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Poly(3-methylene-2-pyrrolidone). Synthesis, characterization and evaluation of cytotoxicity

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ABSTRACT: The homo-and copolymerization of 3-methylene-2-pyrrolidone (3M2P) is introduced. 3M2P is readily polymerized via conventional free radical polymerization, and two reversible deactivation radical polymerization methods (RDRP) including reversible addition fragmentation (chain) transfer (RAFT) and single-electron-transfer living radical polymerization (SET-LRP). Poly(3M2P) has a high thermal stability and a very high T_g. Poly(3M2P) does not dissolve in most common organic solvents, but it has a high aqueous solubility. Cytotoxicity tests reveal that it is non-toxic to cells, even up to concentrations of 1 mg/ml. This adds poly(3M2P) to the family of water-soluble and biocompatible pyrrolidone-based vinyl polymers.

1. Introduction

Vinyl polymers containing a pyrrolidone functionality in the side chain have many important applications in the pharmaceutical, food and cosmetic industry.[1–4] These applications emanate from the properties of the pyrrolidone functionality, which include aqueous solubility, hydrogen bonding capacity, biocompatibility and coordination capacity.[5–10] Most literature reports utilise *N*-substituted vinyl pyrrolidones, the preeminent example being poly(*N*-vinylpyrrolidone) (PVP), the polymer of *N*-vinylpyrrolidone (NVP) (**1**, Figure 1). PVP is fully water soluble in all proportions and is non-cytotoxic.[7,11–13] PVP is synthesized via radical polymerization methods, including reversible deactivation radical polymerization techniques (RDRP), in particular the reversible addition fragmentation chain transfer (RAFT) technique.[14] Other reported pyrrolidone functional vinyl polymers include derivatives of NVP, with 3-alkyl substituents (**2**, Figure 1), and *N*-substituted systems which have spacers between the vinyl functionality and amide nitrogen (**3**, Figure 1). In the latter systems, the polymerizable moiety is (meth)acrylic in nature.[15,16]

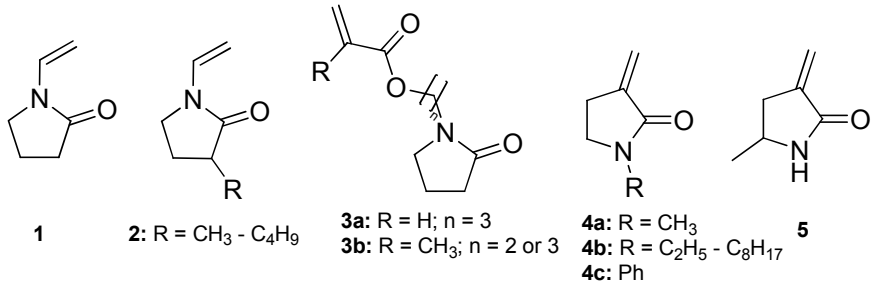


Figure 1. Representative pyrrolidone-based monomers.

A much less reported system of vinyl pyrrolidones is based on monomers in which the vinyl/methylene group is directly attached to the pyrrolidone ring in position 3 (**4** and **5**, Figure 1).[17–20] Monomer **4a** has been polymerized anionically,[21] yielding well defined polymers, and via free radical polymerization (FRP).[19] In the latter study, the aqueous solubility of polymers from **4a** was also demonstrated.[19] In other studies, monomers **4a**-**b**,[17] and **4c**,[18] were polymerized via FRP, and the effect of the *N*-substituent on the thermal properties and aqueous solubility was assessed. Monomer **5** was homo- and copolymerized with styrene and methyl methacrylate via FRP.[20]

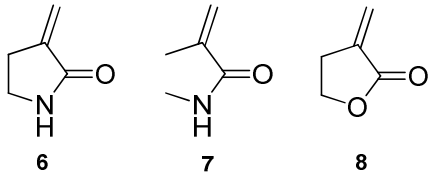


Figure 2. 3M2P (6), *N*-methyl methacrylamide (7) and tulipalin A (8).

