Synthesis and characterization of a novel versatile poly(amic acid) derived from ethylenediaminetetraacetic dianhydride

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1. Introduction

Poly(amic acids) (PAAs) are a class of polymers that are generally formed through a polycondensation reaction between a dianhydride and a diamine [1]. Possessing amides and carboxylic acid groups in the polymer backbone, PAAs have many interesting potential materials applications. However, they are often overlooked and are recognized as intermediate polymers towards the formation of polyimides. In contrast to polyimides, particularly the aromatic polyimides, PAAs are soluble in a range of dipolar aprotic solvents and can easily be processed. In addition, PAAs have the option to form polyimides through a condensation reaction. Over the years, numerous applications of polyimides have been reported [2–6]. Problems of poor solubility and tractability [7,8], often associated with polyimides, have limited their use as potential medical materials. Compared to polyimides, little work has been reported on PAAs as a potentially useful class of materials. There are a few reports describing PAAs application as an interphase in high performance thermoplastic composites [9] and its use for liquid crystal orientation [10]. However, the majority of emphasis in recent years has been placed on PAAs usefulness as a precursor for heat-resistant thin nonplanar polyimide moldings [11] with high refractive index [12] or as a photosensitive polyimide precursor [13]. We are especially interested in PAA’s potential as a material for biomedical applications. Carefully tailored, PAAs may prove to be worthy materials on their own, thereby providing a wide range of material properties. PAAs reported in the literature are mainly composed of aromatic dianhydrides and aromatic diamines [14]. Representative aromatic dianhydrides have included 1,2,4,5-benzenetetracarboxylic dianhydride, 3,3′,4,4′-biphenyl tetracarboxylic dianhydride, 3,3′,4,4′-benzophenone tetracarboxylic dianhydride, 2,3,6,7-naphthalene tetracarboxylic dianhydride etc. and examples of aromatic diamines have included paraphenylenediamine, 4,4′-diaminodiphenylether, 4,4′-diaminodiphenylmethane, etc. We chose to focus on the use of an aliphatic dianhydride and an aliphatic diamine as monomers in PAA synthesis as an alternative to traditional monomers used in PAA synthesis. To date, there have been few reports on this type of PAA and its consideration for materials applications.

In our work on the synthesis and characterization of aliphatic poly(amic acid), the monomers of choice are ethylenediaminetetraacetic dianhydride (EDTAD) and 1,4-diaminobutane (DAB) commonly known as putrescine. EDTAD is biodegradable, often recognized as a chelator and is well known to complex with metal ions [15]. Furthermore, EDTAD has been reported to exhibit very little toxicity since the only reactive group introduced into the polymer backbone is a carboxyl group [16]. Recently, EDTAD has been used in the modification of various hydrogels for drug delivery applications [17]. Similarly, DAB, one of the simplest polyamines found in millimolar concentrations in mammals is produced by the breakdown of amino acids (decarboxylation of ornithine by the enzyme ornithine decarboxylase or decarboxylation of arginine by
the enzyme l-arginine decarboxylase) in both living and non-living organisms [18,19]. In general polyamines have been investigated for anticancer therapy [20]. Putrescine in particular has been shown to be non-toxic, anti-mutagenic and effective in reducing mutation frequency, suggesting further that it plays a vital role in DNA synthesis [21]. Both EDTAD and DAB are soluble in aqueous solutions and their structures predict minimal toxicity.

The use of synthetic polymers for medical devices is presently a targeted area in materials research. The aim of this study was to synthesize, characterize and investigate the relationships between the molecular structure and physical properties of linear PAA.

2. Experimental

2.1. Materials

High purity monomers [1,4-diaminobutane (DAB) and ethylenediaminetetraacetic dianhydride (EDTAD)] were purchased from Sigma–Aldrich and used as received. Solvents N-methyl-2-pyrrolidone (NMP), N,N dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and N,N-dimethylacetamide (DMAc) were also purchased from Sigma–Aldrich in sealed vials and either used directly after opening or were dried over molecular sieves having a nominal pore diameter of 3 Å and filtered prior to use. Additional solvents, anhydrous methanol, anhydrous acetone and ethyl ether were purchased from VWR International. All materials required for phosphate buffer solution (PBS) [potassium hydrogen phosphate (K2HPO4) and potassium dihydrogen phosphate (KH2PO4)] were purchased from Fisher Scientific.

