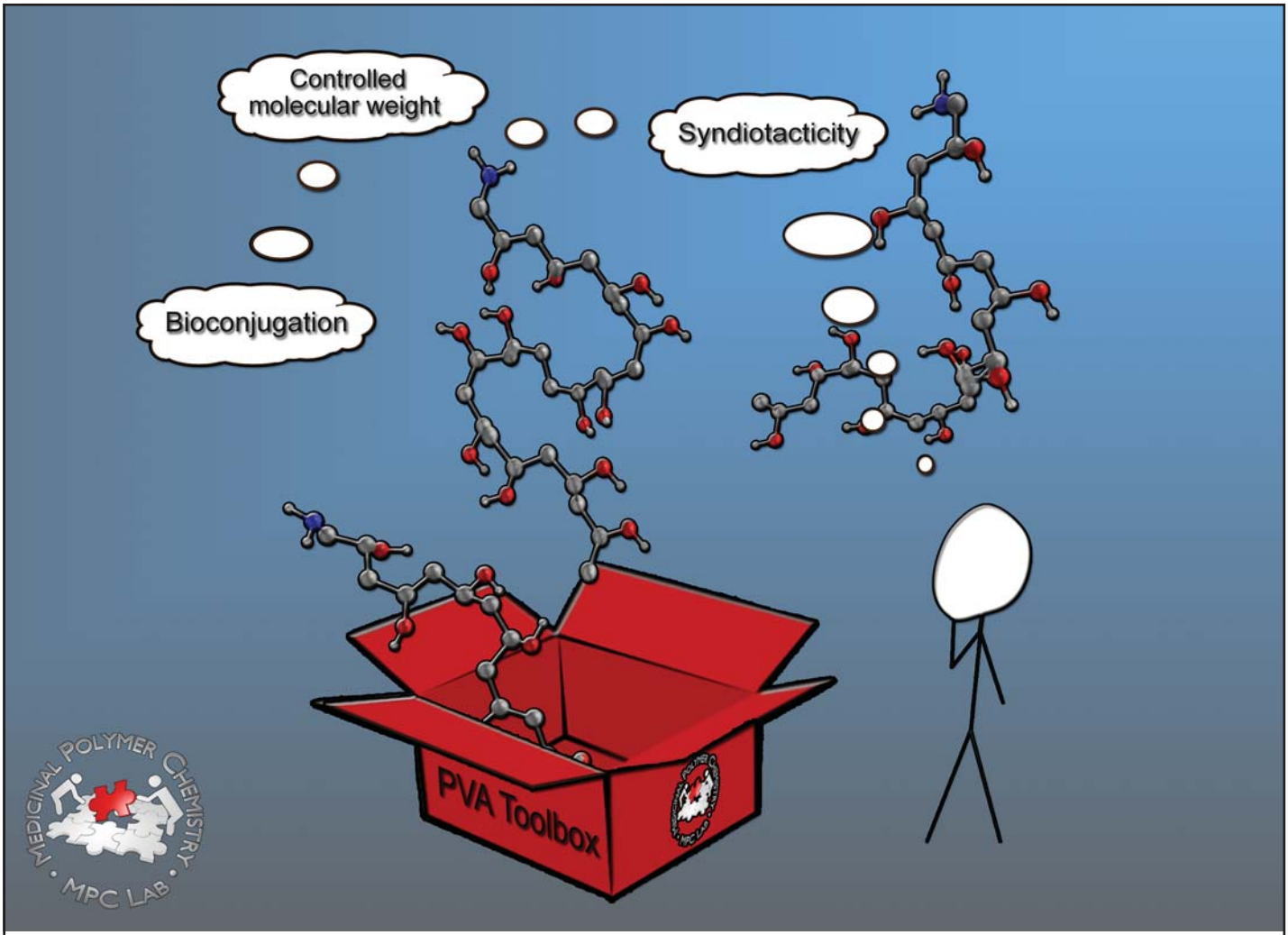

The "Medicinal Polymer Chemistry" lab established and headed by Dr Zelikin at Aarhus University, Denmark, conducts interdisciplinary research with the main focus on surface mediated drug delivery, polymer therapeutics and antiviral research.

