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Abstract
Poly(N-vinylcaprolactam) (PNVCL) and poly(N-vinylpyrrolidone) (PNVP) are water soluble polymers of interest especially in the biomedical field. Moreover, PNVCL is characterized by a lower critical solution temperature close to 36 °C in water, which makes it useful for the design of thermoresponsive systems. In this context, we used the cobalt-mediated radical polymerization (CMRP) and reaction coupling (CMRC) for synthesizing a series of well-defined NVCL and NVP-based copolymers, including statistical copolymers as well as double thermoresponsive diblocks and triblocks. Dynamic light scattering and turbidimetry analyses highlighted the crucial impact of the copolymer composition and architecture on the cloud point temperature (TCP) of each segment and also their influence on the multistep assembly behaviour of block copolymers. Addition of NaCl enabled us to adjust the inter-TCP range of the di- and triblock in which selective precipitation of one block and self-assembly of the cop...
Double thermo-responsive di- and triblock copolymers based on N-vinylcaprolactam and N-vinylpyrrolidone: synthesis and comparative study of solution behaviour†

Anthony Kermagoret, a Kevin Mathieu, a Jean-Michel Thomassin, a Charles-André Fustin, b Roland Duchêne, b Christine Jérôme, a Christophe Detrembleur a and Antoine Debuigne *a

Poly(N-vinylcaprolactam) (PNVCL) and poly(N-vinylpyrrolidone) (PNVP) are water soluble polymers of interest especially in the biomedical field. Moreover, PNVCL is characterized by a lower critical solution temperature close to 36 °C in water, which makes it useful for the design of thermo-responsive systems. In this context, we used the cobalt-mediated radical polymerization (CMRP) and reaction coupling (CMRC) for synthesizing a series of well-defined NVCL and NVP-based copolymers, including statistical copolymers as well as double thermo-responsive diblocks and triblocks. Dynamic light scattering and turbidimetry analyses highlighted the crucial impact of the copolymer composition and architecture on the cloud point temperature (T CP) of each segment and also their influence on the multistep assembly behaviour of block copolymers. Addition of NaCl enabled us to adjust the inter-T CP range of the di- and triblock in which selective precipitation of one block and self-assembly of the copolymer were favoured. Overall, data presented here provide a basis for the synthesis of a broad range of NVCL/NVP based copolymer architectures with a tunable thermal response in water.

Introduction

Development of stimuli-responsive polymers, whose properties and solubility can be triggered by small changes in their environment (temperature, pH, electric or magnetic field, light, etc.), has become an important field of research in the last few years.1–3 These materials have attracted great interest because they sustain a wide variety of applications notably in the biomedical area as bio-sensors, drug or gene delivery carriers or supports for tissue engineering, to name just a few.4–6

Temperature is very popular to regulate the solubility of polymers7–11 because no additive is required, the hydration/dehydration cycle can be repeated endlessly, at least under non-degradative conditions for the polymer chain, and it is easily controllable. Some thermo-responsive polymers are only soluble in water upon heating above a given temperature called the upper critical solution temperature (UCST) while others precipitate when heated above a given temperature called the lower critical solution temperature (LCST).12 Several common polymers exhibit a LCST behaviour in water including poly-oligoethylene glycol(meth)acrylate)13,14 poly(propylene oxide),15,16 poly(vinyl ether)17,18 or poly(2-oxazoline)19,20 but the most studied sequence remains poly(N-isopropylacrylamide) (PNIPAM), which undergoes a coil-to-globule transition at 32 °C.9,21,22 Nevertheless, poly(N-vinylcaprolactam) (PNVCL),21–27 whose LCST (~36 °C) is close to body temperature, has proven to be a valuable alternative to PNIPAM especially for biomedical applications.23,24 In contrast to PNIPAM, PNVCL does not produce toxic low-molecular-weight amines during hydrolysis and offers a higher biocompatibility as proved by cytotoxicity assays.23,24 Moreover, PNVCL does not exhibit a hysteresis phenomenon in the coil-to-globule-to-coil transition in contrast to PNIPAM which forms interchain hydrogen bonds in the collapsed state.21