To extend the versatility of pyrrolidone-functional vinyl polymers, we focused on 3-methylene-2-pyrrolidone (3M2P), (**6**, Figure 2). To the best of our knowledge there are no literature reports that demonstrate the polymerization of 3M2P by chain growth methods such as FRP and RDRP techniques. We drew inspiration from the fact that the lactone analogue, known as tulipalin A (**8**, Figure 2), which can be considered to be the cyclic version of MMA, has been polymerized via FRP,[22] and RDRP techniques including ATRP,[23] yielding well defined polymers with a high T_g and very high thermal stability. We anticipated that 3M2P, which can also be considered to be the cyclic version of *N*-methyl methacrylamide (*N*-MMAAm) (**7**, Figure 2), could also be polymerized via FRP and RDRP techniques, similarly to *N*-MMAAm.[24] The exo-cyclic methylene group is conjugated with the carbonyl group, this should stabilize the propagating radical. Furthermore we anticipated that the subsequent polymers would also be endowed with the desirable qualities of pyrrolidone rings, adding this monomer class to the family of pyrrolidone-based vinyl monomers which have significant academic, industrial and pharmaceutical interest. In this work we demonstrate the radical polymerization of 3M2P, via FRP and RDRP techniques such as RAFT and single electron transfer living radical polymerization (SET-

LRP), to form homopolymers. We demonstrate its copolymerization and block copolymer formation (via RAFT) with 2-(dimethylamino)ethyl methacrylate (DMAEMA). We characterized poly(3M2P)'s thermal and solubility properties, and finally we explored its potential biocompatibility by conducting cytotoxicity tests.

2. Experimental section

2.1 Materials

Ethyl chloroformate (Aldrich, 97 %), potassium carbonate (Fluka, 99 %), 3-amino-1-propanol (Aldrich, 99 %), phosphorus tribromide (Aldrich, 99 %), triphenylphosphine (Acros, 99 %), potassium *tert*-butoxide (Aldrich, 98 %), paraformaldehyde (Aldrich, 95 %), 2-pyrrolidone (Fluka, 99 %), di-tert-butyl dicarbonate (Aldrich, 97 %), 4-dimethylaminopyridine (Merck, 99 %), diethyl oxalate (Aldrich, 99 %), sodium hydride (Aldrich, 95 %), trifluoroacetic acid (Sigma Aldrich, 99 %) were used as received. Solvents; methanol, toluene, dimethylsulfoxide, *N*,*N*-dimethylformamide, diisopropyl ether and acetonitrile were purchased from Sigma Aldrich and used without further purification. PPh_3 was recrystallized from anhydrous acetone. Azobisisobutyronitrile (AIBN) was purchased from Merck and recrystallized from methanol. Me_6TREN and 1,2-dihydroxypropane-3-oxy-2-bromo-2-methylpropionyl were synthesized as described in literature.[25,26] The synthesis of the RAFT agent, 2-hydroxyethyl 2-(butylthiocarbonothioylthio)-2-methylpropanoate is described in the SI. TLC plates (0.20 mm Silica gel 60, with fluorescent indicator UV254) and Silica gel 60(0.063 - 0.2 mm/70 - 230 mesh) were purchased from Machery-Nagel. SnakeSkin® dialysis tubing with a MW cut-off of 3 500 was purchased from Thermoscientific.

2.2 Characterization

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H and ^{13}C NMR spectra were obtained with a Varian VXR-Unity (300 MHz) spectrometer. NMR samples were prepared in deuterated solvents. Proton and carbon chemical shifts are reported in parts per million (ppm) (δ) downfield from tetramethylsilane (TMS) using residual solvent protons as internal standards.

Size Exclusion Chromatography (SEC). Molar mass and dispersities (*Đ*) of the homopolymer, poly(3M2P), were obtained by 1,1,1,3,3,3-hexafluoro isopropanol (HFIP) SEC. The HFIP SEC instrument was equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector, a Waters 2707 autosampler, PSS PFG guard column and two PFG-linear-XL (7 µm, 8 × 300 mm) columns in series. The HFIP contained lithium chloride (2 g/L), the flow rate was 0.8 mL/min and the injection volume was 75 µL. The calculated molar masses were relative to poly(methyl methacrylate) (PMMA) standards with low dispersities, obtained from Polymer Laboratories. Statistical and block copolymers were analysed with a *N*,*N*-dimethylacetamide (DMAc) SEC. The DMAc SEC setup consisted of a Waters 717 plus autosampler connected to a Shimadzu LC-10AT pump with the following column configuration: 1×PSS

GRAM analytical precolumn (10 µm particle size, 8.0×50 mm), 1×PSS GRAM analytical column (10 µm particle size, 100 Å pore size, 8.0×300 mm), 2×PSS GRAM analytical column (10 µm particle size, 3000 Å pore size, 8.0×300 mm.) with a Waters 410 differential refractometer. The system was calibrated using low dispersity PMMA standards.

Electrospray Ionization Mass Spectrometry (ESI-MS). Analysis was performed on a Waters Synapt G2 with electrospray ionization (ESI) in the positive mode. The column used was Waters UPLC C18, 2.1x100 mm. Samples were dissolved in chloroform.

