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Synthesis of randomly aminated polyvinylpyrrolidone and its use in the preparation of hydrolyzable conjugates†

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In this work, a versatile synthetic route to functionalize vinylpyrrolidone (VP) with protected or unprotected aliphatic primary amine groups is described for the first time. Using these monomeric precursors polyvinylpyrrolidones (PVPs) with controlled load of side amine groups randomly distributed along the chains have been prepared. These functionalized VP and PVP systems are active molecules highly desirable for further couplings, *i.e.* they can easily afford the simple preparation of water soluble covalent side chain conjugates onto the PVP backbone. To show the potential of this functionalization, we have prepared functionalized polymers as a controlled release delivering vehicle, employing the bactericide and preservative 2-phenoxyethanol as a hydroxyl-containing model drug.

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Introduction

Polyvinylpyrrolidone (PVP) is a well-known neutral amphiphilic polymer, which is stable, non-toxic and soluble in water as well as in many organic solvents. It is used as a polymeric surfactant,^{1,2} a stabilizer (E1201), a clarifying agent in drinks and it has been approved as a food additive by the FDA.³ Towards the end of the 20th century, it was a classic component of contact lenses. Additionally, when adsorbed or anchored onto surfaces, it resists the non-specific adsorption of proteins.⁴

Polyethylene glycol (PEG), poly *N*-(2-hydroxypropyl)methacrylamide (PHPMA) or polyvinylpyrrolidone are first choice supports to prepare polymer–drug conjugates. Their desirability can be attributed to their solubility in water and protection from the environment through a ‘soft’ and ‘charge-free’ hydrated macromolecular carrier. They typically exhibit high residence time in plasma and low tissue distribution compared to other synthetic polymers.^{5,6} It has been described that these polymer–drug conjugates with antitumor agents can profit from the imperfection of the newly formed tumor vasculature and extravasate in that area in a specific way (the so-called ‘enhanced permeation retention’-EPR effect⁷).

PVP has a chemical limitation in terms of conjugation since the monomer lacks any active functional groups. PEG and PHPMA, on the other hand, have end groups or side chain hydroxyl functions, which allow for direct chemical

modification; several polymer conjugates derived from both can be found in clinical therapies as PEGylated products⁸ or PHPMA based polymer–drug conjugates which are already on the market or in advanced clinical trials.⁹

The functionalization of PVP has therefore been a goal over the last few decades. Other authors have tried to modify the ring lactams of the polymer chains under harsh reaction conditions, which lead to non-controlled modification reactions.^{10–13} End-chain functionalization and conjugation – similar to PEG – may be achieved using special polymerization^{14,15} techniques by using functional transfer agents or controlled polymerization approaches. Pseudo-side-chain type functionalization – similar to PHPMA – may be obtained by copolymerization of VP with a functional vinyl comonomer,^{16–18} although this procedure leads to ‘false’ PVP functionalization. In this case a copolymer system is formed obtaining a heterogeneous backbone due to the different reactivity of the monomeric units (where acrylate/methacrylate/styrene monomers have reactivity rates higher than VPs). A superior alternative seems to be a *homologous bottom-up* route consisting of the modification of VP followed by its easy copolymerization with unmodified monomer.^{19,20} This homologous route, which leads to true side chain PVP conjugates, exhibits great flexibility in the control of its load that may be defined just by adjusting the feed comonomer ratio.

The incorporation of aliphatic primary amines as side substituents on some of the VP units may be extremely useful in many of the aforementioned applications. Aliphatic primary amines are certainly first choice groups for conjugation and bioconjugation,²¹ which may allow for the preparation – on a PVP backbone – of functional foods, functional surfactants or therapeutic nanocarriers similar to the PHPMA derivatives.²² Besides, primary amines are cationizable and therefore their

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incorporation into neutral PVP provides positive charges in aqueous media below the pK_a of the amine. These charges may modulate the interaction of PVP with biological entities such as DNA or cell surfaces, simulating the behavior of other polymers such as chitosan or poly-L-lysine.^{23,24} Other primary amines have been incorporated into contact lens formulations to provide antibacterial properties.²⁵

In this work, a simple and versatile synthetic methodology to prepare VP monomer bearing primary aliphatic amine is described for the first time. The protected form of the new monomer has been transformed by conventional radical copolymerization with VP, followed by deprotection, into novel aminated PVP. It should be noted that the monomer-based 'bottom-up' approach used here allows for great flexibility in the control of the amine load; furthermore, extra components may be easily incorporated by including extra comonomers in the polymerization. Finally, this aminated VP has been used in the design of a novel synthetic route to prepare PVP conjugates with hydroxylic compounds through a hydrolysable ester group, as one example of many possibilities that may provide this monomer. The model compound 2-phenoxyethanol (2PE), a common preservative and bactericide drug, was chosen as a model drug which can be found in many dermatological, cosmetic or pharmaceutical formulations. The bioactive compound release was monitored *in situ* by NMR analysis at different pHs.

Materials and methods

Materials

1-Vinyl-2-pyrrolidone (Sigma-Aldrich) was distilled under reduced pressure before use. Diisopropylamide lithium solution –2.0 M in THF/heptane/ethylbenzene – (Acros Organics), THF anhydrous (Sigma-Aldrich), 1,3-dibromopropane 99% (Sigma-Aldrich), phthalimide potassium salt 98% (Sigma-Aldrich), tetrabutylammonium bromide 99% (Sigma-Aldrich), hydrazine monohydrate 65% (Sigma-Aldrich), acryloyl chloride (Sigma-Aldrich), 2-phenoxyethanol (Sigma-Aldrich) were used as received. The bactericide connected to an acrylate monomer, 2-phenoxyethyl acrylate, was synthesized according to a method reported previously.²⁶

Synthesis of VP derivatives

3-(3-Bromopropyl)-1-vinyl-2-pyrrolidone (VPBr). A freshly distilled solution of VP (10.0 mL, 94 mmol) in anhydrous THF (80 mL) was added dropwise to a commercial solution of lithium diisopropylamide (2.0 M in THF/hexane/ethylbenzene, 80 mL, 160 mmol) in an inert atmosphere at -78°C for 2 h under magnetic stirring. After VP enolate formation (see Fig. 1), 1,3-dibromopropane was added dropwise (94 mmol) in THF (10 mL). This solution was then allowed to warm room temperature and was magnetically stirred for 24 h. At the end of the reaction, monitored by TLC, the solution was hydrolyzed in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (1 : 1, 200 mL). The aqueous layer was extracted with CH_2Cl_2 (2 \times 100 mL), and the organic layers were combined and dried over anhydrous sodium sulfate (Na_2SO_4).

The solvent was evaporated at low pressure. The residue was purified by column chromatography using silica gel as the stationary phase and hexane/ethyl acetate (4 : 1) as the eluent to give a yellow oil as the product. Yield: 30%.