The solution behaviour dependence of PNVCL as a function of temperature has been thoroughly studied25–29 The LCST of this polymer is around 36 °C but it varies with the molar mass and the end-groups of the polymer.25,30–32 In this respect, con-
trolled radical polymerization enables not only tuning of the molar mass of the PNVCL but also design of statistical copolymers with precise NVCL content \(^{33,34}\) and copolymers containing one or more PNVCL segments. \(^{34-43}\) Some CRP techniques have distinguished themselves for the controlled polymerization of \(N\)-vinyl amides and NVCL in particular, \(^{44}\) i.e. the reversible addition fragmentation chain transfer (RAFT/MADIX), \(^{30-33,35-38}\) atom transfer radical polymerization \(^{42,43,45,46}\) and the organometallic-mediated radical polymerization (OMRP) \(^{47,48}\) based on cobalt complexes \(^{34,39-41,49}\). The latter is based on the reversible deactivation of the growing radical chains by the metal complex. In particular, bis-acetylacetonatocobalt(II) (Co(acac)\(_2\)) is a very efficient controlling agent for the polymerization of non-conjugated \(N\)-vinylamides including NVCL (Scheme 1). \(^{49}\) Narrowly distributed PNVCL containing block copolymers with thermally induced self-assembly behaviour, like poly(vinyl acetate)-b-PNVCL and poly(vinyl alcohol)-b-PNVCL, were also prepared by OMRP. \(^{39-41}\) The controlled statistical radical copolymerizations of NVCL with \(N\)-methyl vinyl acetamide (NMVA) or vinyl acetate (VAc) were also achieved by OMRP and the LCST of these copolymers could be precisely tuned up and down by incorporation of precise amounts of vinyl amide or vinyl ester comonomers, respectively. \(^{34}\) After that, a well-defined NVCL-based diblock having two LCSTs could also be prepared by sequential OMRP of NVCL followed by the controlled copolymerization of NMVA with the residual amount of NVCL. \(^{34}\) Although examples of double thermoresponsive diblocks \(^{9,20,34,50}\) and single thermoresponsive triblocks \(^{15,51-55}\) are rather common, only a handful of double thermoresponsive triblocks have been reported \(^{56-59}\) and none of them based on NVCL.

Herein, we report the first synthesis of NVCL-based symmetrical ABA triblock copolymers having two discrete LCSTs. The latter are composed of PNVCL external blocks and a central block made of NVCL and \(N\)-vinyl pyrrolidone (NVP), i.e. PNVCL-b-\(P\)(NVCL-co-NVP)-b-PNVCL (Scheme 2). Briefly, the triblocks were obtained by isoprene-assisted bimolecular radical coupling \(^{60-62}\) of the corresponding double thermo-responsive PNVCL-b-P(NVCL-co-NVP) diblocks formed by OMRP. A series of di- and triblocks with different block lengths were prepared in order to evaluate the relationship between the macromolecular features of the copolymer on the LCSTs and the self-assembly behaviour in water. A great advantage of the radical coupling approach is the fact that triblocks have exactly the same block compositions as the parent diblocks from which they were generated and also the same length for the PNVCL blocks, which allows a direct comparative study of their thermal response.

### Experimental section

**Materials**

\(N\)-Vinylpyrrolidone (NVP, >99%, Aldrich) and isoprene (99%, Aldrich) were dried over calcium hydride, degassed by two freeze-thawing cycles before distillation under reduced pressure and stored under argon. High purity \(N,\!N\)-dimethylformamide (DMF, >99%, Acros) and dichloromethane (CH\(_2\)Cl\(_2\), 99.98%, Acros) are degassed by bubbling. Bis-[acetylacetonato]cobalt(II) (Co(acac)\(_2\)) (>98%, Acros) was stored under argon and used as received. 2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO, 98%, Aldrich) and \(N\)-vinylcaprolactam (NVCL, 98%, Aldrich) were used as received. 2,2’-Azobis(4-methoxy-2,4-dimethyl valeronitrile) (V-70, 96%, Wako) was stored at \(-20\) °C and used as received. The alkyldcobalt(II) adduct initiator ([(Co(acac)\(_2\))\(_2\)–(CH(OAc)–CH\(_2\))\(_2\)–R\(_0\)])\(_0\), R\(_0\) being the primary radical generated by 2,2’-azo-bis(4-methoxy-2,4-dimethyl valeronitrile) (V-70, Wako) was prepared as described previously \(^{63}\) and stored in a CH\(_2\)Cl\(_2\) solution at \(-20\) °C under argon.

**Characterization**

The molar masses (\(M_n\)) and molar mass dispersity (\(D\)) of the P(NVCL-stat-NVP) random copolymers were determined by size-exclusion chromatography (SEC) in dimethylformamide (DMF) containing LiBr (0.025 M) relative to polystyrene (PS) standards at 55 °C (flow rate: 1 mL min\(^{-1}\)) with a Waters 600 liquid chromatograph equipped with a 410 refractive index

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**Scheme 1** OMRP mechanism for \(N\)-vinylamides including NVCL and NVP.

**Scheme 2** General strategy for the synthesis of double thermoresponsive block copolymers by OMRP and radical coupling reaction.
dried under reduced pressure and purified by dialysis overnight in deionized water using a Spectra/Por®1 membrane (6-8 kDa, 8 mL cm⁻¹). The final [NVCL-stat-NVP] copolymer was collected as a white powder after lyophilization of the aqueous solution. The molecular parameters of the copolymer were measured by SEC in DMF using PS as a calibration ($M_n$ SEC = 28 900 g mol⁻¹, $D = 1.05$). The composition of the copolymer previously dissolved in D₂O and dried at 60 °C overnight was determined by ¹H NMR (400 MHz) in CDCl₃ (NVCL/NVP molar ratio in the copolymer = 82/18) (see Fig. S1†).

Similar experiments were carried out with various NVCL/NVP molar ratios (Table 1). The molecular parameters of the copolymers were measured by SEC in DMF using PS as a calibration: NVCL/NVP molar ratio in the copolymer = 73/27, $M_n$ SEC = 24 900 g mol⁻¹, $D = 1.06$; NVCL/NVP molar ratio in the copolymer = 58/42, $M_n$ SEC = 22 000 g mol⁻¹, $D = 1.10$; NVCL/NVP molar ratio in the copolymer = 43/57, $M_n$ SEC = 40 400 g mol⁻¹, $D = 1.12$; NVCL/NVP molar ratio in the copolymer = 28/72, $M_n$ SEC = 29 300 g mol⁻¹, $D = 1.24$; data are presented in Table 1.