Differential Scanning Calorimetry (DSC). Thermal analysis studies were obtained by DSC with a TA Instruments Q100 calorimeter calibrated with an indium standard under N_2 atmosphere. Cooling and heating rates were fixed at 10 °C/min and were obtained from -60 to 300 °C.

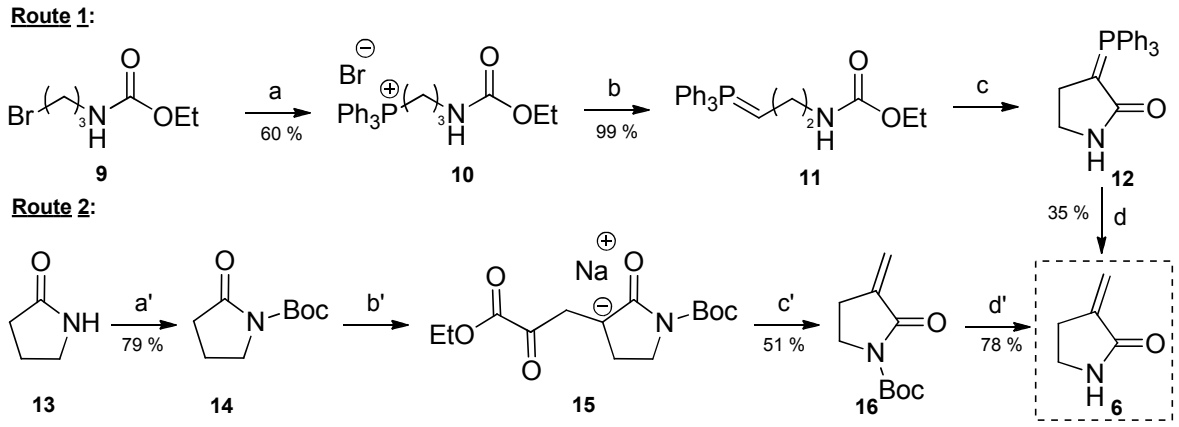
Thermal gravimetric analysis (TGA). Samples were analysed using a Perkin Elmer TGA 7, under an inert nitrogen atmosphere to prevent oxidation. The heating rate was 20 °C per minute up to 900 °C.

Cell Imaging. Fluorescence and light transmission micrographs image acquisition was performed on an Olympus Cell system attached to an IX81 inverted fluorescence microscope equipped with an F-view-II cooled CCD camera (Soft Imaging Systems) using a Xenon-Arc burner (Olympus Biosystems GmbH) as light source.

2.3 Methods

The monomer, 3M2P, was prepared as described in Scheme 1. Further experimental details and characterization are included in the supplementary information (SI).

Scheme 1. Synthesis routes for 3M2P



a) PPh_3, acetonitrile, 100 °C; b) potassium tert-butoxide, toluene, r.t.; c) 50 °C; d) paraformaldehyde, toluene, 50 °C to r.t.; a') di-tert-butyl dicarbonate, acetonitrile, 0 °C; b') diethyl oxalate, sodium hydride, diisopropyl ether, 35 °C; c') paraformaldehyde, dimethylformamide, 100 °C; d') trifluoroacetic acid, dichloromethane.

General FRP of poly(3M2P): Homopolymerizations were carried out in a 5 mL pear-shaped flask; 3M2P (100 mg, 1.03 mmol) and AIBN, in a ratio of 100:1, were dissolved in DMSO (1 mL), and the mixture purged with argon. Then the flask was immersed into an oil bath preheated to either 60 °C or 75 °C to commence the polymerization. The polymer was isolated by slow precipitation from acetone, and was obtained as a white powder. For DSC, TGA and cytotoxicity analyses, poly(3M2P) was dialyzed against water for ~5 days using SnakeSkin®, replacing the water every 12 h, subsequently the polymer was freeze-dried for 24 h. Homopolymers were analysed by ^{1}H NMR spectroscopy (Figure 3) and HFIP SEC (entry 1 and 2, Table 1).

Statistical copolymerization of *N,N*-dimethylaminoethyl methacrylate and 3M2P: A reaction vessel was charged with 3M2P (50 mg, 0.51 mmol) and *N,N*-dimethylaminoethyl methacrylate (DMAEMA) (81 mg, 0.52 mmol) as a comonomer. AIBN (1.7 mg, 0.010 mmol) was added as an initiator in 1.0 mL DMF and the mixture was then purged with argon gas. The vessel was then immersed in an oil bath, preheated to 75 °C, for 24 h. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitation from cold diethyl ether. The copolymers were analysed via ^{1}H NMR spectroscopy and DMAc SEC (Figure 4c and d).