^1H NMR (CDCl_3 , 500 MHz): δ = 7.08 (dd, 1H, $\text{N}-\text{CH}=\text{CH}_2$, J = 16.0 and 9.0 Hz), 4.44 (d, 1H, cis $\text{N}-\text{CH}=\text{CHH}$, J = 9.0 Hz), 4.40 (d, 1H, trans $\text{N}-\text{CH}=\text{CHH}$, J = 16.0 Hz), 3.51 (td, 1H, $\text{CO}-\text{N}-\text{CHH}$, J = 5.0 Hz), 3.47–3.36 (m, 3H, $\text{CO}-\text{N}-\text{CHH}$ and CH_2-Br), 2.53 (qd, 1H, $\text{CH}-\text{CO}$, J = 9.0 and 5.0 Hz), 2.34–2.27 (m, 1H, $\text{CO}-\text{CH}-\text{CHH}$), 2.05–1.91 (m, 3H, $\text{CO}-\text{CH}-\text{CHH}$ and $\text{CH}_2-\text{CH}_2-\text{CH}_2$), 1.79–1.71 (m, 1H, $\text{N}-\text{CH}_2-\text{CHH}$), 1.63–1.54 (m, 1H, $\text{N}-\text{CH}_2-\text{CHH}$). ^{13}C NMR (CDCl_3 , 125 MHz): δ = 174.42, 129.38, 94.36, 42.75, 41.56, 33.26, 30.32, 29.87, 24.44. FTIR (cm^{-1}): 2944, 2881, 1699, 1633, 1426, 1388, 1328, 1268, 981, 851. MS (ESI): calculated m/z 232.033 and 234.031 ($M + 1$)⁺, found m/z 232.032 and 234.030.

3-(3-Phthalimidopropyl)-1-vinyl-2-pyrrolidone (VPPhta). VPBr (8.63 mmol) was dissolved in DMF (10 mL). Phthalimide potassium salt (3.26 g, 17 mmol) was added to the solution, with a catalytic amount of tetrabutylammonium bromide (0.1 g, 0.31 mmol) and magnetically stirred for 40 h at room temperature. The final mixture was then filtered at low pressure and washed with water (3 \times 20 mL) to give a white solid product. Yield: 98%.

^1H NMR (CDCl_3 , 500 MHz): δ = 7.83 (dd, 2H, $\text{C}-\text{H}$, J = 5.5 and 3.0 Hz), 7.70 (dd, 2H, $\text{C}-\text{H}$, J = 5.5 and 3.0 Hz), 7.07 (dd, 1H, $\text{N}-\text{CH}=\text{CH}_2$, J = 16.0 and 9.0 Hz), 4.42 (d, 1H, cis $\text{N}-\text{CH}=\text{CHH}$, J = 9.0 Hz), 4.38 (d, 1H, trans $\text{N}-\text{CH}=\text{CHH}$, J = 16.0 Hz), 3.72 (t, 2H, $\text{CH}_2-\text{N}(\text{CO})_2$, J = 10.0 Hz), 3.49 (td, 1H, $\text{N}-\text{CHH}$, J = 10.0 and 3.0 Hz), 3.37 (dt, 1H, $\text{N}-\text{CHH}$, J = 10.0 and 8.0 Hz), 2.55 (qd, 1H, $\text{CH}-\text{CO}$, J = 9.0 and 5.0 Hz), 2.33–2.27 (m, 1H, $\text{CO}-\text{CH}-\text{CHH}$), 1.96–1.89 (m, 1H, $\text{CHH}-\text{CH}-\text{CH}_2$), 1.82–1.69 (m, 3H, $\text{CH}_2-\text{CH}_2-\text{CH}_2$ and $\text{CO}-\text{CH}-\text{CHH}$), 1.47–1.39 (m, 1H, $\text{CHH}-\text{CH}_2-\text{CH}_2$). ^{13}C NMR (CDCl_3 , 125 MHz): δ = 174.50, 168.33, 133.90, 132.03,

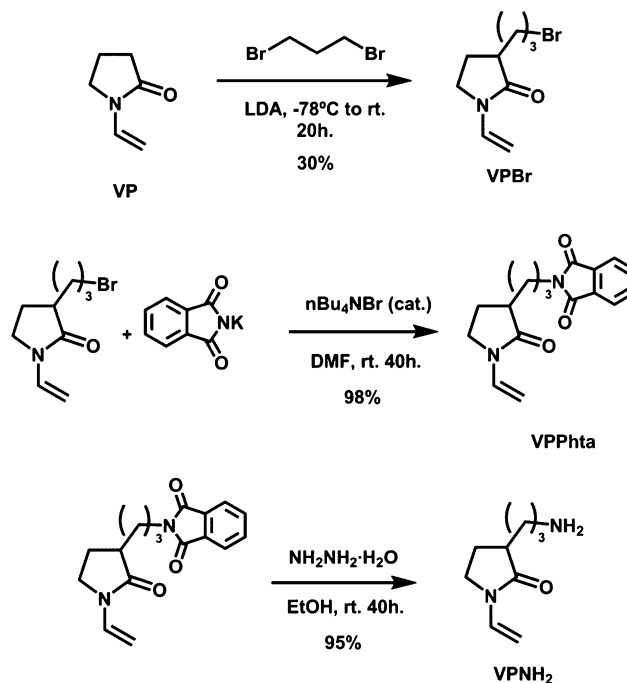


Fig. 1 Procedure to synthesize protected and free aminated VP.

129.42, 123.19, 94.24, 42.75, 41.83, 37.62, 28.31, 26.20, 24.39. FTIR (cm^{-1}): 2943, 2883, 1771, 1698, 1630, 1425, 1394, 1328, 1266, 1051, 982, 851, 718. MS (ESI): calculated m/z 299.139 ($M + 1$)⁺, found m/z 299.139.

3-(3-Aminopropyl)-1-vinyl-2-pyrrolidone (VPNH₂). VPPhta (8.63 mmol) and hydrazine monohydrate ($\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$) (6.40 mL, 130 mmol) were dissolved in EtOH (270 mL), and left to stir at room temperature. The formation of a white solid in the reaction solution indicates that the reaction is proceeding. After 40 hours, the white solid (leaving group) was removed by simple filtration, and the solvent was evaporated at low pressure. 250 mL of diethylether was added to the remainder, upon which more leaving group solid appeared. After filtration, anhydrous MgSO_4 was added and filtered. Solvent was removed at low pressure yielding a pallid yellow semicrystalline liquid. Yield: 95%.

