Research Article

Electrospun Fibres of Polyhydroxybutyrate Synthesized by Ralstonia eutropha from Different Carbon Sources

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The properties of PHB may be affected by the carbon source used in its production and this may affect nanofibres made from this polymer by electrospinning. In this study, P(3-HB) was produced from glucose, rapeseed oil, and olive oil by Ralstonia eutropha H16. Cell growth and polymer production were higher in olive or rapeseed oil supplemented media compared to glucose supplemented media. FT-IR, 1H-, 13C-NMR, and ESI/MS confirmed that the synthesized polymers were P(3-HB). SEM micrograph showed the formation of nanofibres from P(3-HB) samples with the fibre diameters dependent on the source of the carbon used in polymer synthesis and the concentration of the polymer in the electrospinning solution. GPC showed that P(3-HB) from glucose (G-PHB) had a higher molecular weight ($7.35 \times 10^5$ g mol$^{-1}$) compared to P(3-HB) from rapeseed (R-PHB) and olive (O-PHB) oil. Differential scanning calorimetry (DSC) showed that the crystallinity of the electrospun polymers reduces with decreasing polymer concentration with R-PHB having lower crystallinity at all concentrations used. These observations show that more PHB yield can be obtained using either rapeseed or olive oil compared to glucose with glucose producing polymers of higher molecular weight. It also show that electrospinning could be used to reduce the crystallinity of PHB fibres.

1. Introduction

Polyhydroxybutyrate (PHB) is a biodegradable, biocompatible, and nontoxic biopolymer produced by different species of bacteria from soluble substrates and is used by bacteria as carbon and energy reserves in the presence of an excess amount of carbon and a limited availability of nutrients such as phosphorus and nitrogen [1–4]. The synthesis of PHB proceeds best in an environment with a high carbon to low nutrient ratio. After complete utilisation of nitrogen source, growth is no longer possible and the organism channels its energy into the synthesis of the energy reserve material, PHB. Sugars are the most common substrate used for PHB synthesis by bacteria [4].

The biodegradability, biocompatibility, and nontoxic properties of PHB have made it an attractive choice in various applications such as drug delivery and tissue engineering [5]. PHB is also known to possess various nutritional and therapeutic benefits [5–8]. Although the use of PHB has been established in various industrial and biomedical sectors, its commercial success can be threatened by the high cost of production, which is largely due to the cost of the carbon substrate [4]. Due to this factor, most research on PHB production has been focused on identifying cheap and renewable sources of carbon such as cellulose, starch, and plant oils.

Several biochemical pathways have been described for the synthesis of polyhydroxyalkanoates (PHA) within bacteria.
cells from different carbon substrates [4]. *Ralstonia eutropha* (also known as *Cupriavidus necator* or *Ralstonia eutropha*) is about the most studied bacterial species able to synthesize PHA from various carbon sources. These bacteria can accumulate up to 85% PHA per cell dry weight with about 7–12 PHA granules per cell [9]. Plant oils consist of triglycerides in which fatty acids (FA) are attached to glycerol. They have more carbon atoms per weight compared to the most substrate used for PHB production [10] and their complete oxidation has been shown to result in production of more PHB than conventionally used substrates [11]. The FA composition of the oil is however likely to impact on the bacterial growth and PHB synthesis and thus may affect the yield of PHB from *R. eutropha* fermentation. Studies have shown that unsaturated fatty acids inhibit bacterial growth [12–14]. Rapeseed oil and olive oil contain different proportions of saturated, monounsaturated, and polysaturated FAs. It is therefore important to study the effect of different FAs on bacterial growth and PHB yield in order to choose the appropriate plant oil with the right combination of FAs for PHB fermentation.

The high crystallinity of PHB which leads to reduced biodegradability [15] is another factor which contributes to the inability of the polymer to be used in drug delivery and other applications requiring the use of low crystalline materials. Electrospinning has been shown to increase or reduce the crystallinity of polymers by varying electrospinning parameters [16]. In the case of PHB, reducing the crystallinity would improve its properties for application.