General homopolymerization via SET-LRP: To a 25 mL Schlenk flask was added 3M2P (0.10 g, 1.03 mmol), copper wire wrapped around the stirring bar and 2,3-dihydroxypropyl-2-bromo-2-methylpropanoate (2.47 mg, 0.10 mmol) as initiator, in 1.0 mL DMSO. The reaction mixture was degassed by five freeze-pump-thaw cycles whereafter the flask was back-filled with argon. A degassed micro-syringe was then used to transfer the ligand, Me_6TREN (1.19 mg, 1.4 µL, 0.01 mmol), to the already deoxygenized reaction mixture and the Schlenk flask placed in a heated oil-bath at 50 °C. After 24 h, the reaction was stopped by opening the flask to air and cooling, and conversion was determined on the crude polymer mixture using ^{1}H NMR spectroscopy. The reaction mixture was filtered through a short basic aluminium oxide column to remove residual copper, whereafter the polymer was precipitated from acetone to yield a white powder. The homopolymer was analysed via ^{1}H NMR spectroscopy (Figure 2S) and HFIP SEC (entry 4, Table 1 and Figure 4a).

General RAFT homopolymerization procedure of 3M2P: The RAFT agent utilized was 2-hydroxyethyl 2-(butylthiocarbonothioylthio)-2-methylpropanoate (SI). To a 25 mL pear flask was added 3M2P (0.11 g, 1.13 mmol), RAFT agent (1.65 mg, 0.01 mmol) and AIBN (0.18 mg, 0.001 mmol), in 0.8-1.0 mL DMSO. After the reaction mixture was dissolved and degassed with argon gas for 45 min, the polymerization vessel was placed in the heated oil bath at 75 °C for 24h. The reaction was stopped by opening the flask to air, and cooling, conversion was determined, on the crude polymer mixture, using ^{1}H NMR spectroscopy. The polymer was isolated by precipitation from acetone, to yield a yellowish powder. The polymer was analysed via ^{1}H NMR spectroscopy (SI, Figure 1S) and HFIP SEC (entries 3a and 3b, Table 1 and Figure 4a).

Block copolymer consisting of DMAEMA and 3M2P via RAFT: A reaction vessel was charged with DMAEMA (1.0 g, 6.36 mmol), AIBN (3.0 mg, 0.02 mmol), and 2-hydroxyethyl 2-(butylthiocarbonothioylthio)-2-methylpropanoate (31 mg, 0.10 mmol) as the RAFT agent, in 1,4-dioxane (2 mL). The mixture was purged with argon gas, whereafter the vessel was placed in a heated oil bath at 75 °C for 24 h. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitation from cold petroleum ether. The homopolymer was analysed via ^{1}H NMR spectroscopy and DMAc SEC (Figure 4b).

The resulting PDMAEMA macro-RAFT agent was then chain extended with 3M2P. A reaction vessel was charged with PDMAEMA macro-RAFT agent (0.1 g, 0.01 mmol), 3M2P (35 mg, 0.36 mmol), AIBN (0.2 mg, 0.001 mmol) were dissolved in a mixture of 1,4-dioxane (1.0 mL) and DMF (0.2 mL). The mixture was purged with argon gas, and the flask was then immersed in a heated oil bath at 75 °C for 24 h. The reaction was stopped by opening the flask to air, and cooling. The polymer was isolated by precipitation from cooled diethyl ether. The block copolymer was analysed via ^{1}H NMR spectroscopy (SI, Figure 3S) and DMAc SEC (Figure 4b).

Cytotoxicity assay: To determine the cytotoxity of poly(3M2P), different concentrations of dialyzed and freeze-dried poly(3M2P) (1 mg/mL, 1 µg/mL and 1 ng/mL) were dissolved Dulbecco's modified eagle medium (DMEM) containing 1 % penicillin/streptomycin and 10 % fetal calf serum and added to GT1-7 cell (hypothalamic mouse cells) cultures. A control (media and cells) and a positive control (media and cells treated with ethanol) were used as references. Subsequently, the samples were incubated at 37 °C for 4 h, whereafter the cells were stained with fluorescent markers, Hoechst 33342 dye and propidium iodide, respectively (SI, Figure 4S). Fluorescence and light transmission micrographs were obtained and cell viability was determined (Figure 5).