2.2. Synthesis of novel poly(amic acid) films

Poly(amic acid) (PAA) polymers comprising of amide and acid end-groups were synthesized through a condensation polymerization reaction using aliphatic monomers in various aprotic solvents (Fig. 1). A 1 mol equivalent of DAB was placed into a 100 mL, three-necked round bottomed flask and dissolved in an aprotic solvent at a temperature varying from 20 to 37 °C. The apparatus consisted of a condenser, heating element, water bath, stirring bar and magnetic stirrer. A solution containing 1 mol equivalent of EDTAD dissolved in an aprotic solvent was then added to the DAB solution. The PAA polymers were synthesized using four different aprotic solvents (DMF, DMAc, DMSO and NMP). The concentration of PAA solution was varied between 8% and 12% by weight. The temperature of the reaction solution was maintained at 25, 30 or 37 °C (depending on trial) and polymerization was conducted in a nitrogen atmosphere and duplicated for reproducibility. All experiments were conducted at 24 h or until the viscosity of the polymer’s solution increased to the point that uniform stirring of the solution could not be achieved. The synthesized PAA solution was stored at 4 °C in the refrigerator until further use.

2.2.1. Washing, casting and drying

Samples were removed from the refrigerator, precipitated by the addition of methanol, filtered over a Buchner funnel and cast into smooth, circular, glass petri dishes having a diameter of 4 cm. Samples were placed into the fumehood overnight to evaporate the remaining solvent, and then placed into the vacuum oven at 30 °C for 24 h. The dried films or powders were removed from the petri dish, washed three times with methanol and placed into the oven for an additional 72 h.

2.3. Characterization

2.3.1. Fourier transform infrared spectroscopy (FTIR)

Infrared measurements were performed with an attenuated total reflectance (ATR) attachment (Pike Technologies Inc., Madison, WI) using a diamond crystal on a Bruker Vector 22 Fourier Transform Infrared (FTIR) spectrometer (Milton, ON). Spectra were recorded with 256 scans, 4 cm⁻¹ resolution and displayed in absorption mode. The spectra were baseline corrected using OPUS-NT 3.1 software.

2.3.2. Nuclear magnetic resonance (NMR)

Proton nuclear magnetic resonance (¹H NMR) and carbon nuclear magnetic resonance (¹³C NMR) spectra were recorded on an INOVA 600 (Varian 600 MHz). The spectra were obtained by dissolving samples in either deuterated dimethylsulfoxide (DMSO-d₆) or deuterium oxide (D₂O) and by using tetramethylsilane (TMS) as the internal standard (TMS = 0 ppm). The relaxation delay and the angle pulse used for both the ¹H NMR and ¹³C NMR spectra were 1 s and 45°. ACD Labs software was used to process NMR data. In the analysis, phasing parameters and baseline points were determined on the first transformed spectrum. Data transformation and peak height measurements were automated and integration was performed manually.

2.3.3. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis

SEM and EDX measurements of the PAA films were obtained using the LEO-Zeiss 1540XB FIB/SEM which was equipped with an EDX Si-detector (Oxford Instruments). An accelerating voltage of 8 kV was used. During EDX analysis, much care was taken to limit the electron radiation damage on the surface of the polymer sample.
Table 1
Gel permeation chromatography data on poly(amic acid) at varying temperatures and monomer concentrations.