¹H NMR (DMSO-d_6 , 500 MHz): δ = 6.88 (dd, 1H, C-H, 15.0 and 9.0 Hz), 4.45 (d, 1H, trans N-CH=CHH, J = 15.0 Hz), 4.42 (d, 1H, cis N-CH=CHH, J = 9.0 Hz), 3.43 (dt, 1H, N-CHH, J = 12.0 and 3.0 Hz), 3.30 (td, 1H, N-CHH, J = 12.0 and 6.0 Hz), 2.54–2.44 (m, 3H, $\text{CH}_2\text{-NH}_2$ and CH-CO), 2.26–2.19 (m, 1H, CO-CH-CHH), 1.71–1.62 (m, 2H, CO-CH-CHH and CHH-CH₂-CH₂), 1.42–1.24 (m, 3H, $\text{CH}_2\text{-CH}_2\text{-CH}_2$, CHH-CH₂-CH₂). ¹³C NMR (DMSO-d_6 , 125 MHz): δ = 175.74, 129.52, 95.55, 43.22, 42.12, 41.72, 30.50, 28.47, 24.30. FTIR (cm^{-1}): 3341, 2932, 2857, 1684, 1630, 1563, 1470, 1427, 1388, 1326, 1265, 981, 844, 821, 723, 691. MS (ESI): calculated m/z 169.133 ($M + 1$)⁺, found m/z 169.134.

3-(3-((3-Oxo-3-(2-phenoxyethoxy)propyl)amino)propyl)-1-vinyl-2-pyrrolidone (VP-2PE). A solution of VPNH₂ (200.0 mg, 1.19 mmol), 2-phenoxyethyl acrylate (207.0 mg, 1.19 mmol) and AcOH (25 μL) in CHCl_3 (2.5 mL) was stirred at 40 °C for 4 h. The reaction was monitored by TLC. The solvent was evaporated under low pressure and the crude mixture was purified by column chromatography in silica gel, using AcOEt/Hexane/Et₃N (30 : 10 : 2) as the eluent, resulting a white solid. Yield: 90%.

¹H NMR (CDCl_3 , 500 MHz): δ = 7.28 (t, 2H, C-H, J = 7.5), 7.08 (dd, 1H, N-CH=CH₂, J = 16.0 and 9.0 Hz), 6.96 (t, 1H, C-H, J = 7.5), 6.92–8.9 (m, 2H, C-H) 4.45–4.36 (m, 4H, N-CH=CH₂ and COO-CH₂), 4.18–4.16 (m, 2H, COO-CH₂CH₂-O), 3.48 (td, 1H, N-CHH, J = 10.0 and 3.5 Hz), 3.36 (dt, 1H, N-CHH, J = 10.0 and 8.0 Hz), 2.89 (t, 2H, NH-CH₂-CH₂-COO, J = 6.5 Hz), 2.65–2.60 (m, 2H, CH₂CH₂CH₂-NH), 2.58 (t, 2H, CH₂-COO, J = 6.5 Hz), 2.49 (qd, 1H, CH-CO, J = 9.0 and 4.5 Hz), 2.29–2.23 (m, 1H, N-CH₂-CHH), 1.91–1.67 (m, 3H, N-CH₂-CHH, -NH- and CO-CH-CHH), 1.54 (quint, 2H, J = 7.5 Hz), 1.44–1.36 (m, 1H, CO-CH-CHH). ¹³C NMR (CDCl_3 , 125 MHz): δ = 174.90, 172.65, 158.39, 129.48, 121.15, 114.56, 94.14, 65.75, 62.79, 42.45, 44.92, 42.80, 42.20, 34.57, 28.79, 27.56, 24.32. FTIR (cm^{-1}): 2929, 2881, 2825, 1733, 1697, 1630, 1599, 1589, 1494, 1426, 1387, 1327, 1245, 1168, 1120, 1060, 982, 849, 754, 692. MS (ESI): calculated m/z 361.212 ($M + 1$)⁺, found m/z 361.212.

Synthesis of polymers

Poly-VPPhta homopolymer and poly-(VP-co-VPPhta) copolymers. Monomers and initiator (AIBN) were dissolved in 1,4-dioxane at concentrations of 0.6 mol L⁻¹ and 1.5×10^{-2} mol L⁻¹, respectively. Gaseous N₂ was flushed through the polymerizing

solution for 30 min. The polymerization was carried out at 60 °C during 40 h to attain total conversion. The homopolymer and copolymers were isolated and purified by several cycles of precipitation–solution–precipitation in diethylether–chloroform–diethylether.

Poly-VPNH₂ homopolymer and poly-(VP-co-VPNH₂) copolymers. Homo and copolymers with amino groups were obtained by reduction of the corresponding phthalimide containing polymers using hydrazine monohydrate (same amounts as indicated in the monomers synthesis protocol). Products were isolated and purified by dialysis using membranes of cut-off 1000 Da, followed by freeze drying.

Poly-(VP-co-VP-2PE) copolymer. Monomers and initiator (AIBN) were dissolved in 1,4-dioxane at a concentration of 1.0 mol L⁻¹ and 1.5×10^{-2} mol L⁻¹, respectively. Gaseous N₂ was flushed through the polymerizing solution for 30 min. The polymerization was carried out at 60 °C for 40 h to attain total conversion. The copolymer was isolated and purified by precipitation–solution–precipitation cycles using diethylether–chloroform–diethylether.

Methods

¹H and ¹³C NMR spectra were recorded using a VARIAN NMR system (500 and 125 MHz, respectively) in CDCl_3 using tetramethylsilane (TMS) as the internal standard. Gel permeation chromatography (GPC) analyses were carried out with Resipore (250 × 4.6 mm, 3 μm nominal particle size) Polymer Laboratories columns. DMF with 1% LiBr was used as the solvent. Measurements were performed at 70 °C at a flow rate of 0.7 mL min⁻¹ using a RI detector. The molecular weights of the polymers were referenced against polystyrene standards.

Thermal stability of poly-(VP-co-VP-2PE) and PVP were estimated by thermogravimetry analysis registered in a TGA equipment model Q500, using sample weight of 6 mg, temperature range 25 to 500 °C, at 10 °C min⁻¹, under nitrogen atmosphere. Differential scanning calorimetry was carried out in a Perkin-Elmer DSC7 equipment under nitrogen atmosphere, using 12 mg of sample weight with a temperature range of 30–200 °C and a heating rate of 20 °C min⁻¹. Glass transition temperature, T_g , was measured in the second scan and taken as the inflexion point of the transition region of the T_g .

Determination of reactivity ratios. Assuming that the copolymerization is governed by the terminal model,²⁷ the reactivity ratios of the copolymerization of VPPhta and VP in 1,4-dioxane were determined using Copol software.²⁸ The terminal model copolymerization can be used for descriptive purposes although it is well documented that it is only an approximate model.^{29,30} The terminal model considers the reactivity of the growing chain to depend on the nature of the terminal unit, and in the case of binary copolymerizations needs two parameters – namely, the reactivity ratios – to describe the reaction. These reactivity ratios are given by eqn (1) and (2).

$$r_1 = k_{11}/k_{12} \quad (1)$$

$$r_2 = k_{22}/k_{21} \quad (2)$$

where k_{ij} ($i, j = 1, 2$) is the rate constant for the addition of a monomer M_j to a propagating chain end $\sim M_i$. A reactivity ratio value r_1 higher or lower than 1, means that a growing chain end $\sim M_1$ is more reactive towards monomer 1 or 2, respectively.