3. Results and discussion

3.1 Monomer synthesis

The monomer 3M2P was prepared using two known procedures (Scheme 1). In the first approach, 3M2P was accessed by condensing the corresponding phosphorous ylide (**12**, Scheme 1) with paraformaldehyde via a Wittig reaction, as reported by Fotiadu et al. (Scheme 1S), in an overal yield of ~ 20%.[27] In a second approach, similar to that used to synthesize monomers **4a**-**c**,[18,19,21] 3M2P was synthesized by first reacting diethyl oxalate with *N*-Boc-2-pyrrolidone (**14**, Scheme 1) in the presence of sodium hydride, to yield an enolate intermediate (**15**, Scheme 1). Subsequently, **15** was reacted with paraformaldehyde, followed by elimination to give the desired 3-methylene functionality, in an overal yield of ~ 31%. Finally removal of the *N*-Boc protection, afforded 3M2P. Preference was given to the second monomer synthesis route, since it has a fewer, and simpler synthesis steps (see SI for expanded synthesis scheme), and it also afforded a higher overal yield of 3M2P.

3.2 Free radical polymerization of 3M2P

We assessed the homopolymerizability of 3M2P via FRP. Under our optimized conditions, polymerization was carried out in DMSO, and the polymer was isolated by precipitation from acetone, in about 82 % yield. We analyzed the polymer via ^{1}H and ^{13}C NMR (SI, Figure 4S) spectroscopy and HFIP SEC. Figure 3 compares the ^{1}H NMR spectra of the monomer 3M2P and its homopolymer (poly(3M2P)). It is evident that the exo-methylene double bond of 3M2P disappeared after polymerization. The appearance of broad signals in the lower chemical shift region (~1.5 - 3.5 ppm) is an indication of successful polymer formation.

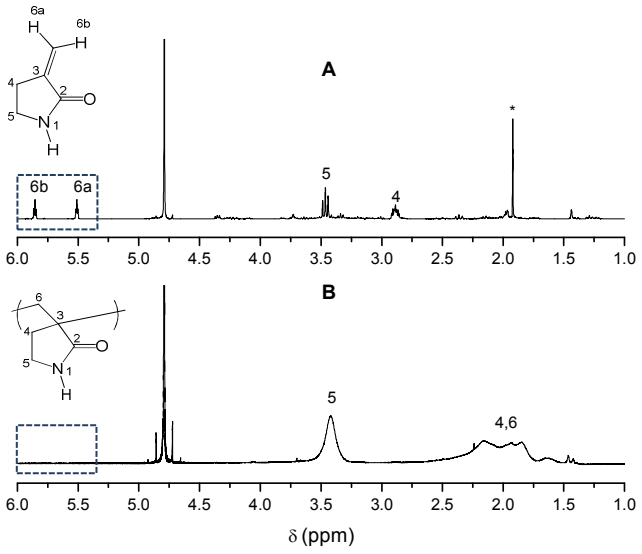


Figure 3. Presaturation ^{1}H NMR spectra of 3M2P (A) and poly(3M2P) (B) in D_2O.

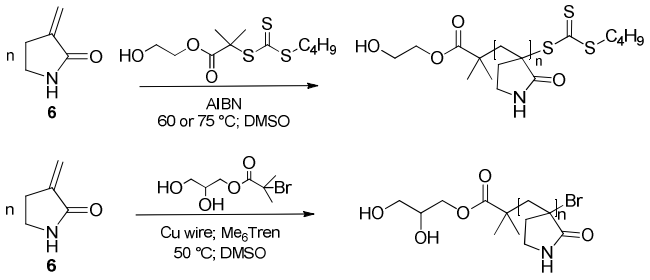
SEC analysis showed that high molar masses were obtained (entries 1 and 2, Table 1), confirming the success of the polymerization. *Đ* values were greater than 1.5, as expected for conventional free radical polymerizations. Poly(3M2P) was insoluble in a wide range of solvents, but it partially dissolved in DMSO and fully dissolved in HFIP and water. A similar solvent resistance was observed for poly(tulipalin A) and it was attributed to structural rigidity of the polymer chain caused by the conformational rigidity of the lactone monomer.[22] Considering the similarity of the ring structures, it is reasonable that poly(3M2P) is also similarly rigid. Additionally poly(3M2P)'s amide/lactam functionality likely participates in intermolecular hydrogen bonding, contributing to its (organic) solvent resistance.

3.3 RDRP of 3M2P

After successfully polymerizing 3M2P via conventional FRP, we then extended the study to assess its polymerizability under RDRP conditions, in view of preparing well-defined polymers with predetermined molar masses, low *Đ*, and defined chain end functionalities. Two RDRP techniques, i.e. RAFT and SET-LRP, were investigated (Scheme 2). We

used the RAFT polymerization, utilizing a trithiocarbonate RAFT chain transfer agent (Scheme 2). As 3M2P can be considered to be the cyclic version of

Scheme 2. Homopolymerization of 3M2P via RDRP techniques



Top: RAFT mediated polymerization and bottom: SET-LRP.