<table>
<thead>
<tr>
<th>Monomer addition</th>
<th>Temp (°C)</th>
<th>Mₚ (g mol⁻¹)</th>
<th>Mₙ (g mol⁻¹)</th>
<th>PDI</th>
<th>Reaction time (h)</th>
<th>Solvent</th>
<th>M (mol L⁻¹)</th>
</tr>
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<tr>
<td>PAAn</td>
<td>37</td>
<td>4483</td>
<td>6124</td>
<td>1.366</td>
<td>0.20</td>
<td>DMF</td>
<td>24</td>
</tr>
<tr>
<td>PAAb</td>
<td>37</td>
<td>4713</td>
<td>6147</td>
<td>1.303</td>
<td>0.15</td>
<td>DMF</td>
<td>24</td>
</tr>
<tr>
<td>PAAc</td>
<td>37</td>
<td>4312</td>
<td>5402</td>
<td>1.253</td>
<td>0.10</td>
<td>DMF</td>
<td>24</td>
</tr>
<tr>
<td>PAAd</td>
<td>25</td>
<td>5417</td>
<td>6503</td>
<td>1.201</td>
<td>0.20</td>
<td>DMF</td>
<td>24</td>
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<tr>
<td>PA Ae</td>
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<td>6410</td>
<td>1.210</td>
<td>0.15</td>
<td>DMF</td>
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</tr>
<tr>
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<tr>
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<td>2.110</td>
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<tr>
<td>PA A d</td>
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<td>2.150</td>
<td>0.20</td>
<td>DMF</td>
<td>24</td>
</tr>
</tbody>
</table>

Mₚ: Number average molecular weight, Mₙ: Weight average molecular weight, PDI: Polydispersity Index = Mₚ/Mₙ, Temp: Temperature, M (mol L⁻¹): Monomer concentration

3.2. Structural characterization

3.2.1. Fourier transform infrared spectroscopy (FTIR)

One of the greatest advantages of such a novel polymer was the presence of cleavable bonds and reactive functional groups such as the acid (COOH) and the amide (–CO–NH–) in the backbone of the polymer chain. This attribute enhances the properties of the polymer by allowing for chemical cross-linking and thus foreseeing the polymers potential versatility. Polymer samples were characterized by ATR-FTIR spectroscopy to identify functional groups in PAA, thereby giving insight to its molecular structure. The spectra of PAA (samples a → f) displayed several sharp peaks in the OH stretch region (Fig. 2; ν1660 cm⁻¹ (amide I; C=O stretch = C–O + C–N), ν1540 cm⁻¹ (amide II; C–N–H stretch = N–H + C–N), ν3290 cm⁻¹ (–NH), and ν2930 cm⁻¹ (–CH) which was consistent with absorption bands found in similar PAA systems [25,26]. The presence of the OH vibration at a wavelength
of 3400 cm$^{-1}$ was difficult to clearly identify as a result of the over-
laying peaks associated with NH and OH vibrations.

### 3.2.2. Proton nuclear magnetic resonance ($^1$H NMR)

High resolution $^1$H NMR and $^{13}$C NMR spectra provided support to the FTIR results and in addition showed valuable information on molecular structure. PAA samples were dissolved in DMSO-$d_6$ (Fig. 3) for $^1$H NMR. Peaks observed on the $^1$H NMR spectrum (600 MHz, DMSO-$d_6$, 1 wt%): $\delta$ (ppm) 2.52–2.57 (triplet, 2 H); 2.62–2.66 (singlet, 8 H); 2.73–2.79 (triplet, 2 H); 3.47–3.5 (singlet, 2H); 3.51–3.58 (singlet, 2H); 7.91 (singlet, 2H); and 12.24 (singlet, 2H) confirmed the structure of PAA. The majority of the EDTAD monomer was seen at 2.63 ppm and 3.67 ppm. The spectrum was compared with the EDTAD monomer. The $^1$H NMR spectrum clearly showed the distinction between methylene protons f and g and its integration values were found to be in agreement with the chemical formula of the repeating unit of PAA. The spectrum displayed two singlet peaks between 3.45 and 3.75 ppm as a result of chemically equivalent protons. In general, downfield chemical shift changes were seen more often when using DMSO-$d_6$ (2.47 ppm) for methylene group hydrogens compared to other deuterated solvents. Since DMSO was a strong hydrogen bond acceptor, using DMSO-$d_6$ resulted in a more dramatic downfield shift for hydroxyl signals (from alcohols and acids) as a result of hydrogen bonding. A broad peak at 12.24 ppm represented the acidic hydrogen from the carboxylic acid. The singlet at 3.13 ppm was the result of the methyl group from the methanol washing and the singlets at 2.85 ppm and 2.70 ppm were the result of the methyl groups from the reaction solvent (DMF) within the sample. Deuterium exchange between the acidic proton and deuterium remained a concern only when D$_2$O was used as the NMR solvent. Amide protons exchange slowly with deuterated solvents and as a result the proton peak is usu-

![Fig. 3. $^1$H NMR spectrum of poly(amic acid).](image)

![Fig. 4. $^{13}$C NMR spectrum of poly(amic acid).](image)
Fig. 5. X-ray photoelectron spectroscopy (XPS) survey scan of poly(amic acid).