The Copol software uses the compositional data of copolymers studied over a wide compositional interval with mole fractions in the monomer feed (F_{VPPhta}) from 0.20 to 0.80, as shown in Table 1 of the supporting information, and determines the reactivity ratios according to the general copolymerization equation by application of the non-linear least squares treatment proposed by Tidwell and Mortimer.³¹ The copolymers were prepared in the same conditions as those described in the previous section. The reaction time was adjusted to obtain conversions lower than 5% weight to satisfy the copolymerization equation (approximately 2 hours). Residual monomers were eliminated by dialysis using membranes of cut-off 1000 Da and 1,4-dioxane as solvent. Finally, polymers were obtained by precipitation in diethylether.

The experimental molar fractions of VPPhta in copolymer (f_{VPPhta}) were obtained by ¹H-NMR in CDCl₃. These f_{VPPhta} were estimated comparing the area of the aromatic band (8.0–7.5 ppm) with that of the group of signals between 3.4–2.9 ppm (corresponding to the hydrogens next to the nitrogen in the lactam cycle) using these equations:

$$A_{8.0-7.5} = 4H_{\text{VPPhta}} \quad (3)$$

$$A_{3.4-2.9} = 2H_{\text{VP}} + 2H_{\text{VPPhta}} \quad (4)$$

Drug release. The hydrolysis assays were carried out in standard phosphate buffered saline solutions (pH 5, 7 and 9) and 37 °C, and were monitored *in situ* by dynamic NMR ARRAY. Deuterated solutions were prepared removing H₂O by freeze-drying and then adding the same volume of D₂O. These experiments were carried out using a Varian 400 NMR facility. To perform quantitative experiments, the following conditions were used: a pulse sequence of 7 μs equivalent to a 90° tip angle and a 120 s delay time were applied in order to allow for the total relaxation of the protons and to process the individual data. The spinning rate of the samples was 7 Hz. The sample temperature was maintained at 37 °C using the heat controller of the NMR equipment. The experimental procedure is described in more detail elsewhere.³² The isolated signals at 4.30 and 4.16 ppm, which were assigned to a $-\text{CH}_2-$ of the polymer conjugate and

free 2PE respectively, were used to determine the hydrolysis ratio by comparing their areas, according to these equations:

$$A_{4.30} = 2H_{\text{conjugate}} \quad (5)$$

$$A_{4.16} = 2H_{2\text{PE}} \quad (6)$$

Therefore the release percentage R is given by

$$R = \frac{H_{2\text{PE}}}{H_{\text{conjugate}} + H_{2\text{PE}}} \times 100 \quad (7)$$

Results and discussion

Fig. 1 shows the synthetic route used in this work to prepare VP functionalized with aliphatic amines. The first step is the preparation of VP functionalized with bromide, this was obtained by the reaction of VP in its 3-position with alkyl dibromides after forming the enolate of the carboxamide with a strong base as lithium diisopropylamide-LDA- at -78 °C. Although similar reactions have been previously described in the literature with alkyl monohalides to prepare mono and dialkyl derivatives in the 3-position with relatively low conversion,^{19,33,34} to the best of our knowledge this is the first time that a VP-alkyl-Br has been prepared. The unwanted dialkylation of the lactam moiety ($\text{VP}(\text{RBr})_2$) or di-substitution of dibromide (VP-R-VP) compounds might also occur, however under these experimental conditions, the formation of these by-products is not favored and they were not detected. Under these conditions, conversions close to 30% were obtained.

Despite the low conversion of this first step, VPBr derivative was converted with very high conversions, under gentle conditions, into protected amines (VPPhta, protected with a phthalimide group) by a simple reaction with phthalimide potassium salt. These protected forms could be easily transformed, also with very high conversion, into the free amines (VPNH_2) by reduction with hydrazine.

To the best of our knowledge, VP has not been functionalized previously with an aliphatic amine. We have previously described the derivatization of VP with much less activated primary and secondary aromatic amines.³⁵ Aliphatic amines such as those described for the first time in this work are certainly highly reactive functional groups with many chemical possibilities to link different active compounds.

VPPhta did homopolymerize and copolymerize with VP in 1,4-dioxane, and the obtained polymers could be deprotected to the free amine species by reduction according to the Scheme shown in Fig. 2 and the ¹H-NMR spectra of Fig. 3 (A-protected polymers, B-deprotected forms).

Four copolymers poly-(VP-co- VPNH_2) with F_{VPPhta} from 0.20 to 0.80 as indicated in Table 1, as well as the homopolymer poly-(VPPhta), have been prepared at high conversion. Spectra of Fig. 3A show signals corresponding to poly-(VP-co-VPPhta) copolymers. The signals assigned to the VPPhta units, such as the aromatics bands at 8.0–7.5 ppm and the $-\text{CH}_2\text{Phta}$ band at 3.8–3.5 ppm, increase when increasing the F_{VPPhta} . Fig. 3B shows how aromatic bands disappeared when the reduction

Table 1 Feed (F) and copolymer (f) molar fractions of VPPhta in the polymerizations carried out at high conversion, and GPC data of the obtained copolymers

F_{VPPhta}	f_{VPPhta}	Mn (Da)	PDI
0.20	0.17	15 300	1.6
0.40	0.37	17 100	1.6
0.60	0.60	20 000	1.8
0.80	0.75	18 300	1.6
1.00	1.00	17 700	1.6

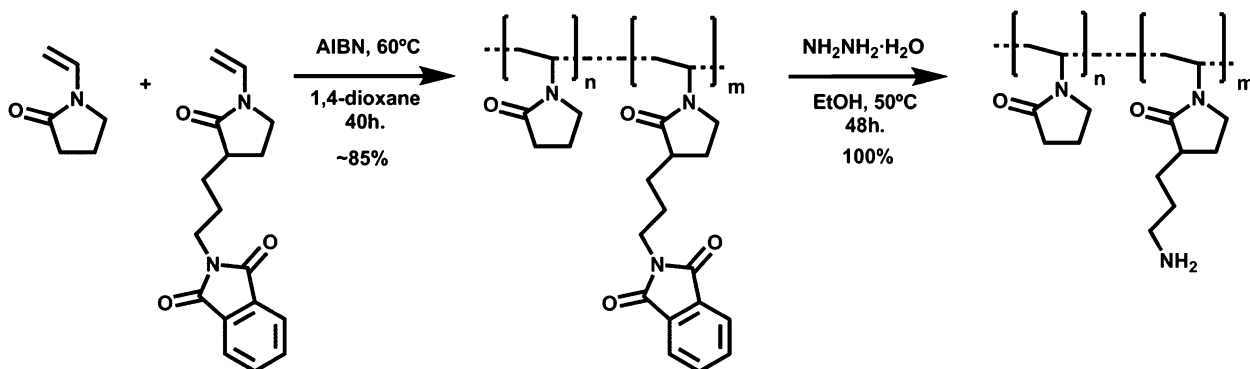


Fig. 2 Synthesis of poly (VP-co-VPNH₂) copolymers and poly VPNH₂ homopolymer.