Table 1. Experimental results for the homopolymerization of 3M2P via FRP and RDRP techniques

#	Technique	T (°C)	Time (h)	Target M_n	Conversion (%)	$M_{n,NMR}$ (g mol^{-1})	$M_{w,SEC}$ (g mol^{-1})	$M_{n,SEC}$ (g mol^{-1})[e]	$Đ$[f]
1	FRP	60	24	-[a]	82[b]	-[d]	156 300	67 200	2.33
2	FRP	75	16	-[a]	83[b]	-[d]	97 100	52 300	1.86
3a	RAFT	60	40	20 000	> 70[c]	18 800	20 000	16 300	1.22
3b	RAFT	75	36	20 000	> 65[c]	13 900	20 100	14 900	1.35
4	SET-LRP	50	24	10 000	> 80[c]	8 000	12 900	11 200	1.16

a) Monomer:AIBN (100:1). b) Conversion determined gravimetrically. c) Conversion was determined from ^{1}H NMR spectroscopy. d) Chain-ends were not visible on ^{1}H NMR spectroscopy. e) HFIP SEC calibrated with PMMA standards. f) Dispersity.

N-MMAAm, a trithiocarbonate RAFT agent was selected, as such Z-groups are known to have high chain transfer constants and have been shown to control monomers with a stabilized propagating radical such as methacrylamides.[28] RAFT mediated polymerizations were carried out in DMSO with AIBN as initiator. Characterization by SEC, in HFIP (Figure 4a), and by ^{1}H NMR spectroscopy (SI, Figure 1S) gave M_n values which correlate reasonably well (entries 3a and 3b, Table 1). Additionally $Đ$ values are low, typical of well-behaved RAFT mediated polymerizations. SET-LRP was selected for polymerization of 3M2P via a transition metal catalyzed process (Scheme 2). SET-LRP is a versatile and mild RDRP technique for controlling the polymerization of vinyl monomers such as methacrylamides.[29,30] It can be readily carried out in polar solvents such as DMSO and water, which enhances the disproportionation of Cu^I to Cu^0, essential to SET-LRP.[29–31] We conducted our SET-LRP of 3M2P in DMSO based on the earlier successful FRP and

RAFT of 3M2P in DMSO, and we obtained a well-defined polymer with low *Đ* of 1.16 (entry 4, Table 1). Representative SEC traces for the polymer prepared by SET-LRP, are shown in Figure 4b and no bimodality was evident. Conversions of the RDRP experiments could not be determined accurately, as polymerizations were performed on a very small scale.

3.4 Statistical and block copolymerizations

To further enhance the application prospects of poly(3M2P), the copolymerization of 3M2P with other vinyl monomers was investigated. We selected DMAEMA as comonomer as it forms a tertiary propagating radical similar to 3M2P, also it is miscible with polar solvents that dissolve 3M2P. We copolymerized 3M2P with DMAEMA via FRP, producing a polymer with a M_n of 15 700 g/mol and a *Đ* of 1.78 (Figure 4c). Despite the initial feed of 50:50, the final polymer had a 39 % incorporation of 3M2P monomer. It is likely that DMAEMA has a higher reactivity ratio than 3M2P, hence the greater incorporation in the final polymer. The copolymerizability of 3M2P with a readily available 'more activated' monomer (MAM) is significant because the most frequently used pyrrolidone monomer, NVP, does not copolymerize readily with MAMs.[12]

Block copolymer formation was pursued by chain extending poly(DMAEMA), synthesized with a trithiocarbonate RAFT agent, with 3M2P. Block copolymer formation was confirmed by SEC and ^{1}H NMR spectroscopic analysis. With the former technique, the SEC trace shifted to a lower elution volume, indicating an increase in the molar mass of the polymer (Figure 4d). The *Đ* value (≈ 1.26) was also reasonably low. We also evaluated the block copolymer formation via ^{1}H NMR spectroscopy (Figure 3S), and we observed the appearance of signals attributable to poly(DMAEMA) and poly(3M2P) in the block copolymer.

3.5 Thermal properties

We further assessed poly(3M2P) by characterizing its thermal properties, using TGA and DSC. The TGA thermogram revealed a 20 % weight loss near 100 °C (SI, Figure 5S), attributable to water loss, suggesting that poly(3M2P) is very hygroscopic. TGA analysis showed that poly(3M2P) has a high thermal stability, with the main thermal decomposition occurring between 400-500 °C.

