Macromolecular design of poly(vinyl alcohol) by RAFT polymerization

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Reversible Addition - Fragmentation chain Transfer polymerization (RAFT) is employed herein to obtain the first example of poly(vinyl alcohol), PVA, with controlled molecular weight and terminal amine groups thus presenting a flexible tool for materials design and bioconjugation. Furthermore, we demonstrate that RAFT control can be used to facilitate syndiotactic chain propagation and obtain PVA with the highest reported content of syndiotactic dyad (~ 78%).

Over the past two decades, rational macromolecular design has become an indispensable tool which provides for an overall success of polymers in diverse biotechnological and biomedical applications. Among the polymers with particular success, poly(vinyl alcohol) stands out as a candidate material with over 50 years of diverse applications, FDA approval for several uses, and clinical applications in humans, specifically as embolic bodies. Early studies revealed that at similar molecular weights, pharmacokinetics of PVA is near identical to that of PEG, a benchmark polymer in biomedicine. Further to this, a bioconjugate of PVA with superoxide dismutase was among the first examples of polymer-protein conjugates with dramatically enhanced pharmacokinetics. Despite all this, PVA and materials derived thereof have largely failed to satisfy the demands of (nano)biomaterials and as such are not in the focus of biomedical research. This failure is due to that with PVA, even the most fundamental aspects of macromolecular design remain unaccomplished, and to date, surprisingly, synthesis of PVA with controlled molecular weight and narrow polydispersity, in conjunction with bioconjugation, is yet to be demonstrated. Herein, we specifically address these shortcomings and demonstrate the utility of Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization technique to gain control over PVA molecular weight and stereochemistry and obtain samples with facile means of bioconjugation. To the best of our knowledge, macromolecular design using PVA, as described below, has no prior precedents and we expect that presented results will lead to novel opportunities in using this FDA approved polymer in (nano) biotechnology and biomedicine.

The synthesis of PVA differs from that of typical vinyl polymers in that this polymer has no true monomer and is obtained via hydrolysis of a precursor polymer, typically poly(vinyl acetate). This was first accomplished in 1930s, and since then vinyl acetate (VAc) remains a popular monomer of choice in elucidating mechanism and kinetics of polymerization techniques, including RAFT. Despite this, few reports have focused on synthesis of PVA from VAc obtained via RAFT (or other controlled radical polymerization technique), and while solitary successful reports include the syntheses of PVA with linear and multi-arm architectures, none of these demonstrate synthesis of PVA with molecular weights controlled over a broad range.

To accomplish this, we capitalize on the features inherent with RAFT polymerization, namely control over polymer molecular weight via the ratio of monomer to RAFT agent and polymerization time. Xanthate RAFT agents have previously been shown to afford good control over polymerization of VAc with typical polydispersity indexes 1.2-1.3. Indeed, with the use of 5-phthalimidomethyl-O-ethyl xanthate, judicious choice of polymerization parameters allowed the synthesis of PVA with molecular weights spanning two orders of magnitude, from 3,000 to 121,000 Da, preserving good polydispersity, D (Fig. 1). We note that GPC characterization is presented herein for PVAc, a polymer precursor for PVA, and not for PVA itself, to avoid complication of molecular weight analyses arising from supramolecular association of PVA, i.e. gelation. Nevertheless, all PVAc samples were successfully deacetylated to produce the target polymer, PVA (Table 1).

The second and equally important goal of the proposed design in the synthesis of PVA relates to producing samples of PVA amenable for facile bioconjugation. While existing opportunities in bioconjugation using hydroxyl groups are significantly disadvantaged compared to classic site of conjugation (amine,
Figure 2. Degree of phthalimide removal from the PVAc terminus achieved by hydrazine hydrate taken at varying molar equivalents to Phth group in methanol at 60 °C over 30 min (●) or 60 min (○). Insert: Fluorescence microscopy image of a surface adhered PVA physical hydrogel obtained via microtransfomring technique and using a 4.5 kDa sample of PVA fluorescently labelled through the terminal amine functionality. Sample is imaged in PBS in the hydrated state, scale bar: 20 µm.

Figure 3. 1H NMR spectra (d6-DMSO, 25 °C) of synthesized samples of PVA with varying degree of syndiotacticity, R% = \[ \{ r + m \} / \{ \{ r + m \} + m + r \} \] \), where \( r \) and \( m \) signify \( \text{racemo} \) and \( \text{meso} \) configuration of adjacent hydroxyls on a PVA chain, \( r \), \( m \) and \( r \) are syndiotactic, isotactic and atactic diads. \( R = 53\% \) is a value typical of atactic PVA samples; samples with \( R > 58\% \) are typically considered syndiotactic; \( R = 74\% \) is the highest value previously reported for PVA in literature.9,10