Electrospun fibres have shown desirable properties such as increased fibre length with reduced diameter and pore size, large surface area, and high porosity [17]. Owing to these and several other properties, there is increasing interest in the use of electrospun fibres in various industrial and biomedical sectors. These composite fibres have a wide range of applications from filtration in engineering industry to various medical applications, such as wound dressing, tissue engineering, and medical prostheses [18–20]. Applications of electrospun fibres have been comprehensively reviewed by Lu and Ding [20].

This research thus looked into the effect of producing PHB from glucose, rapeseed oil, or olive oil on the various media, large surface area, and high porosity [17]. Owing to these and several other properties, there is increasing interest in the use of electrospun fibres in various industrial and biomedical sectors. These composite fibres have a wide range of applications from filtration in engineering industry to various medical applications, such as wound dressing, tissue template, tissue engineering, and medical prostheses [18–20]. Applications of electrospun fibres have been comprehensively reviewed by Lu and Ding [20].

This research thus looked into the effect of producing PHB from glucose, rapeseed oil, or olive oil on the various properties of the polymer and its corresponding effect on the morphology of the nanofibres made from the polymer. The chemical structure of the polymer remains the same irrespective of the carbon source. However, the molecular weight was dependent on the carbon source used. The diameter of the fibre made by electrospinning was also dependent on the source of carbon used for polymer production.

2. Materials and Methods

2.1. Chemicals. K$_2$HPO$_4$ and KH$_2$PO$_4$ were purchased from Fischer Scientific Ltd., Loughborough, UK, while KNO$_3$, (NH$_4$)$_2$SO$_4$, MgSO$_4$·7H$_2$O, NaCl, CaCl$_2$, CuSO$_4$·5H$_2$O, MnSO$_4$·H$_2$O, ZnSO$_4$·5H$_2$O, FeSO$_4$, and (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O were purchased from BDH Chemicals, UK. Chloroform and n-hexane (HPLC grade) were obtained from Sigma Aldrich, UK.

2.2. Microorganism. *R. eutropha* H16 (NCIMB 10442, ATCC 17699) was obtained from National Collection of Industrial and Marine Bacteria, Aberdeen, UK. Stock culture was freeze-dried and kept at −20°C. Before use, cultures were resuscitated and grown overnight at 30°C. The organism was then subcultured on tryptone soya agar (TSA) and incubated at 30°C for 24 h.

2.3. Carbon Sources. Three different sources of carbon were used individually for PHB production; these included glucose (Lab M Ltd., UK), olive oil, and rapeseed oil purchased from local supermarkets. Both oils were sterilized by autoclaving at 121°C for 15 min while glucose solution was sterilized at 110°C for 20 min at 10 psi. Spread plating of 1 mL of oil or glucose on TSA showed no colonies after incubation at 30°C for 24 h.

2.4. Growth Media. Tryptone soya broth (TSB) and TSA were both purchased from Lab M Ltd., UK, and prepared following the manufacturer’s instructions under aseptic conditions. Basal salt medium (BSM) was prepared as described by Verlinden et al. [1]. The pH values of TSB and BSM were 7.3 ± 0.3 and 6.8 ± 0.2, respectively. Ringer’s solution (Lab M, UK) was made up with distilled water and used at 1/4 strength. All media were sterilized by autoclaving at 121°C for 15 min.

2.5. Fermentations. Single colonies of *R. eutropha* were used to inoculate 20 mL of TSB and incubated aerobiocally for 24 h in a rotary incubator (New Brunswick Scientific, UK) at 30°C and 150 rpm. These were then used as starter cultures for PHB fermentation. The cultures were checked for purity by gram staining and microscopic observations at magnification of 1000.

Fermentations for each carbon source were carried out in triplicate using 500 mL Erlenmeyer flasks. Five grams of the oils (~10 mL) or 5 g of glucose (10 mL glucose solution) was added to labelled flasks, respectively, each containing 220 mL of BSM. The sterile media with oils were sonicated (Bandelin Electronic, Berlin) for 7 min at 0.5 active and passive intervals with a power of 60%. The media were then inoculated with 20 mL of the starter culture, thus giving a total volume of 250 mL with a concentration of 20 g/L of the respective carbon source and 8% (v/v) of the starter culture in all fermentation media. All flasks were incubated in a rotary incubator for 48 h at 30°C and 150 rpm.