**Synthesis of the PNVL-block-P(NVCL-stat-NVP) diblock copolymer C₁₁₃(C₄₀P₁₇₂).** A bent 50 mL flask containing NVCL (15.0 g, 107.7 mmol) was connected to a 100 mL Schlenk flask equipped with a magnetic bar and degassed by three vacuum-argon cycles. The NVCL was melted at 40 °C under vacuum to eliminate oxygen. A solution of alkyl-cobalt(III) initiator ([Co(acac)₂(CH(OAc)-CH₂)₄R₀] in CH₂Cl₂) was introduced under argon into the 100 mL Schlenk flask (1.0 mL of a 0.1 M stock solution, 0.1 mmol) and the dichloromethane was evaporated to dryness under reduced pressure at room temperature. The melted NVCL at 40 °C was then transferred onto the CMRP initiator before addition of distilled NVP (0.77 mL, 0.81 g, 7.3 mmol) under argon (NVP/NVL molar feed ratio = 10/90, $M_{nth}$ 100% = 100 000 g mol⁻¹). The reaction mixture was stirred at 40 °C and the monomer conversion was monitored by ¹H NMR spectroscopy in CDCl₃. After 5.5 h, the monomer conversion reached 24% for NVCL and 17% for NVP. A volume of 1.5 mL of a degassed solution of TEMPO in DMF (1.5 mL of 0.32 M stock solution, 0.48 mmol) was injected into the medium in order to stop the polymerization and eliminate the cobalt complex from the copolymer chain-end, according to a previous report.⁵⁴ The mixture was dissolved in approximately 20 mL of DMF and the polymer was recovered by precipitation in diethylether (Et₂O, 200 mL). The polymer was then filtered, dried under reduced pressure and purified by dialysis overnight in deionized water using a Spectra/Por®1 membrane (6-8 kDa, 8 mL cm⁻¹). The final P[NVCL-stat-NVP] copolymer was collected as a white powder after lyophilization of the aqueous solution. The molecular parameters of the copolymer were measured by SEC in DMF using PS as a calibration ($M_n$ SEC = 28 900 g mol⁻¹, $D = 1.05$). The composition of the copolymer previously dissolved in D₂O and dried at 60 °C overnight was determined by ¹H NMR (400 MHz) in CDCl₃ (NVCL/NVP molar ratio in the copolymer = 82/18) (see Fig. S1†).

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<table>
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<tr>
<th>Entry</th>
<th>In the feed (mol%)</th>
<th>[monomers]/[R-Co(acac)₂]</th>
<th>Time (h)</th>
<th>Conv (%)</th>
<th>$M_n$ (g mol⁻¹)</th>
<th>$D$</th>
<th>In the copolymer (mol%)</th>
<th>NVP/NVL</th>
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<td>24</td>
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<td>3</td>
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<td>759</td>
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<td>803</td>
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<td>34</td>
<td>29 300</td>
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</table>

a Determined by ¹H NMR 250 MHz in CDCl₃. b Determined by SEC in DMF containing LiBr (calibration PS). c Determined by ¹H NMR 400 MHz in CDCl₃.
ation was pursued at 40 °C (comonomer feed NVP/NVCL: 63/37). After 2 h, the mixture became viscous. Based on the 1H NMR spectrum of the reaction mixture in CDCl₃, the NVP consumption reached 19% and the cumulative NVCL conversion was 43%. A volume of 25 mL of DMF was added to decrease the viscosity. Half of the solution was further used for the synthesis of the triblock (see the procedure below) while the rest was transferred using a cannula to another 100 mL Schlenk flask containing 100 mg of TEMPO (0.64 mmol, degassed by three vacuum-argon cycles) in order to produce the cobalt-free PVNCL-block-P(NVCL-stat-NVP) diblock. After treatment with TEMPO, the diblock was precipitated in diethylether (250 mL), filtered, dried and purified by dialysis using a Spectra/ Por®1 membrane (6–8 kDa) in deionized water. The PVNCL-block-P(NVCL-stat-NVP) copolymer was collected as a white powder after lyophilization of the aqueous solution. SEC-MALLS measurements were carried out in DMF in order to determine the molecular parameters of the copolymer (dn/dc of PVNCL-block-P(NVCL-stat-NVP) = 0.0992 mL g⁻¹, Mₙ MALLS = 43,100 g mol⁻¹, D_MALLS = 1.05 and D = 1.08 with PS calibration). The composition of the P(NVCL-stat-NVP) block was determined by 1H NMR (81/19: NVCL/NVP) and allowed to attribute the degree of polymerization of each segment of the copolymer (PVNCL₁₃₂-block-P(NVCL₄₀-stat-NVP)₁₇₂). Synthesis of PVNCL-block-P(NVCL-stat-NVP)-block-PVNC