Further to polymer molecular weight, polymer syndiotacticity is a characteristic decisive for the properties of PVA, specifically a capacity of the polymer to form physical hydrogels.11 Herein, we report the first data which suggest that the RAFT mechanism can be used to facilitate a syndiotactic chain propagation and present characterization of samples of PVA with degree of syndiotacticity exceeding the highest values previously reported in literature.18,19 Aiming to obtain oligomeric samples of PVA and using an increased concentration of RAFT agent (monomer-RAFT ratio, [M]/[RAFT] = 33), we observed that at 60 °C, a high concentration of the RAFT agent resulted in an expected retardation of polymerization. Resulting PVA samples were characterized by a degree of syndiotacticity \( R = 53 \pm 2\% \), Figure 3. Surprisingly, a decrease in polymerization temperature to 50 °C resulted in a drastic change in tacticity of chain propagation and afforded PVA samples with an average degree of syndiotacticity \( R = 69 \pm 8\% \) (average tacticity values for both polymerization conditions are quoted based on three independent experiments). Notably, within the studied range, the temperature alone did not effect a syndiotactic chain propagation, as evidenced by a polymerization at 37 °C at [M]/[RAFT] ratio of ~ 200, which resulted in an atactic sample of PVA (Table 1). This data strongly suggest that a high concentration of a RAFT agent, together with appropriate choice of temperature, is imperative for syndiotactic chain propagation.
a phenomenon which may have ramifications far exceeding the scope of this work.

Compared to other methods which afford syndiotactic PVA (bulky monomers, fluorooacohols solvents etc), the method is significantly advantaged in using readily available monomer and convenient polymerization temperature (as opposed to custom monomers\textsuperscript{20,21} and protocols carried out at e.g. -78 °C\textsuperscript{25}) as well as a facile saponification step to yield PVA (in contrast with e.g. vinyl pivalate\textsuperscript{22}). An inherent limitation of the synthesis of s-PVA as described above, namely the relatively low molecular weight of the samples (<1 kDa), can be overcome by employing other tools of macromolecular design, such as a comb architecture of PVA,\textsuperscript{23} which is a subject of ongoing research. An important aspect in the recovery of s-PVA relates to a significant change in the product solubility observed with increasing chain length. From a different perspective, syndiotactic chain propagation in VAc is essentially arrested the utility of this polymer in biomedicine. Despite an extensive history of applications, macromolecular materials properties of this highly syndiotactic sample of PVA.

Conclusions

Despite an extensive history of applications, macromolecular design of PVA remained elusive, and the data presented above are, to the best of our knowledge, the first example of the synthesis of PVA with defined molecular weight and facile sites for bioconjugation. The importance of this lies in that pharmacokinetic parameters of PVA (blood residence time etc)\textsuperscript{6} are governed by the polymer molecular weight, and while PVA exhibited a pronounced tendency to self-associate, as evidenced by a low solubility; we are now investigating solution and materials properties of this highly syndiotactic sample of PVA.

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Notes and references

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Table 1. Characteristics of the PVA samples obtained through RAFT polymerization of VAc. Monomer conversion and R values were estimated from the \textsuperscript{1}H NMR spectra of polymerization mixture (d3-chloroform) and PVA (d6 DMSO, 25 °C). Number average molecular weights for PVA were calculated from VAc conversion (theoretical value, Mn\textsubscript{theo}) and from GPC data obtained for the parent sample PVAc (Mn\textsubscript{theo}). For entries 4 and 5, R values are average of 3 independent polymerizations, experimental conditions are listed for representative runs.

<table>
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<tr>
<th>[M] / [RAFT]</th>
<th>Temp, °C</th>
<th>Conv, %</th>
<th>Mn\textsubscript{calc}, kDa</th>
<th>Mn\textsubscript{GPC}, kDa</th>
<th>D</th>
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<tr>
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<td>80</td>
<td>6.9</td>
<td>10</td>
<td>1.29</td>
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</tr>
<tr>
<td>4 33</td>
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<td>65</td>
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<td>1.5</td>
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<tr>
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<td>1.20</td>
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12 For all polymerizations, VAc was purified by cryogenic distillation and polymerized in bulk. The reaction mixtures were degassed by freeze-pump-thaw cycles and incubated at a specified time and temperature, after which the synthesized polymer was recovered via precipitation into heptane. Removal of the phthalimide protecting group was accomplished via a treatment using hydrazine monohydrate in methanol at 60 °C. Hereafter, aqueous or methanolic NaOH was added and the mixture was stirred overnight at room temperature. Resulting PVA was collected as a white precipitate. This procedure yielded a complete saponification in all samples tested, allowing a one-pot conversion from PVAc to PVA-NH\textsubscript{2}.
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15 Surfac ed adhered, patterned physical hydrogels were assembled via a microtransfer moulding technique using a poly(dimethylsiloxane) stamp and a 24 h stabilization in 0.5 M solution of sodium sulfate, as described in detail in Ref. 16
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