Samples were collected aseptically from the fermentation broth at 0, 3, 24, 27, and 48 hours of incubation for viable cell count to determine bacteria growth. Results obtained are expressed in log$_{10}$ CFU mL$^{-1}$.

2.6. Extraction of PHB. After 48 h, fermentation was stopped and cultures were centrifuged (Hermle Labortecnik, Z300K) for 15 min at 6,000 rpm. The supernatant was discarded and the biomass kept overnight at −20°C before lyophilisation. Frozen bacterial pellets were lyophilised
(Edwards freeze-drier, Modulyo) at –40°C and at 5 MBAR for 72 h. Lyophilised dry biomass was weighed. Obtained cell dry weight (CDW) was transferred into an extraction thimble. PHB was extracted by Soxhlet extraction (with HPLC grade chloroform) running for 5 h. The hot solution of polymer was concentrated by evaporation. Subsequently, the solution was precipitated in n-hexane (Sigma Aldrich). The polymer precipitated as a white substance. The precipitated polymer was separated from solution by filtration (Whatman no. 1 paper) and air-dried before being weighed. The percentage of PHB produced (wt/wt) by the bacteria was calculated using the following equation:

\[
\%\text{PHB} = \frac{\text{weight of extracted polymer}}{\text{cell dry weight}} \times 100. \tag{1}
\]

2.7. Electrospinning. The polymer solutions were electrospun using a custom built electrospinning setup, which consisted of a standard 10 mL syringe (Cole Palmer Ltd., UK) containing the polymer solution. The polymer solution was driven out of the syringe using an NE-300 single syringe pump (Pump Systems Inc., USA). A metal 25-gauge syringe needle (Cole Palmer Ltd., UK) was attached to the syringe. These needles were ground down to produce a blunt tip allowing for the formation of a more stable Taylor cone prior to use. A vertical orientated design was implemented with the needle tip and collecting plate were connected to a high voltage power supply (model 73030P, Genvolt Ltd., UK).

The applied voltage was set at 7 kV and kept constant for all experiments. A flow rate of 1 mL h\(^{-1}\) was used in order to create a stable system whereby the fluid forced into the drop equalled the average rate at which the fluid was carried away by the jet. In all experiments, the distance between the needle tip and collecting plate were connected to a high voltage power supply (model 73030P, Genvolt Ltd., UK).

Three separate solution concentrations of the different PHB samples were made (1.5, 2, and 2.5% wt/v). To ensure reproducibility, experiments for each concentration were carried out in triplicate.

2.8. Characterisation Methods

2.8.1. Fourier Transform Infrared Spectroscopy (FTIR). Samples of PHB were identified using a Nicolet FTIR spectrometer (Magna-IR 860). FTIR spectra of both nonelectrospun and electrospun PHB samples were compared to observe any difference in the peaks.

2.8.2. Nuclear Magnetic Resonance (NMR) Spectroscopy. \(^1\)H NMR spectra were recorded with a Bruker Avance II operating at 600 MHz, with 64 scans, 2.65 s acquisition time, and 11 μs pulse width. \(^{13}\)C NMR spectra were recorded with a Bruker Avance II operating at 150.9 MHz, with 20,480 scans, 0.9088 s acquisition time, and 9.40 μs pulse width. \(^1\)H NMR and \(^{13}\)C NMR spectra were run in CDCl\(_3\) at room temperature with tetramethylsilane (TMS) as internal standard.

2.8.3. Electrospray Mass Spectrometry (ESI-MS\(^n\)) Analysis. ESI-MS\(^n\) was performed using a Finnigan LCQ ion trap mass spectrometer (Finnigan, San Jose, CA, USA). The samples were dissolved in methanol/chloroform (2:1, v/v). The solutions were introduced into the ESI source by continuous infusion at 10 μL min\(^{-1}\) using the instrument’s syringe pump. The LCQ ESI source was set to an operating voltage of 4.5 kV, and the capillary was heated to 200°C. Nitrogen was used as the nebulising gas. For ESI-MS/MS experiments, monoisotopic ions of interest were isolated in the ion trap and activated using helium damping gas in the mass analyser to promote collisions. The RF amplitude was set such that the peak height of the molecular ion decreased by at least 50%. Analyses were performed in positive-ion mode.