A series of PVNCL-block-P(NVCL-stat-NVP) diblock and PVNCL-block-P(NVCL-stat-NVP)-block-PVNC copolymers with different block lengths and compositions have been prepared accordingly (see Tables 2 and S1 for molecular characteristics and polymerization conditions). In order to distinguish the PVNCL-block-P(NVCL-stat-NVP) and PVNCL-block-P(NVCL-stat-NVP)-block-PVNC copolymers based on their composition, C₆₀(C₄P₁₉₁) and C₆₀(C₂₃P₂₅₂) labels were used where C and P correspond to NVCL and NVP, respectively, and n and l denote the number of NVCL units and m the number of NVP units.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics of the NVCL and NVP containing di- and triblock copolymers prepared by CMRP and CMRC</th>
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<td>110,500</td>
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<td>8</td>
<td>128,000</td>
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</table>

a Exact molar mass and dispersity determined by DMF-SEC using a MALLS detector and an RI detector with PS calibration, respectively.
b Determined by 1H NMR taking into account the exact molar mass of the PVNC segment. c C and P designate NVCL and NVP, respectively. The numbers associated with these letters correspond to the degree of polymerization of each monomer. Detailed polymerization conditions are provided in Table S1.
equipped with a He–Ne laser with a wavelength of 633 nm. The measurements were performed in water at 90° angle at a concentration of 1 g L$^{-1}$. Data were analysed using the CONTIN method, which is based on an inverse-Laplace transformation of the data and gives access to a size distribution histogram.

**Results and discussion**

**Copolymer synthesis by CMRP**

This section is exclusively dedicated to the synthesis of the NVCL and NVP containing statistical, diblock and symmetrical triblock copolymers. The control of the radical copolymerization of this pair of $N$-vinylamides using Co(acac)$_2$ as mediating species will be presented as well as the macromolecular features of each copolymer like composition, block length, dispersity, etc. The solution behaviour and the thermal response of these copolymers will be addressed and compared in the next section. Nevertheless, when reading through the synthesis section, it is interesting to keep in mind that increasing the NVP content in a PVNV backbone is expected to increase the LCST of the polymer segment because PVNP is more hydrophilic than NVCL and does not exhibit LCST in pure water.$^{65}$

First, the controlled statistical radical copolymerization of NVCL and NVP was performed in bulk at 40 °C by CMRP using a low molecular weight alkyl-cobalt($n$) adduct (R–Co(acac)$_2$) as the initiator. The latter consists of oligoPVAc (average number of 4 VAc units) capped by Co(acac)$_2$ whose synthesis and ability to initiate the CMRP of $N$-vinylamides has been previously reported.$^{49}$ Upon heating, homolytic cleavage of the R–Co(acac)$_2$ bond occurs releasing the initiating fragment (R') and the metallic controlling agent which reversibly traps the growing radical chains. Table 1 summarizes the NVP/NVCL statistical copolymers prepared in this study. Several comonomer feed ratios were tested and the monomer/initiator molar ratio was adjusted to target the P(NVCL-stat-NVP) copolymer of 100 000 g mol$^{-1}$ at full monomer conversion. Although CMRP of NVCL remains efficient above 60%,$^{49}$ the above mentioned NVP/NVCL copolymerizations were stopped between 20% and 35% in order to ensure an optimal control of the polymerization and well-defined NVP/NVCL statistical copolymers with molar masses ranging between 20 000 and 40 000 g mol$^{-1}$ were produced accordingly. In this case, the dispersity of the copolymers slightly increases with the NVP content in the feed. This observation is consistent with the better control reported for the NVCL homopolymerization compared with the NVCL under the same experimental conditions.$^{49}$ The compositions of the resulting copolymers were determined by $^1$H NMR (see Fig. S1†). The NVP content was systematically a bit higher in the copolymer than in the feed, which is in good agreement with the reactivity ratio reported in the literature ($r_{\text{NVCL}} = 1.7$, $r_{\text{NVP}} = 2.8$).$^{66}$

Next, CMRP and CMRC methods were used to produce novel double thermoresponsive NVCL and NVP-based diblocks and triblocks (Scheme 3). Inspired by a recent one-pot procedure for the copolymerization of NVCL and $N$-vinylmethacrylamide,$^{64}$ our general strategy consists of the controlled radical homopolymerization of NVCL from R-Co(acac)$_2$ in bulk at 40 °C up to 27% of conversion followed by injection of a precise amount of NVP which copolymerizes with the residual NVCL leading to the targeted PNVCL-block-P(NVCL-stat-NVP)-Co(acac)$_2$. After dilution with dimethylformamide, the resulting PNVCL-block-P(NVCL-stat-NVP)-Co(acac)$_2$ copolymer solution was divided into two equal parts. One of them was treated with TEMPO in order to eliminate the metal at the chain-end of the PNVCL-block-P(NVCL-stat-NVP) diblock. The other portion was reacted at room temperature with an excess of isoprene compared with the cobalt promoting the radical coupling of the diblock by CMRC and the formation of the corresponding PNVCL-block-P(NVCL-stat-NVP)-block-PNVCL triblock. Briefly, the allyl radical resulting from the addition of one isoprene unit at the copolymer chain-end is neither prone to propagate at room temperature nor to react with the Co(acac)$_2$. Consequently, massive dimerization of these allyl radicals terminated chains occurs.$^{60-62,67}$ Importantly, the degree of polymerization of the PNVCL block and composition of the P(NVCL-stat-NVP) segment are identical in the triblocks and the parent diblocks, which is serious advantage for comparing the thermal properties of both architectures, as will be discussed later.