Title: Macromolecular design of poly(vinyl alcohol) by RAFT polymerization

Poly(vinyl alcohol) is a unique polymer with an outstanding history of biomedical applications yet an even greater unrealized potential. For decades, making this polymer with controlled molecular weight and equipped with sites for facile bioconjugation remained an elusive task. The Zelikin lab accomplished this using RAFT polymerization and is now on the way to use this polymer in engineering of physical hydrogels for applications in biomedicine.

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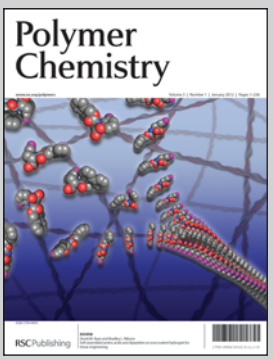

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COMMUNICATION

Macromolecular design of poly(vinyl alcohol) by RAFT polymerization

Anton A. A. Smith,[a] Thomas Hussmann,[a] Joseph Elich,†[a] Almar Postma,[b] Marie-Helene Alves[a] and Alexander N. Zelikin*[a]

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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.[1,2] 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,[3] and clinical applications in humans, specifically as embolic bodies.[4,5] Early studies revealed that at similar molecular weights, pharmacokinetics of PVA is near identical to that of PEG,[6] 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 improved pharmacokinetics.[7] Nevertheless, despite early successes, currently PVA does not appear in the focus of biomedical research, largely due to that polymer science has failed to accomplish fundamental aspects of macromolecular design, namely a synthesis of PVA with controlled molecular weight and narrow polydispersities. Indeed, while pharmacokinetics[6] and materials properties[8] of PVA are highly dependent on the polymer molecular weight, materials science and biomedicine still rely on the use of PVA samples with polydispersity indexes at and above 2.[8] Furthermore, opportunities in bioconjugation with PVA also remain scant, as compared to the polymers in the spotlight of polymer therapeutics. 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 of 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 vinylic polymers in that this polymer has no true monomer and is obtained *via* hydrolysis of a precursor polymer, typically poly(vinyl acetate), PVAc. 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.[9] Despite this, few reports have focused on synthesis of PVA from PVAc obtained *via* RAFT (or other controlled radical polymerization techniques), and while solitary successful reports include the syntheses of PVA with linear[10] and multi-arm architectures,[11] none of these demonstrated synthesis of PVA with molecular weights controlled over a broad range.

To accomplish this, we capitalize on the features inherent with the living character of 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.[12] Indeed, with the use of *S*-phthalimidomethyl-*O*-ethyl xanthate, judicious choice of polymerization parameters allowed the synthesis of PVAc with molecular weights spanning two orders of magnitude, from 3000 to 121 000 Da, preserving good polydispersity, Đ (ref. 13) (Fig. 1). We note that GPC characterization is presented herein for PVAc, a polymer precursor for PVA, and not for PVA, 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 the classic site of conjugation (amine, thiol, *etc.*), incorporation of the latter as polymer terminal groups has recently been established *via* a choice of an appropriate RAFT agent. This notion provided an impetus for using a RAFT agent which contains a phthalimide (Phth), specifically within the so-called "R" group in the structure of RAFT agent. Phth presents a protected amine moiety with a facile deprotection using hydrazine, a strategy which has been previously realized in the context of polymer terminal groups[14] to achieve *e.g.* fluorescent labeling of polymer samples.[15] We hypothesized that, if performed on PVAc, the target polymer (PVA), unlike

[a]*Department of Chemistry, Aarhus University, Aarhus, C 8000, Denmark. E-mail: zelikin@chem.au.dk*
[b]*CSIRO—Materials Science and Engineering, Clayton, VIC, Australia*
† On leave from Cornell University, Ithaca, NY, USA