2.8.4. Differential Scanning Calorimetry (DSC). DSC experiments were carried out in a Perkin-Elmer DSC7 instrument. For each sample ~5 mg of the extracted polymer was placed in a covered aluminium pan. Covered empty pan was used as a reference. The DSC traces were performed from 30 to 200°C at a heating rate of 10°C min\(^{-1}\) and were initially held at 30°C for 5 min to stabilise the equipment. The sample was then held at 200°C for 1 min and then cooled from 200 to 30°C at the same rate.

Percentage crystallinity was calculated by the equation

\[
C (\%) = \frac{H^m_f}{H_{f100}^m} \times 100, \tag{2}
\]

where C is the percentage crystallinity, \(H^m_f\) is the measured heat of fusion (J g\(^{-1}\)), and \(H_{f100}^m\) is the enthalpy of fusion for fully crystalline PHB (146 J g\(^{-1}\)) [21].

2.8.5. Gel Permeation Chromatography (GPC). The molecular weight of PHB was determined by GPC. Solution of the sample was prepared by adding 10 mL of CHCl\(_3\) to 20 mg of sample. The mixture was left overnight to dissolve and then filtered through a 0.2 μm polyamide membrane. The sample was analysed at a flow rate of 1.0 mL min\(^{-1}\) using PLgel guard plus 2 mixed bed-B columns (Cirrus GPC version 3.2). Polymer Laboratories software was used to produce the GPC calibration curve from which the weight average molecular weight (\(M_w\)) and the number average molecular weight (\(M_n\)) were obtained. The Dispersity Index (DI) of the polymers was calculated using the formula DI = \(M_w/M_n\) [22].

2.8.6. Scanning Electron Microscopy (SEM) Morphology. A Philips XL 30 ESEM FEG was used in order to observe high resolution images of electrospun PHB nanofibres. The samples were coated with platinum using a Polaron SC 7640 sputter coater at a current of 25 mA for 3 min. Average fibre diameter values were derived by measuring 120 random fibre diameters from each sample using ImageJ software (National Institutes of Health, USA).

2.9. Statistical Analysis. GraphPad Prism 5 was used for the statistical analysis of all data. Two-way ANOVA with the
Table 1: Average cell dry weight (CDW) and PHB production by *R. eutropha* NCMBI 14402 grown in BSM at 30 °C and 150 rpm for 48 hours with olive oil, rapeseed oil, and glucose used as carbon sources.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Average CDW (g/L)</th>
<th>Average P(3-HB) (g/L)</th>
<th>P(3-HB) (% wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed oil</td>
<td>2.90 ± 0.1</td>
<td>1.06 ± 0.1</td>
<td>36.54</td>
</tr>
<tr>
<td>Olive oil</td>
<td>3.45 ± 0.15</td>
<td>1.26 ± 0.05</td>
<td>36.66</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.07 ± 0.1</td>
<td>0.13 ± 0.03</td>
<td>12.00</td>
</tr>
</tbody>
</table>

**FIGURE 1:** Growth analysis of the shake flask culture of *R. eutropha* NCMBI 10442 grown in BSM at 30 °C and 150 rpm using rapeseed oil, olive oil, and glucose as sources of carbon for the production of PHB. Results are an average of triplicates.

Bonferroni multiple comparison test was used to analyse differences in *R. eutropha* growth using the different carbon sources and also to analyse the differences in fibre diameter of PHB obtained. A *P* value of < 0.05 was taken as significant.