Table 2 summarizes the molecular parameters ($M_n$, $D$, $P$) of the NVCL precursors, the PNVCL-block-P(NVCL-stat-NVP) diblocks and the final PNVCL-block-P(NVCL-stat-NVP)-block-PNVCL triblocks. Detailed polymerization conditions are provided in the experimental section and in Table S1.† The exact molar mass of the polymers was obtained using a DMF-SEC chromatograph equipped with a multi-angle laser light scattering (MALLS) detector and the $D$ using a PS calibration. The composition of the P(NVCL-stat-NVP) statistical segment in the
diblock and the triblock was determined by $^1$H NMR by taking into account the degree of polymerization of the PNVCL block that it is linked to.

The low dispersity of the polymers, the composition of the statistical segment and the increase of molar mass at each step of the process provide a reliable basis to conclude on the efficacy of this synthetic approach. The overlay of the chromatograms in Fig. 1 confirms the efficiency of the PNVCL-block-P(NVCL-stat-NVP) by chain extension carried out from the PNVCL-Co(acac)$_2$. Although not quantitative, the radical coupling reaction is efficient because the molar mass of a large majority of the chains double when treating the PNVCL-block-P(NVCL-stat-NVP)-Co(acac)$_2$ with isoprene. Indeed, a limited amount of unreacted diblocks (15% by deconvolution of the SEC chromatograms) contaminates the final PNVCL-block-P(NVCL-stat-NVP)-b-PNVCL triblock (Fig. 1). The latter results from partial deactivation of the diblock chain-end before coupling.

In this way, we designed a series of NVCL and NVP di- and triblocks with various block lengths and compositions. Because copolymers with two discrete LCSTs are targeted, we ensured that the statistical P(NVCL-stat-NVP) segments of copolymers are NVP-rich (see Table 2) to make sure that their transition temperature sufficiently differs from the LCST of the PNVCL block.

**Thermoresponsive behaviour of the copolymers**

In this section, the solution behaviour and the thermal response of the copolymers prepared above will be addressed starting with the statistical P(NVCL-stat-NVP) copolymers. As indicated in Table 1, the latter differ by their NVP/NVCL composition but their molar mass remains in the same range. The cloud point temperature ($T_{CP}$) of the aqueous solution of each copolymer (0.1 wt%) was measured by turbidimetry. The transmittance versus temperature plots, also called cloud point curves are shown in Fig. S2.† A heating rate of 1 °C min$^{-1}$ was used and we considered that the cloud point was reached when half of the transmittance of the solution was lost. The $T_{CP}$ of each P(NVCL-stat-NVP) copolymer was then plotted as a function of their NVP molar fraction (Fig. 2). For the sake of the comparison, the $T_{CP}$ of a homoPNVCL (37 °C) was added on the graph. Because NVP is more hydrophilic than NVCL that contains two additional aliphatic carbons in the lactam ring, the higher is the NVP content in the copolymer, the higher is the phase transition temperature (Fig. 2). For example, a $T_{CP}$ of 72 °C was obtained when the NVP molar fraction in the copolymer was 0.57 (entry 5, Table 1). Note that no phase transition was detected below 100 °C for the copolymer containing 72 mol% of NVP (entry 6, Table 1). Although the dependence of the transition temperature with the NVP content does not appear to be linear, it is now possible to precisely tune the $T_{CP}$ of a P(NVCL-stat-NVP) copolymer by adjusting its composition.

With these data in hand, we investigated the solution behaviour of the PNVCL-block-P(NVCL-stat-NVP) diblocks and PNVCL-block-P(NVCL-stat-NVP)-b-PNVCL triblocks. Theoretically, both types of copolymers should be double thermoresponsive, the $T_{CP}$ of the PNVCL block(s) being inferior to the one of the NVP/NVCL statistical segments. Moreover, the transition temperature of one block could be influenced by the adjacent segment(s). Therefore, the impact of the copolymer architecture and composition on the $T_{CP}$ of each segment are key issues addressed in the present work as well as the organization of the di- and triblock copolymers in solution at different temperatures. Overall, an increase of the temperature is expected to change the solubility of the blocks in a stepwise fashion, inducing a multistep self-assembly behaviour described in Scheme 4. Below (region I) and above (region III), both $T_{CP}$s, the copolymer chains should be fully soluble and aggregated in water. At intermediate temperatures (region II,
For most copolymers (see Table 2), the statistical P(NVCL-stat-NVP) segment is longer than the PNVCL block. Therefore, it is reasonable to expect the formation of spherical micelles composed of dehydrated PNVCL core stabilized by a P(NVCL-stat-NVP) corona, the latter consisting of loops in the case of the triblocks.

The thermal response of the block copolymers presented in Table 2 was systematically analysed by combination of turbidimetry and DLS. The study of the C132[C40P172] and C132[C80P344]C132 copolymer pair is presented in detail hereafter (Fig. 3 and 4) while the transition temperatures of all the copolymers determined by a similar approach are collected and compared in Table 3.