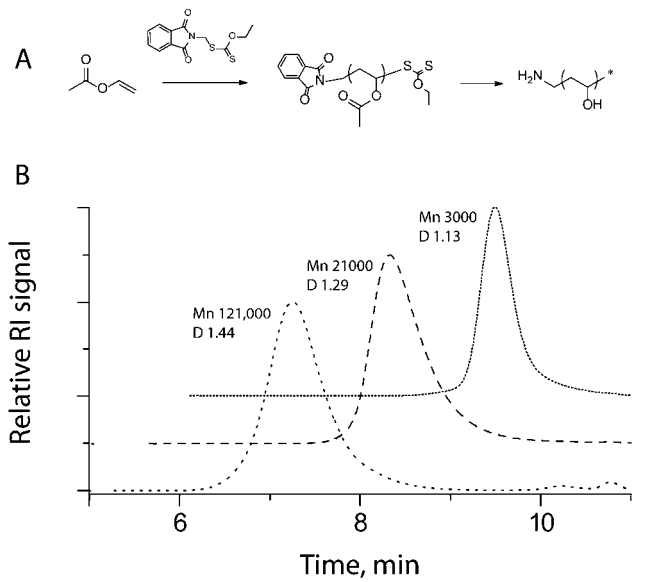

Fig. 1 (A) Schematic illustration of the synthesis of amine-functionalized PVA *via* RAFT mediated polymerization of VAc, removal of the terminal phthalimide protecting group and saponification. (B) Gel permeation chromatography elution profiles for three representative samples of PVAc obtained *via* RAFT polymerization. Subsequent hydrazinolysis and saponification yielded amine-terminated PVA samples with calculated molecular weights (left-to-right) of 60, 10 and 1.5 kDa.

its pristine counterpart, would be equipped for convenient, site-specific bioconjugation. To investigate this in detail, hydrazinolysis of PVAc was conducted using varied relative amounts of hydrazine in methanol at 60 °C for 30 min (Fig. 1). Under these conditions, 50 mole equivalents of hydrazine were sufficient for a completion of reaction, as evidenced by a disappearance of Phth characteristic peaks in the NMR spectra. With longer reaction times (1 h), the relative content of hydrazine could be reduced to 20 mole equivalents, a feature which becomes increasingly important when treating oligomeric samples of PVAc. In optimizing reaction conditions for the final step to obtain the target polymer, saponification of PVAc into PVA, we found that avoiding water as a reaction medium and using methanolic NaOH was pivotal for a better polymer recovery, specifically for low molecular weight samples. The final polymer samples were analyzed *via* NMR in d_6-DMSO, and recorded spectra were identical to those typically reported for PVA.

We emphasize that while macromolecular design *via* a RAFT agent "Z" group (-OEt in the chosen RAFT agent) is appropriate for PVAc, a subsequent saponification step is expected to remove these terminal functionalities from PVA. Formation of a terminal thiol group upon removal of thioester functionality, as widely used in bioconjugation using a variety of RAFT-derived polymers,[12] for PVA is also complicated by dominating side reactions.[10] These two notions that make the proposed R-group design unique. Compared to rather scant opportunities in bioconjugation using PVA hydroxyl groups, we believe that terminal amine functionalities and controlled molecular weights render this polymer synthesis route highly attractive for production of PVA for bioconjugation and design of functionalized hydrogels. To provide an initial illustration to novel opportunities in materials design associated with amine-functionalization of PVA, a 4.5 kDa sample of H_2N-PVA was reacted with rhodamine isothiocyanate (RITC), a model low molecular weight cargo. The resulting polymer was used to assemble surface adhered, patterned PVA hydrogels, towards their applications in guided cell adhesion and surface mediated drug delivery.[16] Functionalized PVA hydrogels are unique in that both physical (matrix elasticity) and chemical (low fouling and/or pro-cell adhesion functionalization) cues are available to elicit a desired cellular response at an interface. The presented fluorescent image (Fig. 2, inset) reveals that synthesized samples of PVA, despite a low molecular weight, exhibit outstanding gelation capability and retention of model cargo, thus contributing towards a novel platform in the design of intelligent biointerfaces.[17]

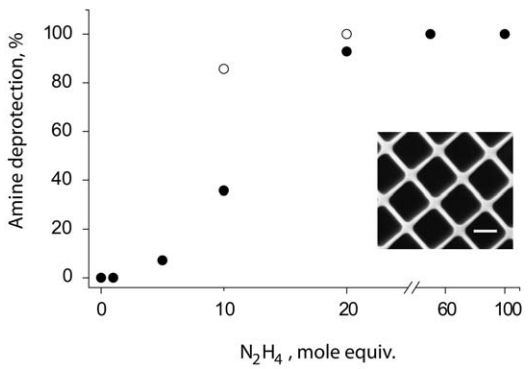

Fig. 2 Degree of phthalimide removal from the PVAc terminus achieved by hydrazine hydrate taken at varying molar equivalents to Phth group in methanol at 60 °C over 30 min (filled circles) or 60 min (open circles). Inset: fluorescence microscopy image of a surface adhered PVA physical hydrogel obtained *via* a microtransfer molding 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.