### 3. Results and Discussion

#### 3.1. Fermentations

*R. eutropha* H16 growth curve for all three substrates used is presented in Figure 1. After 48 h of fermentation, the viable cell numbers for both rapeseed and olive oils increased by approximately 2 log$_{10}$ from the initial concentration while the viable cell number for glucose fermentation increased with less than 1 log$_{10}$. ANOVA analysis showed no significant difference (*P* > 0.05) between the viable cell numbers of rapeseed and olive oil fermentations at various growth times. However, these were significantly higher (*P* < 0.05) than those of glucose fermentation from 3 to 48 h of growth (Figure 1). Furthermore, the CDW obtained from olive oil fermentation was significantly higher (*P* < 0.05) than that obtained from rapeseed oil while the difference in the CDW obtained using either oil was highly significant (*P* < 0.0005) compared to glucose, with both oils having a higher CDW (Table 1). As observed in Figure 1, cell growth was always higher in the oil supplemented media compared to that of glucose. This indicates that the oil resulted in an increased growth rate of the cells, thus accounting for the significantly higher growth of *R. eutropha* in oil supplemented media compared to glucose supplemented media. Supplementation of fermentation media with vegetable oils has been shown to stimulate growth in both plant and microbial cells [23, 24]. According to Jones and Porter [24], the stimulation of microbial growth by plant oil is achieved by increasing the rate of nutrient consumption.

Polymer yield per gram of carbon source and percentage production per CDW (wt/wt) were similar for olive oil and rapeseed oil. However, these were significantly higher (*P* > 0.005) when glucose was used as the carbon source for polymer production (Table 1). Similar results have been obtained with soybean oil as a source of carbon for PHA production by *R. eutropha* or *Pseudomonas stutzeri*. In both cases, soybean oil had higher PHA yield per gram and higher percentage PHA/CDW (wt/wt) compared to glucose [25, 26]. Plant oils possess more carbon atoms than sugars [1, 10]. This together with the stimulated cell growth provides possible explanations for the increased accumulation of PHB within the cells grown in the oils compared to that grown in glucose supplemented media.

#### 3.2. Polymer Identification

Polymers extracted from all three carbon sources were analysed by FT-IR, $^1$H-, and $^{13}$C-NMR. Results obtained indicated the presence of pure polyhydroxybutyrate (PHB) in all three cases (Figures 2, 3, and 4). Further analysis using ESI/MS$^n$ was carried out to confirm the presence of P(3-HB) monomers. It is known that PHA undergoes controlled chemical degradation by several methods. For example, in the presence of selected acetic acid salts proceeding via an ElcB mechanism Kawalec et al. obtained oligomers which have the same composition and sequence distribution of monomer units as starting biopolymesters [27]. Therefore, ESI-MS$^n$ analysis was performed to establish the chemical structure of PHB synthesized by *R. eutropha* from the different carbon sources. ESI-MS spectrum of oligomers obtained as a result of O-PHB degradation in the presence of potassium acetate is presented in Figure 5 and is similar to that obtained from rapeseed oil and glucose. Peaks in ESI-MS spectrum showed a peak-to-peak mass increment of 86 Da, which is equal to the mass of the 3-hydroxybutyrate repeating unit; the series of ions corresponds to the sodium adduct of oligo(3-hydroxybutyrate) with crotonate and carboxyl end groups. Figure 6 shows the ESI-MS/MS spectrum obtained for the precursor ion at m/z 969; the fragmentation of this ion, which occurs as a result of statistical breaking of the ester bonds along the polyester chain, leads to the formation of...
Figure 2: FTIR spectra of electrospun and nonelectrospun samples of PHB obtained from (a) glucose, (b) olive oil, and (c) rapeseed oil.

Figure 3: $^1$H NMR spectrum of PHB produced from rapeseed oil.

Figure 4: $^{13}$C NMR spectrum of PHB produced from rapeseed oil.
one set of oligo(3-hydroxybutyrate) product ions terminated by carboxyl and crotonate end groups. The product ion at $m/z$ 883 can be formed due to the loss of crotonic acid from either the carboxyl or the crotonate end of this ion. The ESI-MS$^+$ analysis thus indicated that the polymer produced from the different carbon sources is primarily 3-hydroxybutyrate units.