Fig. 3 shows the evolution of the transmittance (dotted line) and of the relative scattered light intensity ($I/I_0$) measured by DLS upon heating the aqueous solutions of C132[C40P172] and C132[C80P344]C132. For the diblock, two marked transitions are observed. The first one, indicated by the increase of the scattered intensity (full line in the upper graph of Fig. 3), appears around 45 °C. This transition corresponds to the selective precipitation of the PNVCL block ($T_{CP1}$). The latter occurs at higher temperature compared to a homoPNVCL ($T_{CP} \sim 37 °C$) because in the diblock the PNVCL is linked to a more hydrophilic sequence of P(NVCL-stat-NVP). The second phase transition was clearly identified by a sharp loss of transmittance at 70 °C, which is due to the dehydration and precipitation of the NVP containing segment. Importantly, the $T_{CP}$ of the statistical P(NVCL-stat-NVP) sequence of the copolymer (70 °C) is drastically lowered by the more hydrophobic, and already collapsed, PNVCL block. Indeed, as mentioned above, an isolated NVCL/NVP statistical copolymer with the same composition ($F_{NVCL} = 0.81$) should not exhibit a $T_{CP}$ since we did not detect any for the P(NVCL-stat-NVP) with a NVP molar fraction of 0.72. The DLS size distribution histograms recorded at different temperatures are presented in Fig. 4. At 35 °C (region I), the sample mainly consists of free chains, corresponding to the main peak being observed around a few nm. The second population, around 100 nm, is probably due to a small fraction of loose aggregates but is negligible being absent from the number-weighted size distribution graph (not shown). At 70 °C (region III), rather large objects are formed (~300 nm) suggesting complete aggregation of the copolymer chains. At 50 °C, so in region II corresponding to temperatures between the two $T_{CP}$s, micelles with an average hydrodynamic radius of 20 nm and a low polydispersity index (0.02) were observed. The morphology of the object has been tentatively studied by transmission electron microscopy (TEM) but aggregation occurred during the deposition on the TEM grid and evaporation of the thermostated solution (55 °C). Considering the copolymer composition, those are probably core–shell spherical micelles. Micelles with a very similar size were observed at several temperatures within region II (see Fig. S3, † for additional DLS curves). Interestingly, in the previously studied PNVCL-block-P(NVCL-stat-NMVA) copolymers, a transition region corresponding to the formation of large and ill-defined objects was
Two phase transitions were observed. The first one corresponds to the precipitation of the central P(VCL) block at both extremities of the P(NVCL-stat-VP) diblock and the corresponding P(NVCL-stat-VP)-b-PNVCL triblock aqueous solutions (1 g L\(^{-1}\)) at various temperatures (I below \(T_{\text{CP1\&2}}\), II between \(T_{\text{CP1}}\) & \(T_{\text{CP2}}\), III above \(T_{\text{CP1\&2}}\)). Additional DLS curves of the copolymers at other temperatures are provided in Fig. S3.†

Table 3 \(T_{\text{CPs}}\) of the NVCL/NVP-based di- and triblocks

<table>
<thead>
<tr>
<th>Copolymers</th>
<th>(T_{\text{CP1}}) (^{\text{a}}) (°C)</th>
<th>(T_{\text{CP2}}) (^{\text{b}}) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(<em>{68})(C(</em>{28})P(_{110}))</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>C(<em>{60})(C(</em>{36})P(<em>{28}))C(</em>{68})</td>
<td>52</td>
<td>58</td>
</tr>
<tr>
<td>C(<em>{112})(C(</em>{40})P(_{172}))</td>
<td>45</td>
<td>70</td>
</tr>
<tr>
<td>C(<em>{132})(C(</em>{55})P(_{154}))</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>C(<em>{216})(C(</em>{31})P(_{154}))</td>
<td>40</td>
<td>67</td>
</tr>
<tr>
<td>C(<em>{236})(C(</em>{10})P(<em>{208}))C(</em>{236})</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>C(<em>{282})(C(</em>{54})P(_{297}))</td>
<td>35</td>
<td>63(^{d})</td>
</tr>
<tr>
<td>C(<em>{282})(C(</em>{36})P(<em>{308}))C(</em>{282})</td>
<td>40</td>
<td>68(^{d})</td>
</tr>
</tbody>
</table>

| Copolymer aqueous solutions (1 g L\(^{-1}\)). \(T_{\text{CP1}}\) values correspond to the onset temperature of the increase of the scattered light intensity measured by DLS (as shown in Fig. 3). \(T_{\text{CP2}}\) is measured at 50% of transmittance loss on the turbidimetry curves. \(T_{\text{CP}}\) was detected below 95 °C. Determined in 2 M NaCl aqueous solution.