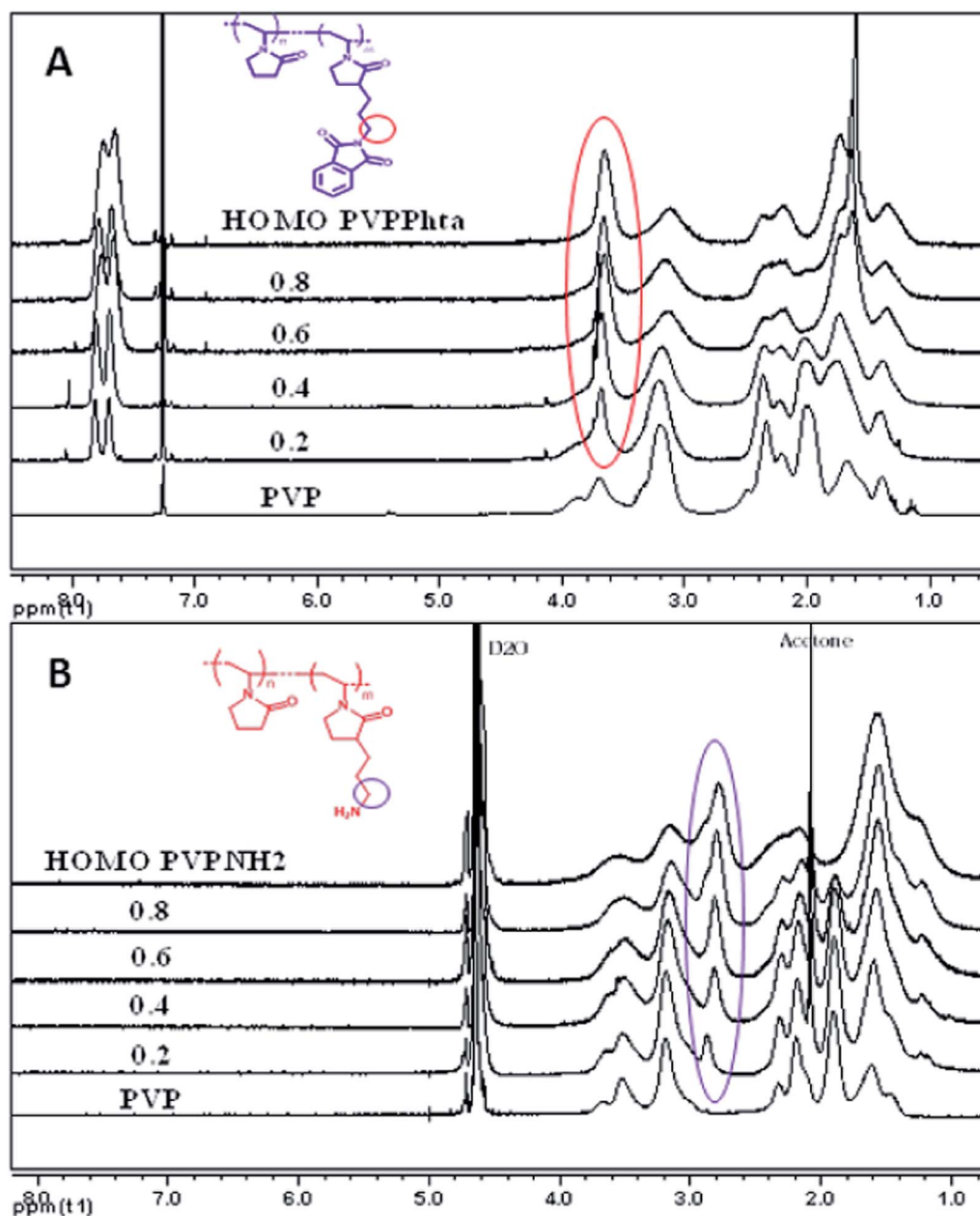


Fig. 3 (A) ¹H-NMR spectra of poly (VP-co-VPPha) copolymers and homopolymers in CDCl₃ and (B) poly (VP-co-VPNH₂) copolymers and homopolymers in D₂O. PVP spectra have been included for comparative purposes.

was complete in each polymer, as well as a displacement of the 3.8–3.5 ppm band ($-\text{CH}_2\text{Phta}$) to 2.9–2.6 ppm ($-\text{CH}_2\text{NH}_2$).

VPNH_2 did not polymerize by conventional radical reaction either in organic solvents (1,4-dioxane and DMF) or in water. A primary aliphatic amine group is actually an active group that may suffer from different secondary reactions in radical polymerization, such as Michael additions or transamidation in the case of (meth)acrylics, or it may react with radicals, thus preventing polymerization. In order to avoid some of these problems, commercial methacrylic monomers bearing amine groups are usually provided as hydrochloride salt.

The copolymer compositions of polymerizations carried out at high conversion determined as indicated in experimental section are very similar to the feed values indicating that both monomers are properly incorporated into the macromolecular chains (Table 1). Their GPC analysis has shown some relatively low M_n ranging between 15 000 and 20 000 Da.

The values of the reactivity ratios, $r_{\text{VPPha}} = 0.44$ and $r_{\text{VP}} = 0.98$, determined as indicated in the experimental section from the compositional values of reactions carried out at conversion lower than 5% weight (Table 1 of ESI†), indicate that growing radicals are slightly more reactive towards VP. In any case, their values are not far from unity which is in agreement with the structural homology of both polymerizable double bonds, leading randomly distribution along the chains. Fig. 1 of ESI† shows how the compositional diagram obtained using $r_{\text{VPPha}} = 0.44$ and $r_{\text{VP}} = 0.98$ adjust to the experimental compositional data.

Focusing on the usefulness of VPNH_2 in the *bottom up* approach for obtaining PVP–drug conjugates by (1) preparing the precursor VP–drug and (2) copolymerizing it with VP, in this work we have designed a route to couple the amine to a hydroxyl group containing-drug through a hydrolysable spacer as indicated in Fig. 4.

This synthetic design proposes the transformation of the hydroxyl group-containing drug to an acrylic ester, which is susceptible to undergoing Michael addition with the aliphatic amine, leading to a β -amino ester (more labile than others aliphatic esters). Alkyl acrylates are known to be good Michael acceptors and easily accept weak nucleophiles such as amines.³⁶ This reaction forms an ester with a much higher sensitivity towards hydrolysis than is normally shown by other aliphatic

esters. This has been reported to be related to the position of the amine in the β position.³⁷

This synthetic approach has been put into practice by selecting VPNH_2 and 2-phenoxyethanol (2PE) as the hydroxyl group-containing model drug. The VP-2PE conjugate formed is depicted in Fig. 5.