3.3. Morphology of Electrospun Nanofibres. The SEM micrographs presented in Figure 7 showed that fibres were successfully produced from each of the PHB samples at all three solution concentrations used. Fibre morphology was found to be dependent both on the source of the polymer used and on the concentration of the electrospinning solution. The average fibre diameter (Figure 8) increased in all three PHB samples when solution concentration was increased. In the case of G-PHB there was an increase of $\sim$400 nm when comparing fibres produced from a 1.5% (wt/v) solution to a 2% (wt/v) solution. Furthermore, differences in average fibre diameter were observed between electrospun G-PHB, O-PHB, and R-PHB from solutions of the same concentration (Figures 7 and 8). R-PHB produced the thinnest fibres from a 1.5% (wt/v) solution with a value of 431.29 nm, followed by G-PHB at 586.05 nm, while O-PHB had the largest diameters with an average of 760.65 nm. ANOVA analysis confirmed that the differences were significant ($P < 0.05$). This pattern was however lost at higher solution concentrations of 2% and 2.5% (wt/v) with no significant differences observed in the diameter of fibres from all three polymer sources.

Previous research has shown that fibre diameters increased with increasing polymer concentration based on a power law relationship [28]. The viscosity of a solution is directly related to the polymer concentration and increasing the polymer concentration results in increased viscosity.
Figure 7: SEM images of PHB nanofibres using different concentrations of PHB from various carbon sources: (a), (b), and (c) = 1.5%, 2%, and 2.5% G-PHB; (d), (e), and (f) = 1.5%, 2%, and 2.5% O-PHB; (g), (h), and (i) = 1.5%, 2%, and 2.5% R-PHB, respectively.
Table 2: The $M_w$, $M_n$, and DI of electrospun and nonelectrospun PHB from olive oil, rapeseed oil, and glucose ($M_w$: weight average molecular weight, $M_n$: number average molecular weight, and DI: dispersity = $M_w$/

<table>
<thead>
<tr>
<th>PHB</th>
<th>$M_w$ ($\times 10^5$ g/mol)</th>
<th>$M_n$ ($\times 10^5$ g/mol)</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-PHB</td>
<td>5.92</td>
<td>4.15</td>
<td>1.4</td>
</tr>
<tr>
<td>O-PHB (electrospun)</td>
<td>7.54</td>
<td>5.6</td>
<td>1.4</td>
</tr>
<tr>
<td>R-PHB</td>
<td>5.79</td>
<td>4.05</td>
<td>1.4</td>
</tr>
<tr>
<td>R-PHB (electrospun)</td>
<td>7.4</td>
<td>6.28</td>
<td>1.2</td>
</tr>
<tr>
<td>G-PHB</td>
<td>7.35</td>
<td>6.07</td>
<td>1.2</td>
</tr>
<tr>
<td>G-PHB (electrospun)</td>
<td>8.05</td>
<td>6.59</td>
<td>1.2</td>
</tr>
</tbody>
</table>

In higher concentration solutions, the number of polymer chains is higher and therefore more chain entanglements are formed causing the higher viscosity. Chain entanglements act to oppose the elongation and thinning forces caused by the electric field; therefore fibres formed from higher concentration polymer solutions tend to have higher diameters. All PHB solutions at a concentration of 1.5% (wt/v) demonstrated a lower viscosity, which may have caused the jet to split into multiple jets during the electrospinning process [28–30]. The formation of secondary jets acts to reduce the diameter of the PHB fibres [18]; thus the significantly reduced fibre diameters are produced from 1.5% (wt/v) solution concentrations. Average fibre diameters between the three PHB samples were similar when electrospun from 2% and 2.5% wt/vol solutions, however, varied significantly at 1.5% (wt/v) concentration (Figures 7 and 8). This is thought to be a result of the viscosity of the solutions. At 2% and 2.5% (wt/v) the viscosity was high enough for stable electrospinning to take place resulting in the formation of bead free fibres (Figure 7). The 1.5% (wt/v) solution was unstable due to insufficient viscosity resulting in an unstable jet with many beads formed. In this unstable system, fibre diameters can vary widely due to intermittent formation of secondary jets or “splitting” of solution from the needle tip.