Observed prior to micellization upon heating the copolymer solution. In contrast, the present PNVCL-block-P(NVCL-stat-NVP) diblock exhibits a sharp unimers/micelles transition. Next, we examined the corresponding C\(_{132}\)(C\(_{80}\)P\(_{344}\))C\(_{132}\) triblock under the same dilution condition (1 mg mL\(^{-1}\)). Again, two phase transitions were observed. The first one corresponds to the \(T_{\text{CP}}\) of the PNVCL external blocks of the copolymer and occurs around 40 °C as emphasized by the increase of the scattered light intensity measured by DLS (full line in the lower graph of Fig. 3). The second transition corresponds to the precipitation of the central P(NVCL-stat-NVP) segment of the triblock and is identified at 50 °C on the turbidimetry curve (dotted line in the lower graph of Fig. 3). The DLS size histograms presented in Fig. 4 and S3 show that the triblock copolymer mainly exists as free chains below the first \(T_{\text{CP}}\) and is fully aggregated above the second \(T_{\text{CP}}\), as for the diblock. In the inter-\(T_{\text{CP}}\) region, a major population around a hydrodynamic radius of about 100 nm is observed. This size seems too large to correspond to simple flower-like micelles, and the associated objects are probably small aggregates of micelles. The ABA triblock copolymer is indeed more prone to aggregation than the diblock because the A blocks can precipitate into two different micellar cores, inducing the formation of bridges between micelles. Moreover, the self-assembly of the triblock in water is more difficult to control because the inter-\(T_{\text{CP}}\) window (region II) is much narrower compared with the diblock. Although the composition of the blocks of C\(_{132}\)(C\(_{80}\)P\(_{172}\)) and C\(_{132}\)(C\(_{80}\)P\(_{344}\))C\(_{132}\) are strictly identical, their respective \(T_{\text{CP}}\)s vary from one architecture to another and are lower in the triblock than in the diblock (see Table 3). Doubling of the molar mass of the copolymer and of the statistical P(NVCL-stat-NVP) sequence by radical coupling might contribute to this effect. Nevertheless, considering previous reports and the present range of molar mass variation, this factor should only be responsible for a decrease of a few degrees of the \(T_{\text{CP}}\). By contrast, the presence of a more hydrophobic NVCL block at both extremities of the P(NVCL-stat-NVP) segment of the triblock should strongly decrease its transition temperature (from 70 °C in the diblock to 50 °C in the triblock). On the other hand, the hydrophobicity of the polymer chain linked to the PNVCL block, i.e. P(NVCL-stat-NVP) in the diblock and P(NVCL-stat-NVP)-block-PNVCL in the triblock, is globally higher in the triblock than in the diblock. This might account for the lower \(T_{\text{CP}}\) of the PNVCL segment in the triblock (40 °C) compared to the one in the diblock (45 °C) (see Table 3).

Following the same approach, we studied the thermal response of the aqueous solution of the other diblocks and triblocks mentioned in Table 2, which differ by their block length and compositions. All turbidimetry and DLS curves are provided in the ESI (Fig. S3–S10†). The key transition temperatures of each copolymer are reported in Table 3 and discussed hereafter. Except for C\(_{282}\)(C\(_{27}\)P\(_{297}\)) and C\(_{282}\)(C\(_{34}\)P\(_{304}\))C\(_{282}\), all copolymers exhibited a double thermostresponsive behaviour in water. Again, the \(T_{\text{CP}}\)s of both the PNVCL block and of the NVP containing sequence were lower in the triblock than in the parent diblock. Moreover, the transition temperature of the longer PNVCL block is less impacted by the hydrophilic P(NVCL-stat-NVP) segment. For example, considering the series of diblocks, the \(T_{\text{CP}}\) of the PNVCL was recorded at 50 °C, 45 °C and 40 °C for degrees of polymerization (DP) of PNVCL equal to 68, 132 and 236, respectively (Table 3). As a reminder, the \(T_{\text{CP}}\)s of homoPNVCL is around 37 °C. Interestingly, between \(T_{\text{CP1}}\) and \(T_{\text{CP2}}\), C\(_{68}\)(C\(_{27}\)P\(_{191}\)), C\(_{112}\)(C\(_{40}\)P\(_{72}\)) and C\(_{132}\)(C\(_{55}\)P\(_{154}\)) formed well-defined micelles whose size increases with the length of the PNVCL segment. The \(R_{\text{m}}\) of the micelles is equal to 15 nm, 20 nm and 30 nm for DP of 68, 132 and 236, respectively (Fig. S5, S3 and S7†). On the other hand, the study of the C\(_{68}\)(C\(_{14}\)P\(_{382}\))C\(_{68}\) and C\(_{236}\)(C\(_{14}\)P\(_{308}\))C\(_{236}\) triblocks confirmed the difficulty to form well defined objects and to avoid aggregation.
For C_{282}(C_{27}P_{297}) and C_{282}(C_{54}P_{594})C_{282}, no precipitation was observed in water below 95 °C because the NVP content in their statistical block was too high (F_{NVP} = 0.92), suppressing the second T_{CP}. However, the T_{CP} of the PNVCL segment in the diblock (Fig. 5) and in the triblock (Fig. S8†) was detected around 40 °C. These copolymers mainly exist as free chains above 40 °C, but as micelles below 40 °C and self-assembled above the transition temperature. In water and in 2 M NaCl aqueous solutions as a function of temperature, C_{282}(C_{27}P_{297}) led to assemblies with a hydrodynamic radius between 25 and 35 nm, which probably correspond to flower-like micelles, as well as larger aggregates (R_h ~ 200 nm) (Fig. S9†).