Table 1 Characteristics of the PVA samples obtained through RAFT polymerization of VAc. Monomer conversion and *R* values were estimated from the ^{1}H NMR spectra of polymerization mixture (d_3-chloroform) and PVA (d_6-DMSO, 25 °C). Number average molecular weights for PVA were calculated from VAc conversion (theoretical value, M_n^{calc}) and from GPC data obtained for the parent sample PVAc (M_n^{GPC}). For entries 4 and 5, *R* values are the average of 3 independent polymerizations, experimental conditions are listed for representative runs

	[M]/[RAFT]	Temp./°C	Conv. (%)	M_n^{calc}/kDa	M_n^{GPC}/kDa	Đ	*R* (%)
1	3900	60	50	84	60	1.44	53
2	3900	60	13	22	19	1.27	53
3	200	60	80	6.9	10	1.29	53
4	33	60	65	0.9	1.5	1.13	(53 ± 2)
5	33	50	53	0.75	1.0	1.1	(69 ± 8)
6	200	37	67	5.8	7.0	1.2	53

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.[18] 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 a degree of syndiotacticity exceeding the highest values previously reported in the literature.[19,20] Aiming to obtain oligomeric samples of PVA and using an increased concentration of RAFT agent (monomer-to-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 $R = 53 \pm 2\%$, Fig. 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 are quoted based on three independent experiments for each set of conditions). Notably, within the studied range, temperature alone did not affect a syndiotactic chain propagation, as evidenced by a polymerization at 37 °C at a [M]/[RAFT] ratio of ~200, which resulted in an atactic sample of PVA (Table 1). These data strongly suggest that a high concentration of a RAFT agent, together with appropriate choice of temperature, is imperative to facilitate a 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, fluoroalcohol solvents, *etc.*), the revealed approach is significantly advantaged in using a readily available monomer and a convenient polymerization temperature (as opposed to custom monomers[21–23] and protocols carried out at *e.g.* −78 °C (ref. 24)) as well as a facile saponification step to yield PVA (in contrast to hydrolysis of *e.g.* vinyl pivalate[25]). 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,[26] 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 increased content of syndiotactic dyad. In a process not dissimilar to crystallization employed for low molecular weight compounds, we took advantage of this phenomenon and were able to isolate a sample of s-PVA with a syndiotacticity of 78% (overall polymer recovery of 15%). To the best of our knowledge, this is the highest value of syndiotacticity for PVA ever reported. Despite a low molecular weight, this sample exhibits 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.

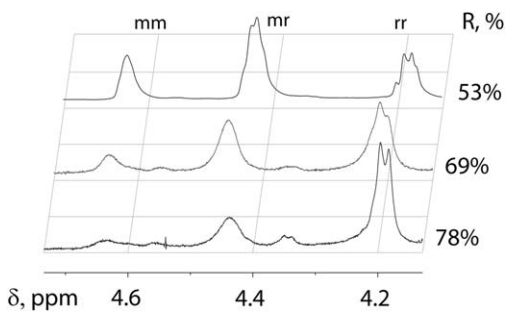

Fig. 3 ^{1}H NMR spectra (d_6-DMSO, 25 °C) of synthesized samples of PVA with varying degrees of syndiotacticity, $R\% = [rr + mr/2]/[rr + mr + rr]$, where *r* and *m* signify *racemo* and *meso* configuration of adjacent hydroxyls on a PVA chain, *rr*, *mm* and *mr* are syndiotactic, isotactic and atactic dyads. $R = 53\%$ 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 the literature.[19]

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 weights, narrow polydispersities and facile sites for bioconjugation. The importance of this lies in that pharmacokinetic parameters of PVA (blood residence time, *etc.*)[6] are governed by the polymer molecular weight, and while PVA showed promise in polymer therapeutics,[7] the lack of well-defined methods of polymer synthesis and bioconjugation essentially arrested the utility of this polymer in biomedicine. From a different perspective, syndiotactic chain propagation in RAFT-controlled polymerization of PVAc and the synthesis of PVA with the highest ever degree of syndiotacticity, reported herein for the first time, is important for both fundamental and applied polymer sciences and opens broad possibilities for macromolecular design of PVA.

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