal denaturation. However, polymerizations conducted at 100 °C did not have the expected effect of protein denaturation. In fact the apparent rate constant associated with the polymerization continued to increase. To test the thermal tolerance of N435, we continually increased the reaction temperature in ten degree intervals to a maximum of 150 °C and performed 24 h polymerization cycles. The expectation was that at elevated temperatures the enzyme would become completely and irreversibly denatured. To our surprise each batch of N435 polymerized the disiloxane monomers with increasing proficiency reaching a maximum at 130 °C; above 130 °C the rate of polymerization slowly decreased. Furthermore, there appeared to be a complete loss of catalytic activity when polymerizations were carried out at 150 °C. These observations suggest that the optimal polymerization temperature of disiloxane containing monomers by N435 is approximately 130 °C, higher than that reported for the polymerization of poly(caprolactone) and poly(pentadecalactone) [28].

The ratio of the resonances at δ 3.66 and 4.02 were compared to determine the degree of monomer conversion after 24 h of polymerization (Fig. 6). As the reaction temperature was increased from 35 °C to 90 °C there was a steady increase in monomer conversion. For polymerizations conducted above 90 °C, monomer conversion seemed to plateau between 85% and 90%. We were concerned that a potential cause for the apparent plateau was a reduced capacity of the enzyme to process the disiloxane-containing substrates. This hypothesis was tested by separating the enzyme from the reaction mixture and assaying an aliquot of the enzyme for residual activity.

3.4. Residual activity of N435

The residual activity of the immobilized enzyme was quantified by monitoring the production of octyl palmitate from 200 mM 1-octanol and 200 mM palmitic acid in isooctane at 35 °C for 1 h (Fig. 7) [13]. A typical assay was performed using 5.0 mg of recovered lipase beads in 1 mL solution of the ester precursors; reactions were carried out at 35 °C for 60 min with the aid of magnetic stirring. Control assays which consisted of fresh N435 that had never been used, and an enzyme-free control reaction were included for comparison. Octyl-palmitate assays were monitored by ^{1}H NMR

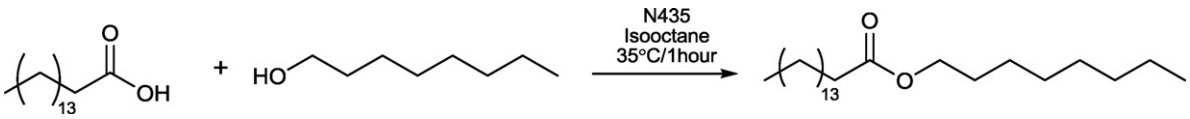


Fig. 7. The esterification of 1-octanol and palmitic acid to octyl palmitate was used to determine the residual activity of N435 after each polymerization reaction.

again using the change in the intensity of the resonances for the *O*-methylene protons in 1-octanol and octyl palmitate. In the absence of any enzyme esterification between 1-octanol and palmitic acid was not observed; no observable resonance visible at 4.02 ppm was apparent. On the contrary, the virgin enzyme processed 93% ($n = 3$) of the 1-octanol within the allotted time period. All subsequent assays were normalized to this value to give the relative residual activity. Every batch of recovered enzyme that was assayed, even those used at 100 °C, retained in excess of 95% of their relative activity compared to the virgin enzyme. Binns et al. [29] reported the residual activity of CALB to range between 6% and 88% after the polymerization of adipic acid with a range of simple diols. In effect, the residual activity of the enzyme increased with the decreasing polarity and increasing hydrophobicity of the reaction medium. The highest residual enzyme activity was seen when 1,5-pentane diol and 1,6-hexane diol were used. Poojari et al. recovered between 5% and 50% residual activity after the polymerization of 1,3-bis(3-carboxypropyl)-1,1,3,3-tetramethyldisiloxane and 1,4-butanediol, 1,6-hexanediol or 1,8-octanediol [13]. The more hydrophobic 1,8-octanediol did not affect the residual activity of the enzyme as compared to the other diol monomers. Two solvents were used by these researchers to isolate the acrylic beads in hopes of determining if there was a solvent effect on the residual activity. When tetrahydrofuran was the isolation solvent the enzyme retained a higher amount of activity compared to the enzyme that was isolated with toluene. The Gross group reported the retention of approximately 80% of the residual activity from a batch of N435 after 48 h of polymerizing adipic acid and 1,8-octanediol at 70 °C [14].

There appears to be a significant relationship between the hydrophobicity of the monomers and the amount of residual enzymatic activity of the enzyme catalyst. This is perhaps not surprising given the native environment in which lipases have evolved to function. Silicones are known to be some of the most hydrophobic materials, and as such disiloxane polyesters should offer a suitable environment for lipases to function. In fact *Candida rugosa* lipase entrapped within a silicone elastomer retained high activity over extended periods of time even when stored at room temperature [30,31]. Furthermore, the free form of the lipase displayed higher activity when assayed in silicone oil (D_5) compared to simple hydrocarbons [30,31].