Fig. 6. Core-level spectrums of (a) Carbon (C), (b) Oxygen (O) and (c) Nitrogen (N) for poly(amic acid). Note: The chemical formula with an asterisk (*) indicates the specific atom which is being observed. The asterisk is present only when two or more atoms of the same element are present in different bonding situations.
the survey spectra; % Carbon = 58.0 ± 5.8, % Oxygen = 27.7 ± 1.8% and % Nitrogen = 14.3 ± 1.4. These values were in good correlation with the theoretical values; % Carbon = 52.5, % Oxygen = 30.0 and % Nitrogen = 17.5 which were calculated based on the molecular weight of the repeating unit of PAA. Experimental results showed a higher amount of carbon in the material and a lower amount of oxygen and nitrogen. This higher amount of carbon was most probably due to contamination by other carbon rich compounds in the experimental environment.

3.2.5. Energy dispersive X-ray (EDX) analysis

The percent compositions of the bulk polymer samples were determined using EDX and compared to the compositions of the surface as determined by XPS and were found to be in good agreement (Fig. 7). Quantitative analysis for the experimental compositions of the atoms were determined for five polymer samples; % Carbon = 56.0 ± 4.7, % Oxygen = 30.5 ± 1.4 and % Nitrogen = 13.3 ± 2.3. These values were relatively close to both the XPS data and theoretical percent compositions stated above. The percent compositions for both carbon and nitrogen were statistically significant (t-test; p < 0.05) this was most probably due to radiation damage by the electron beam and surface charging of the samples.

3.2.6. Gel permeation chromatography (GPC)

GPC in combination with ultraviolet and differential refractive index (RI) detectors were used to determine the molecular weights and molecular weight distributions of PAA. The number average molecular weights ($M_n$), weight average molecular weights ($M_w$) and polydispersity indices (PDI) where PDI = $M_w/M_n$, for all the poly(amic acid) samples based on PEG/PEO standards are illustrated in Table 1. Polymerization led to low molecular weight PAA samples having $M_n$ values ranging from 4000 to 6000 g mol$^{-1}$ within 24 h and PDI values averaging close to 1.3. Table 1 displays some expected trends; the higher the reaction temperature and concentration, the higher the molecular weight but the broader the molecular weight distribution. A narrow distribution is plausible at an optimal temperature of 25°C, a reaction time of 24 h and a monomer concentration of 0.20. Polymerization with monomer concentration greater than 0.20 was never conducted since dissolving EDTAD in a polar aprotic solvent was rather difficult. In general all samples were of relatively low molecular weights compared to the molecular weights of polymers synthesized by the traditional condensation polymerization methods. Samples having low molecular weights in the past have often been attributed to water residue inside the reaction vessel, monomer concentration, reaction time and reaction temperature. However, in this particular reaction the 1:1 stoicheometric ratio of the monomers also had a significant impact on the molecular weights of the polymer samples. Despite every effort to change the above factors, polymer samples remained below 10,000 g mol$^{-1}$. At this molecular weight range, perhaps the PAA polymer may be suitable for use as a coating material for medical devices such as a coronary stent to improve biocompatibility and in addition when bioactive molecules are conjugated into PAA, act as a drug delivery medium.

4. Conclusions

A series of linear PAA polymers containing acid and amide side chains with a $M_n$ in the range of 4000–6000 g mol$^{-1}$ have been successfully prepared and verified using several analytical tools; FTIR, $^1$H NMR, $^{13}$C NMR, XPS, EDX and GPC. Chemical structure and physical properties were established. The presence of cleavable bonds (functional groups: –COOH, –CO–NH–) in the polymer chain enhanced the properties of the polymer by providing versatility. To investigate the PAA polymer further, cell compatibility studies using aortic endothelial cells are being conducted for its potential use in cardiovascular applications [28]. In addition, chemical cross-linking studies are also underway to obtain high molecular weight PAA polymers.

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References