3.4. Molecular Analysis of Polymer Fibres. The GPC results for electrospun and nonelectrospun P(3-HB) from all three carbon sources are presented in Table 2. The $M_w$ values for P(3-HB) were the highest when glucose was used as a carbon source at $7.35 \times 10^5$ g/mol. The $M_w$ values obtained for P(3-HB) from rapeseed and olive oil were comparable at $5.79 \times 10^5$ and $5.92 \times 10^5$ g/mol, respectively. Results show a high uniformity in the molecular weight distribution of P(3-HB) from glucose giving rise to a low DI of 1.2 compared to P(3-HB) from rapeseed or olive oil with DI of 1.4 (Table 2). All P(3-HB) samples demonstrated an increase in $M_w$ after being electrospun (Table 2). Higher molecular weight PHB has previously been reported from fructose compared to virgin, soybean, or waste edible oil [31, 32]. The presence of glycerol in plant oils has been suggested as a probable cause of the lower molecular weight PHB from plant oils as glycerol is known to terminate PHA chain elongation by acting as a chain transfer agent [32]. The increase of electrospun PHA molecular mass compared to nonelectrospun samples may be due to some fractionation of the polymer into the fibre mats preparation, which also indicates that no PHB degradation took place during the process.

3.5. Thermal Analysis of Polymer Fibres. DSC traces are shown in Figure 9. The $T_m$ for PHB is known to be $\sim 2^\circ$ C and did not appear in any of the traces. No significant differences ($P < 0.05$) were found in the $T_m$ and $T_c$ of PHB samples from rapeseed oil (R-PHB), olive oil (O-PHB), and glucose (G-PHB) before and after electrospinning (Table 3). However, the crystallinity of electrospun fibres was reduced with decreasing polymer solution concentration (Table 3). These were lower compared to nonelectrospun samples (Table 3). The crystallinity of electrospun R-PHB was as low as 41.9% (Table 3) compared to nonelectrospun samples with a crystallinity of $\sim 52.2$% (Table 3). Furthermore, electrospun O-PHB and R-PHB had lower crystallinity compared to G-PHB at lower solution concentrations (Table 3). Finally, the DSC traces obtained from electrospun G-PHB, O-PHB, and R-PHB all demonstrated a decrease in crystallinity when the solution concentration was reduced.
Figure 9: DSC trace for heating and cooling of electrospun and nonelectrospun samples of PHB at solution concentrations of 1.5%, 2%, and 2.5% (wt/v). (a), (b) represent the DSC trace on heating and cooling of G-PHB. (c), (d) represent the DSC trace on heating and cooling of R-PHB. (e), (f) represent the DSC trace on heating and cooling of O-PHB.
The degree of crystallinity of the electrospun P(3-HB) may be influenced by the flight time of the jet. When the P(3-HB) solution initially leaves the jet, the polymer molecules are more ordered [33]. The flight of the jet can be influenced by voltage and solution concentration. A higher voltage would result in a decrease in the time it takes for the jet to reach the collecting plate after leaving the needle tip [34]. However, as the voltage was kept constant throughout the experiments at 7 KV, the solution concentration was the only variable. PHB solutions with a higher concentration of 2.5% (wt/v) will demonstrate a decrease in the jet flight time in comparison to 2% and 1.5% (wt/v). This is because greater solution viscosity will act to resist bending instabilities brought about by the applied voltage causing the jet to travel in a straight line for a longer period of time. On this basis the crystallinity for electrospun PHB nanofibres at 2.5% (wt/v) concentration was found to be dependent on the source of carbon used in polymer synthesis and the concentration of the polymer in the electrospinning solution. Also, increase in molecular mass and decrease in crystallinity were observed following electrospinning.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

### Acknowledgments

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### References


<table>
<thead>
<tr>
<th>Polymer</th>
<th>Nonelectrospin</th>
<th>Polymer solution concentrations (%wt/v) during electrospinning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_m$ (°C)</td>
<td>$T_c$ (°C)</td>
</tr>
<tr>
<td>O-PHB</td>
<td>173.2</td>
<td>121.6</td>
</tr>
<tr>
<td>R-PHB</td>
<td>172.2</td>
<td>120.8</td>
</tr>
<tr>
<td>G-PHB</td>
<td>172.5</td>
<td>118.6</td>
</tr>
</tbody>
</table>

Table 3: Thermal properties of electrospun fibres of PHB from olive oil (O-PHB), rapeseed oil (R-PHB), and glucose (G-PHB) with solution concentrations of 1.5%, 2%, and 2.5%: $T_m$ = melting temperature and $T_c$ = crystallisation temperature on cooling, and C = crystallinity.