3.5. Repeated use of N435

In light of the residual activity of N435, even after prolonged exposure to elevated temperatures, it was of interest to determine the long-term thermal tolerance of a single batch of the immobilized catalyst. We were interested to determine the number of reaction cycles that a single batch of N435 could be used in before it became unsuitable for catalysis. To accomplish this, a series of 24 h reaction cycles at 100 °C were performed. The change in both the second order rate constant and the degree of monomer conversion were used to determine the effect of long term reaction sequences. Between each consecutive use of the catalyst, the reaction mixture was cooled to room temperature and washed with diethyl ether to cleanse the acrylic beads of any residual polymer. The acrylic beads were separated from the crude reaction mixture by filtering through a medium porosity glass fritted filter.

The change in the apparent second order rate constant and monomer conversion for each of 10 successive uses of the same batch of N435 is presented in Fig. 8. With the exception of the first three trials in which the apparent rate constant remained constant, each successive use of the immobilized enzyme led to some loss in catalytic activity. The apparent rate constant decreased by approximately 50% after six reaction cycles and by the tenth reaction cycle more than 80% of the initial enzyme activity had been lost. Despite the loss in catalytic activity, when the polymerizations were allowed to continue for the full 24 h reaction cycle, monomer conversion continued to reach high levels, typically in the range of 80–93%. These results can be compared to a previous study where a single batch of N435 was used for the ring opening polymerization (ROP) of ε-caprolactone to produce polycaprolactone (PCL) in toluene at 70 °C over 4 h reaction cycles [32]. In that study fresh N435 was shown to have lower activity for the first reaction cycle than in subsequent cycles. It was postulated that swelling of the acrylic resin allowed for lipase molecules that were immobilized on the interior of the solid support to become available to the medium and participate in the ROP of PCL [32].

The increased thermal tolerance of the immobilized lipase can be potentially attributed to the favourable interaction between the solid support and the enzyme, or the interaction between the enzyme and the hydrophobic disiloxanes. The lipase is potentially protected from thermal denaturation due to many favourable, although relatively weak, interactions it may have with the solid support (*i.e.* hydrogen bonding and van der Waals interactions) which combine to impart stability to the enzyme [33]. Enzymes are also known to maintain structural rigidity in organic solvents, as long as the solvent is hydrophobic and non-polar, which limits its molecular motion and in turn thermal denaturation [34]. Both of these factors are under consideration for the observed residual activity of N435 after use at higher temperatures. The substrate of immobilization may form electrostatic interactions with the lipase imparting a protective effect against denaturation. Even though a hydrophobic solvent was not used here, *per se*, the hydrophobicity

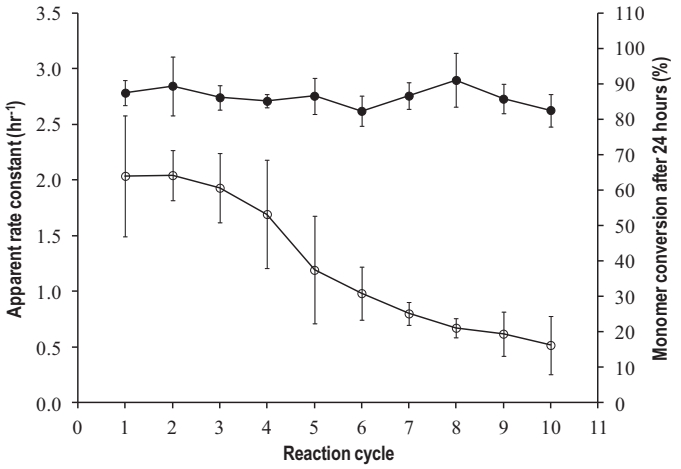


Fig. 8. The effect of multiple reaction cycles for a single batch of N435. The apparent rate constant (open circles) and total monomer conversion after 24 h (black circles) are shown for each of ten consecutive 24 h reaction cycles at 100 °C.

of the disiloxanes could be hypothesized to function in a similar role, and contribute to the stabilization of the lipase.

4. Conclusions

Enzymatic catalysis using N435 has been applied to the synthesis of disiloxane containing polyesters under solvent-free conditions. The structural microenvironments of the disiloxane polyesters were probed by NMR and FTIR spectroscopies. Furthermore, the spectral data suggest that N435 does not exhibit catalytic activity towards the disiloxane linkage; disiloxane cleavage was not observed. Differential scanning calorimetry provided data for the thermal transitions within these systems; the T_g of the polymeric materials was determined to be −114 °C, slightly higher than that expected for typical polydimethylsiloxane, but lower than that expected for aliphatic polyesters. Well defined melting points or cold crystallization data could not be detected given the dynamic nature of the siloxane backbone of the polymers.

Reaction temperature and the observed rate constant showed a positive correlation, however this relationship was not linear. Increases in the reaction temperature from 35 °C to 100 °C led to increases in the apparent rate constant as well as the M_n of the systems. At 150 °C the enzyme elicited a diminished degree of activity, with the apparent rate constant being depressed when compared to those polymerizations conducted at lower temperatures. After 24 h at 150 °C the enzyme had lost all catalytic activity however it is currently not known where during the 24 h reaction cycle that this catastrophic denaturation occurs. A single batch of N435 could be reused for ten 24 h reaction cycles without showing a significant decrease in monomer conversion even though the reaction rates dropped precipitously, nearly 80%, over ten reaction cycles.

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