This synthetic route uses high conversion couplings. In fact, the acylation reaches high yields and the Michael addition is nearly quantitative. Carrying out a global synthetic analysis for the preparation of the VP-2PE conjugate, the limiting reaction in terms of conversion is the first step of functionalization of VP with Br since the other steps are almost quantitative.

VP-2PE has exhibited a good copolymerizability with pure VP. A copolymer labeled poly-VP-2PE₂₀, with a nominal VP : VP-2PE ratio of 4 : 1 (20% mol of VP-2PE in the polymer) was prepared by conventional radical polymerization.

The relative molecular weight of the copolymer was determined by GPC *versus* polystyrene standards. Values of number average molecular mass (M_n) of 38 400 Da and PDI of 2.0 were obtained.

The $^1\text{H-NMR}$ spectrum of poly-VP-2PE₂₀ in Fig. 6 shows broad peaks that are typical for polymeric species. In addition, it shows peaks characteristic of the VP-2PE unit, such as the aromatic one or the CH_2 signals at 6.82, 4.45 and 4.18 ppm respectively. Comparison of the area of the isolated peak centered at 4.45 ppm with that of the group of signals between 3.4 and 2.9 ppm (assigned previously) was used to determine the experimental copolymer composition according to these equations:

$$A_{4.45} = 2H_{\text{VP-2PE}} \quad (8)$$

$$A_{3.4-2.9} = 2H_{\text{VP}} + 2H_{\text{VP-2PE}} \quad (9)$$

A copolymer composition of $f_{\text{VP-2PE}} = 0.17$ was obtained, which is very close to the monomer feed ratio ($F_{\text{VP-2PE}} = 0.20$), which indicates that both units are incorporated properly along the copolymer chains. The similar reactivity of VP and VP-2PE is to be expected for this copolymerization due to the electronic and structural similarity of the polymerizable vinyl groups.

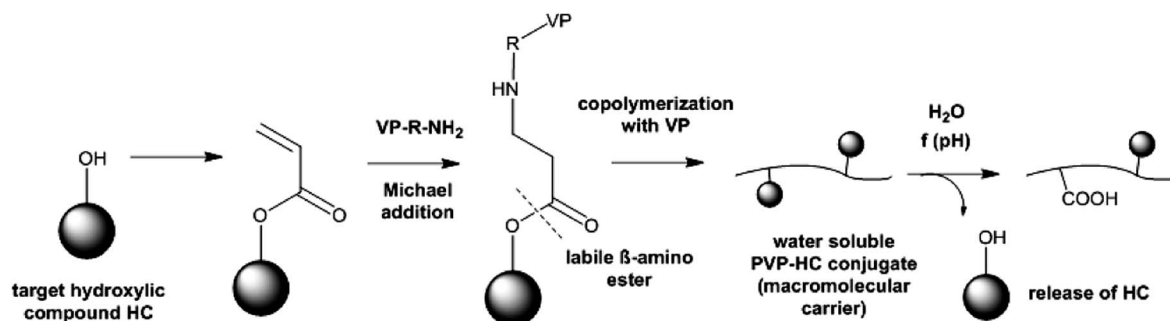


Fig. 4 Synthetic route designed in this work to prepare hydrolysable conjugates of PVP with hydroxyl group-containing drugs from the aminated VP.

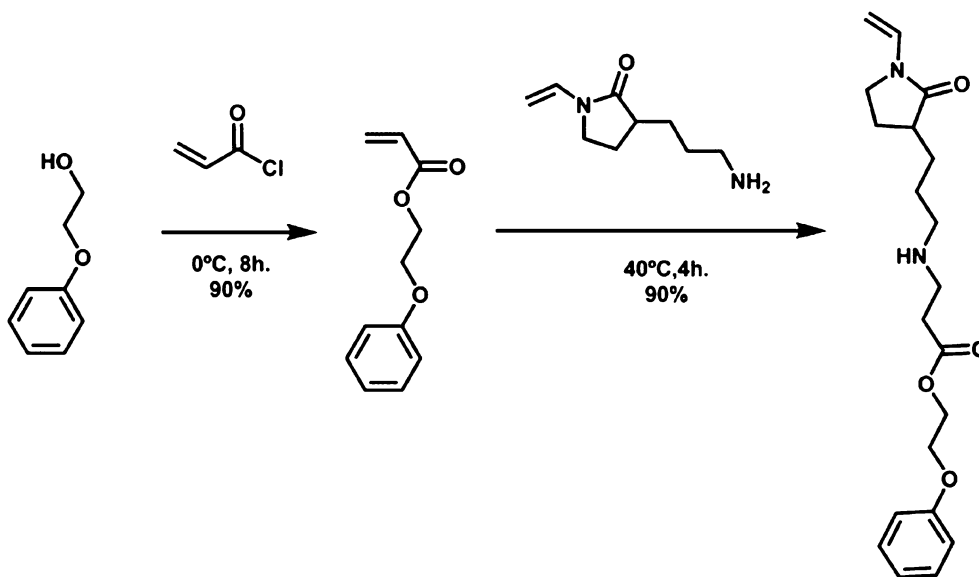


Fig. 5 Synthetic scheme of the VP-2PE conjugate.

TGA and DCS analysis of the copolymer poly VP-2PE₂₀ have confirmed the incorporation of VP-2PE (see Fig. 2 and 3 in Supporting Information). On one hand the TGA analysis has

shown a 15% weight loss in the interval 270–310 °C not present in the thermogram of control PVP, which has been assigned to the loss of 2PE following the ester hydrolysis. The 15 weight