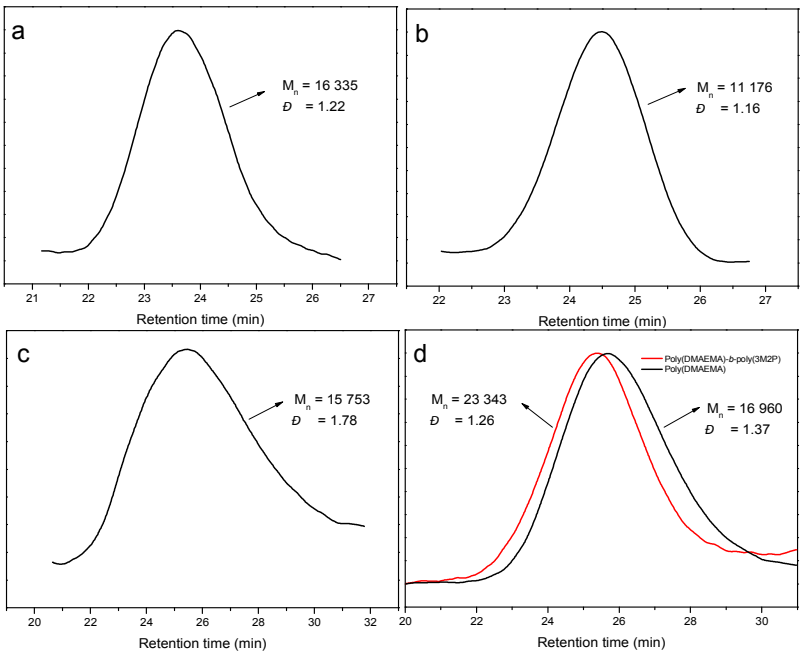


Figure 4. SEC traces of: a) poly(3M2P) prepared RAFT (entry 3a, Table 1), b) poly(3M2P) prepared via SET-LRP (4, in Table 1), c) random copolymer of DMAEMA and 3M2P prepared via FRP, and d) Poly(DMAEMA) and poly(DMAEMA)-block-poly(3M2P) prepared via RAFT.

The DSC trace revealed a glass transition temperature (T_g) at 285 °C (SI, Figure 6S). Poly(tulipalin), which is poly(3M2P)'s lactone analogue, has its thermal decomposition at 320 °C, and a T_g of 195 °C.[22] The polymers from monomers **4a** and **4b** (Figure 1) exhibit T_g values ranging from 110 °C to -5 °C, with the T_g values decreasing with increasing length of the *N*-alkyl substituent.[17] The enhanced thermal stability and higher T_g of poly(3M2P) is likely due to a combination of the rigidity imposed by its cyclic nature, and presumed intermolecular hydrogen bonding interactions of the lactam's amide linkage. Poly(3M2P) did not exhibit LCST behavior, in aqueous solution, within the accessible experimental range, 0-80 °C.

3.6 Cytotoxicity

The water-solubility of poly(3M2P) makes it an attractive polymer for biomedical applications, therefore we evaluated its cytotoxicity at different concentrations. These studies were performed on GT1-7 cultured hypothalamic mouse cell lines in Dulbecco's modified eagle medium (DMEM). The cells were incubated for 4 h, and afterwards, were stained with Hoechst *33342* dye (blue) and propidium iodide (red) prior to recording fluorescence and light microscopy images. Hoechst *33342* dye is used to stain cells for fluorescence microscopy studies, the counterstain, propidium iodide only penetrates the cell membranes of dead cells and is used to determine cell viability.[32]

Figure 5. Fluorescence and light transmission micrographs of cells stained with Hoechst *33342* (column a) dye and propidium iodide (column b), and an overlay (column c). Scale bar: 100 µm.