The polymer solubility, and LCST of thermoresponsive macromolecules in particular, can be strongly influenced by the presence of salts in the aqueous solution. In particular, hydration and phase behaviour of PNVCL and PNVP in water are known to be impacted by the presence of salts which decreases their LCSTs. This salting out effect was here used to change the T_{CP} of the PNVCL segment of C_{282}(C_{27}P_{297}) and of C_{282}(C_{54}P_{594})C_{282} and to possibly decrease the solubility of the statistical P(NVCL-stat-NVP) segment, revealing a second T_{CP} below 100 °C and providing thus double thermoresponsiveness to the copolymer. In a 2 M NaCl aqueous solution, C_{282}(C_{27}P_{297}) indeed showed two T_{CP}s (25 °C and 63 °C, Fig. 5) and micelles with a hydrodynamic radius of about 35 nm were observed in the inter-T_{CP} window (Fig. S10†). Addition of salt also shifted downwards the T_{CP} of C_{282}(C_{54}P_{594})C_{282} and for the first time the temperature range of the inter-T_{CP} window was rather large (between 25 °C and 67 °C) (Fig. S8 and S10†). In conclusion, the salting out effect thus appears to be a very useful tool to adjust the transition temperature of PNVCL and NVP-based double thermoresponsive block copolymers.

Finally, the reversibility of the temperature induced phase transitions was investigated by turbidimetry for C_{282}(C_{54}P_{594})C_{282} in a 2 M NaCl aqueous solution. For the diblock, the T_{CP} observed upon heating and cooling the solutions were quite similar and hysteresis did not exceed a few degrees (Fig. S11†). Moreover, the objects formed in solution, when increasing and decreasing the temperature, were similar (Fig. S12†) although the micelles detected in the inter-T_{CP} region were larger when cooling the solution. Indeed, the R_h of the micelles observed at 45 °C were around 35 nm and 60 nm in the heating and cooling phases, respectively (Fig. S12†). In such experiments, kinetically frozen micelles are most probably formed considering the abrupt character of the LCST transition. It is therefore not surprising to observe micelles of different sizes during heating and cooling, since it is well-known that the preparation protocol may strongly influence the micellar characteristics in the case of kinetically frozen micelles. In the case of the triblock C_{282}(C_{54}P_{594})C_{282}, as indicated by the turbidimetry curves (Fig. S11†), the re-solubilisation of the copolymer, when cooling the solution was difficult, which prevent us from performing a size analysis by DLS. Nonetheless, its double thermo-responsive character is demonstrated in both the heating and cooling cycles.

**Conclusion**

A series of novel well-defined thermoresponsive NVCL and NVP-based copolymers, including statistical, diblock and triblock copolymers, were prepared by cobalt-mediated radical polymerization (CMRP) and cobalt-mediated reaction coupling (CMRC). Due to the high level of control on the molecular parameters of the polymers it was possible to compare their thermal properties based on their composition, block length, and architecture. Increasing the NVP content in statistical NVCL/NVP copolymers was shown to increase their lower critical solution temperature in water, 0.7 being approximately the maximum NVP molar fraction for maintaining the T_{CP} of such statistical copolymers below 90 °C. With these data in hand, four pairs of double thermoresponsive di- and triblock composed of PNVCL and P(NVCL-stat-NVP) blocks were obtained by sequential CMRP of NVCL and NVCL/NVP polymerizations followed by radical coupling with isoprene. In both types of architectures, the thermal response of PNVCL and of the

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**Fig. 5** Transmittance (1 °C min⁻¹) (dotted line) and scattered intensity measured by DLS (full line) for the C_{282}(C_{27}P_{297}) diblock (1 g L⁻¹) in water and in 2 M NaCl aqueous solutions as a function of temperature.
P(NVCL-stat-NVP) segment influenced each other, the LCST of the PNVCL block being increased by the more hydrophilic P(NVCL-stat-NVP) block and vice versa. As a general trend, the inter-$T_{CP}$ region was narrower and occurred at a lower temperature for the triblock because the central NVCL/NVP block is linked to PNVCL at both extremities. In the case of the diblocks, narrowly dispersed micelles were formed from unimers upon heating, and total collapse occurred above the second $T_{CP}$, confirming the multistep assembly behaviour of the copolymers. For the triblocks, the self-assembly was more difficult to control due to their enhanced tendency to aggregation, linked to their ABA structure, and the narrower inter-$T_{CP}$ window. Moreover, the NaCl salting-out effect was successfully used to adjust the $T_{CP}$s of these types of copolymers to the extent that the $T_{CP}$ of a NVP rich statistical segment became detectable below 100 °C, making the corresponding di- and triblock copolymers doubly thermoresponsive. Finally, the double thermo-responsive character of the NVCL and NVP-based copolymers was also emphasized in both the heating and cooling cycles. As a result, the data presented here provide a basis to synthesize a broad range of double thermo-responsive NVCL- and NVP-based di- and triblock copolymers with tunable LCSTs and highlight the crucial impact of the architecture of the copolymer on their thermal phase transition.

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Notes and references