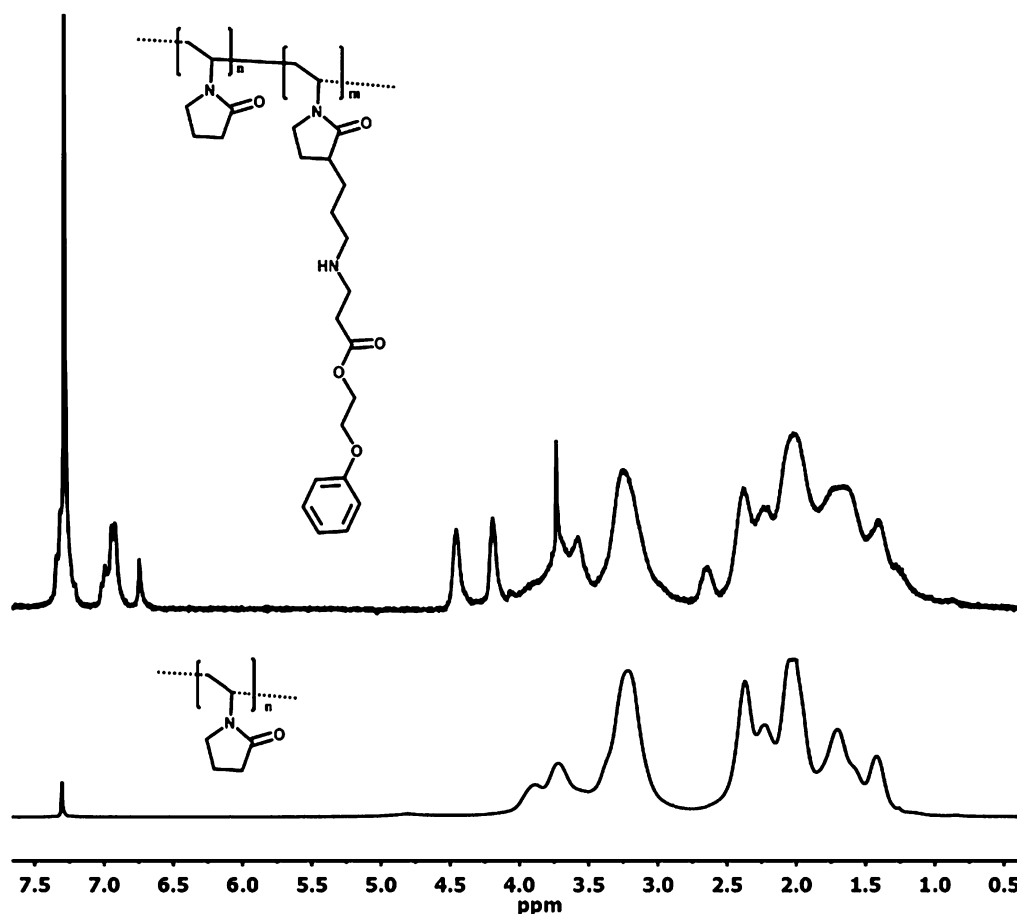


Fig. 6 ¹H-NMR spectrum of the conjugate Poly VP-2PE₂₀, recorded in CDCl₃. The spectrum of PVP has been included for comparative purposes.

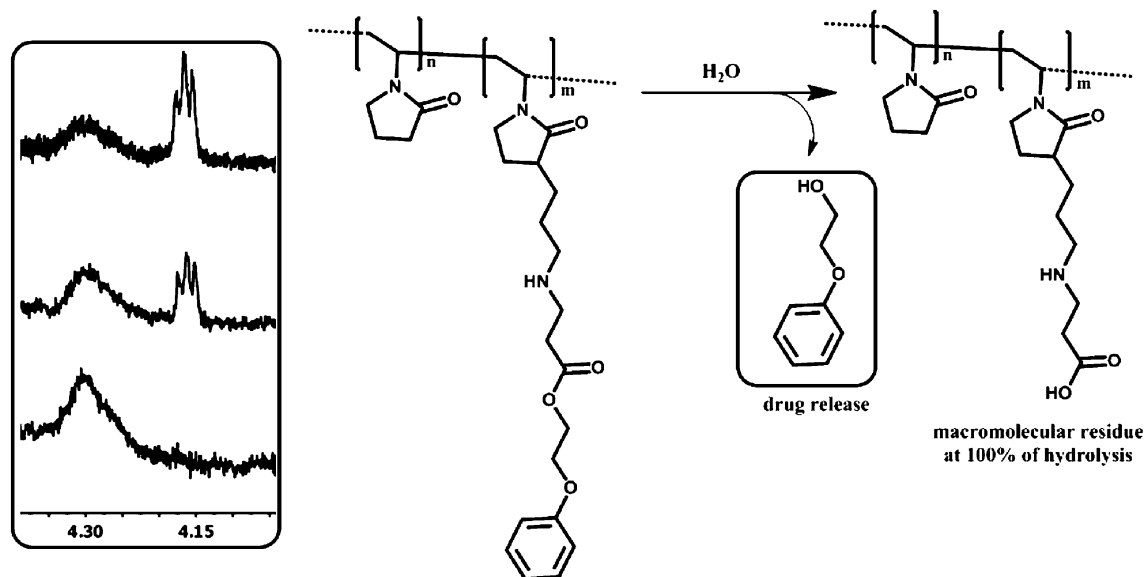


Fig. 7 Scheme of the hydrolytic process and release of 2PE (right). Details of the ¹H-NMR regions used to determine the release rate for three spectra recorded at 0, 8 and 24 hours at pH 9 (left).

percent is in agreement with this loss for the composition determined from NMR, $f_{VP-2PE} = 0.17$ (Fig. 2 and 3 of ESI†). This result confirms the lability of the ester. On the other hand DSC analysis has shown a T_g at 78 °C, much lower than control PVP (T_g of 170 °C). This is in agreement with the presence of VP-2PE, which bears a side chain with two flexible spacers between the pyrrolidone ring, the ester and the aromatic ring. The hydrolytic behavior of the poly VP-2PE₂₀ conjugate has been studied by proton NMR *in situ* at 37 °C using different buffer solutions in D₂O. Fig. 7 (right) schematically shows the hydrolytic process. A VP unit bearing a side β-amino carboxylic acid is formed as a residue after the hydrolysis and the 2PE release.

The degradation could be monitored *in situ* by analyzing the region between 4.5 and 4.0 ppm (see Fig. 7 left). In this region, the hydrolysis produces the reduction of a broad polymeric $-CH_2-$ signal centered at 4.30 ppm, while a resolved triplet of

another non-polymeric $-CH_2-$ peak at 4.16 ppm shows a correlative increase. These signals, assigned to a $-CH_2-$ group of the VP-2PE unit and to free 2PE respectively, have been used to determine the release rate, which is depicted in Fig. 8 at different pHs. A more detailed explanation of this calculation can be found in the Experimental section.

As expected, there is a clear dependency between the rate of hydrolysis and the pH of the medium. The half-life times of the kinetics change from hours at pH 9 to days at pH 7, and months at pH 5, due to the well-known sensitivity of the ester group to basic media and to the higher ester activation by the β-amino group in its neutral state.

Conclusions

A versatile synthetic route to functionalize VP with protected and deprotected primary aliphatic amine groups has been described for the first time. This unprecedented functionalization may open new goals to obtain – on a pure PVP backbone – functional foods, surfactants or therapeutic nanocarriers, owing to the multiple applications of PVP. This monomer with an aliphatic amine group itself is of high interest as it leads to PVP functionalized with amines useful for different applications. Also, we are currently evaluating these synthesized polymers of poly (VPNH₂) homopolymer and poly (VP-co-VPNH₂) as gene vectors, obtaining promising results.