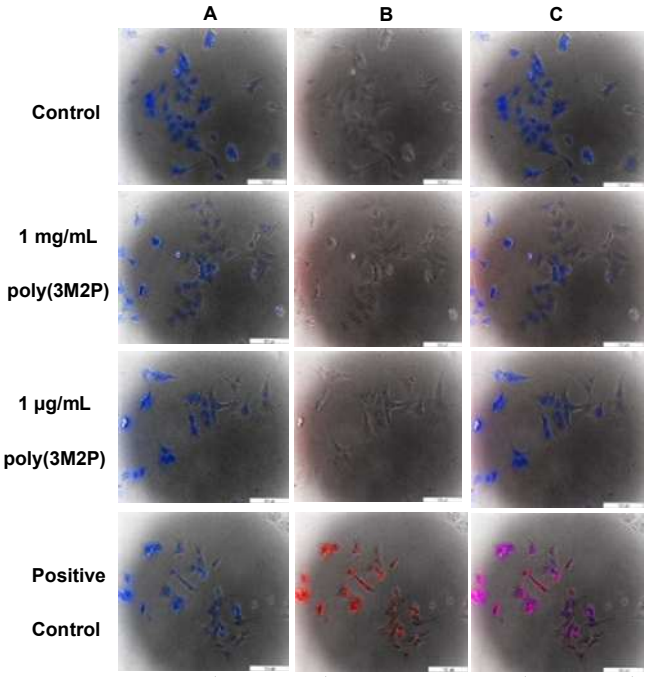


Figure 5 shows fluorescence and light transmission micrographs of cells stained with Hoechst 33342 dye (column **A**), propidium iodide (column **B**) and an overlay of Hoechst 33342 dye and propidium iodide (column **C**); for the control (cells in media), a positive control (cells treated with ethanol in media) and cells exposed to 1 µg/mL and 1 mg/mL poly (3M2P). The cells in the control micrograph are stained blue, but were impermeable to the red propidium iodide (**B**, Figure 5) indicating that they were viable, as expected. The cells in the positive control stained red with propidium iodide, as expected indicating that they were not viable, micrograph **C**, shows a pink colour, as a result of the overlay of the red and blue stains. The micrographs of the polymer-cell solutions with concentrations of 1 mg/mL and 1 µg/mL of P(3M2P), did not stain red, indicating that they were not penetrated by propidium iodide, and that the presence of polymer, even up to high concentrations of 1 mg/mL, was non-toxic to the cells. This suggests that poly(3M2P) is non-cytotoxic, and therefore it is a promising candidate for use in the biomedical field.

4. Conclusion

In summary, we have presented the polymerization of 3M2P, via FRP and RDRP techniques, i.e. RAFT and SET-LRP. Poly(3M2P) is highly–water soluble, but is insoluble in most common organic solvents except in HFIP and in DMSO (partially). Polymerization via the RAFT and SET-LRP techniques produced well-defined polymers with low dispersities. We successfully prepared random and block copolymers of poly(DMAEMA) and poly(3M2P) via RAFT mediated polymerization. Thermal analysis by TGA revealed that poly(3M2P) is very hygroscopic and has a very high thermal stability, whilst DSC revealed a very high T_g of 285 °C, which we attributed to the combination of 3M2P's cyclic rigidity and inter-chain hydrogen bonding interactions. We also showed that poly(3M2P) is non-cytotoxic even up to concentrations of 1 mg/mL suggesting its potential as a biomedical polymer. Finally, the ready polymerizability of 3M2P via various RDRP techniques, and the apparent non-cytotoxicity of poly(3M2P), makes this polymer an interesting alternative to traditional pyrrolidone functional polymers such as PVP.

ASSOCIATED CONTENT

Experimental details on the synthesis and characterization of the monomer, 3M2P; polymerization details and characterization; molecular weights of all polymers analysed via ^{1}H NMR spectroscopy and SEC; TGA and DSC thermograms; cytotoxic studies.

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Notes

The authors declare no competing financial interest.

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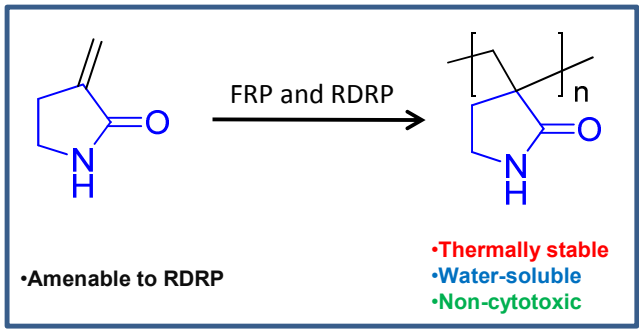
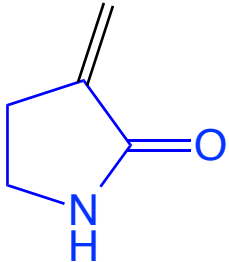
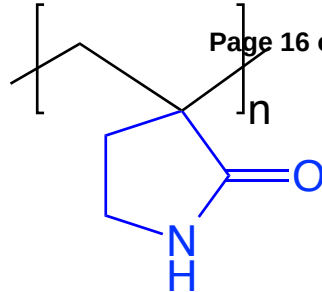




FRP and RDRP



amenable to RDRP

- thermally stable
- water soluble
- non-cytotoxic