The preparation of a VP-drug conjugate by Michael addition using this aminated monomer was performed as an example of the many coupling possibilities afforded by the primary amine. PVP-drug conjugates were synthesized by coupling 2-phenoxyethanol through a hydrolysable spacer. The synthetic design of this coupling may be extended to other hydroxyl containing drugs. The poly VP-2PE₂₀ conjugate allows for a pH modulated drug release, increasing the hydrolysability with pH. As PVP is

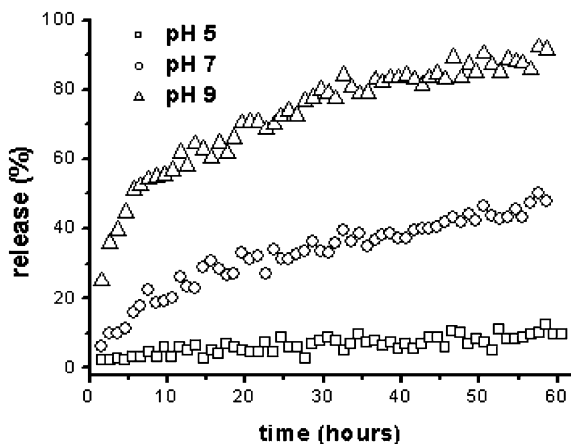


Fig. 8 Release percentage, R , versus time at different pHs obtained by *in situ* ¹H-NMR monitoring as explained in the text.

an approved food and drink additive, this dependence with the pH may be used to prepare some PVP conjugates for oral delivery to be administered in drinks or food.

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References

- 1 Y. Xiong, I. Washio, J. Chen, H. Cai, Z.-Y. Li and Y. Xia, *Langmuir*, 2006, **22**, 8563.
- 2 D. Wang, V. L. Dimonie, E. D. Sudol and M. S. El-Aasser, *J. Appl. Polym. Sci.*, 2002, **84**, 2721.
- 3 www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.55, March, 2012.
- 4 Z. Wu, H. Chen, X. Liu, Y. Zhang, D. Li and H. Huang, *Langmuir*, 2009, **25**, 2900.
- 5 M. L. Nucci, R. Shorr and A. Abuchowski, *Adv. Drug Delivery Rev.*, 1991, **6**, 133.
- 6 Y. Kaneda, Y. Tsutsumi, Y. Yoshioka, H. Kamada, Y. Yamamoto, H. Kodaira, S. Tsunoda, T. Okamoto, Y. Mukai and H. Shibata, *Biomaterials*, 2004, **25**, 3259.
- 7 Y. Matsumura and H. Maeda, *Cancer Res.*, 1986, **46**, 6387.
- 8 G. Pasut and F. M. Veronese, *Adv. Drug Delivery Rev.*, 2009, **61**, 1177.
- 9 R. Duncan, *Adv. Drug Delivery Rev.*, 2009, **61**, 1131.
- 10 B. U. Specht and W. Brendel, *Biochim. Biophys. Acta, Enzymol.*, 1977, **484**, 109.
- 11 W. Albrecht, J. Schauer, T. Weigel, K. Richau, T. Groth and A. Lendlein, *J. Membr. Sci.*, 2007, **291**, 10.
- 12 A. J. M. D'Souza, R. L. Schowen and E. M. Topp, *J. Controlled Release*, 2004, **94**, 91.
- 13 R. Devarajan, V. Arunachalam, M. Kumaraswamy, I. Tajuddin and T. Joghee, *J. Appl. Polym. Sci.*, 1992, **44**, 1473.
- 14 G. Pound, J. M. McKenzie, R. F. Lange and B. Klumperman, *Chem. Commun.*, 2008, 3193.
- 15 C. Baldoli, C. Oldani, S. Maiorana, P. Ferruti, E. Ranucci, M. Bencini and A. Contini, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 1683.
- 16 Y. Yamamoto, Y. Tsutsumi, Y. Yoshioka, H. Kamada, K. Sato-Kamada, T. Okamoto, Y. Mukai, H. Shibata, S. Nakagawa and T. Mayumi, *J. Controlled Release*, 2004, **95**, 229.
- 17 Y. Abe, H. Shibata, H. Kamada, S. I. Tsunoda, Y. Tsutsumi and S. Nakagawa, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 251.
- 18 S. Kim, S. Wan Kim and Y. H. Bae, *Biomaterials*, 2005, **26**, 3597.
- 19 J. U. Engström, L. J. Lindgren and B. Helgee, *Macromol. Chem. Phys.*, 2007, **207**, 536.
- 20 M. G. Tardajos, M. Nash, Y. Rochev, H. Reinecke, C. Elvira and A. Gallardo, *Macromol. Chem. Phys.*, 2012, **213**, 529.
- 21 N. Larson and H. Ghandehari, *Chem. Mater.*, 2012, **24**, 840.
- 22 R. Duncan, *Adv. Drug Delivery Rev.*, 2009, **61**, 1131.
- 23 S. Hong, P. R. Leroueil, E. K. Janus, J. L. Peters, M.-M. Kober, M. T. Islam, B. G. Orr, J. R. Baker Jr and M. M. Banaszak-Holl, *Bioconjugate Chem.*, 2006, **17**, 728.
- 24 M. Prasitsilp, R. Jenwithisuk, K. Kongsuwan, N. Damrongchai and P. Watts, *J. Mater. Sci.: Mater. Med.*, 2000, **11**, 773.
- 25 D. G. Vanderlaan and S. B. Orr, U.S. Pat. 5.514.732, Johnson and Johnson Vision Products, May, 1996.
- 26 Y. Iwasawa, E. Katakura, Y. Ochiai, M. Ogawa, S. Okamoto, K. Ozawa and H. Takahashi, Pat. US 4036984 A, 1977.
- 27 F. R. Mayo and F. M. Lewis, *J. Am. Chem. Soc.*, 1944, **66**, 1594.
- 28 A. Gallardo, M. R. Aguilar, G. A. Abraham and J. San Román, *J. Chem. Educ.*, 2004, **81**, 1210.
- 29 T. Fukuda, A. Goto, Y. Kwak, C. Yoshikawa and Y. D. Ma, *Macromol. Symp.*, 2002, **182**, 53.
- 30 M. Coote and T. P. Davis, *Prog. Polym. Sci.*, 1999, **24**, 1217.
- 31 P. W. Tidwell and G. A. Mortimer, *J. Polym. Sci., Part A: Gen. Pap.*, 1965, **3**, 369.
- 32 M. R. Aguilar, A. Gallardo, M. d. M. Fernández and J. S. Román, *Macromolecules*, 2002, **35**, 2036.
- 33 L. White, S. Jönson, C. Hoyle and L. J. Mathias, *Polymer*, 1999, **40**, 6597.
- 34 J. U. Engström and B. Helgee, *J. Comb. Chem.*, 2006, **8**, 355.
- 35 M. Perez-Perrino, R. Navarro, M. Gomez-Tardajos, A. Gallardo and H. Reinecke, *Eur. Polym. J.*, 2010, **46**, 1557.
- 36 B. D. Mather, K. Viswanathan, K. M. Miller and T. E. Long, *Prog. Polym. Sci.*, 2006, **31**, 487.
- 37 Y. B. Lim, Y. H. Choi and J. S. Park, *J. Am. Chem. Soc.*, 1999, **121**, 5633.