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# CO<sub>2</sub>-switchable thermoresponsiveness of poly(*N,N*-(diethylamino)ethyl acrylamide)-based homo- and copolymers in water

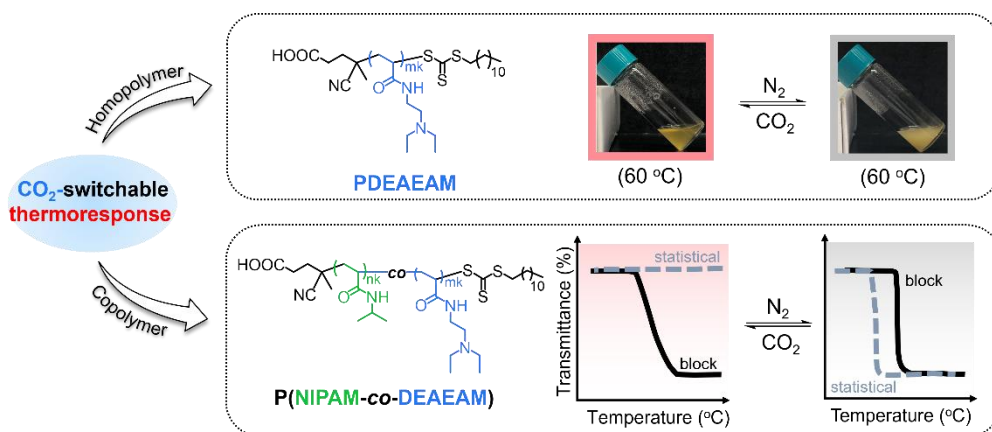
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## GRAPHICAL ABSTRACT



The insertion of CO<sub>2</sub> switchable *N,N*-(diethylamino)ethyl acrylamide monomer enable to tune easily and reversibly the properties of PNIPAM-based polymers.

## ABSTRACT

The properties of thermo-sensitive polymers can be easily and reversibly switched using addition/removal of carbon dioxide (CO<sub>2</sub>) as an innovative and ecological trigger. *N,N*-(diethylamino)ethyl acrylamide (DEAEAM) was (co)polymerized with *N*-isopropylacrylamide (NIPAM) by Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization to obtain copolymers with various compositions and architectures. The properties (solubility, aggregation behavior...) of these polymers in aqueous solution in the presence of CO<sub>2</sub> was studied via scattering, microscopy and turbidimetry measurements. The copolymerization of *N,N*-(diethylamino)ethyl acrylamide (DEAEAM) monomer with NIPAM enables to produce either a statistical copolymer or a series of diblock copolymers and to tune the cloud point temperature of obtained copolymers either by changing the composition or the architecture (statistical, block) or by CO<sub>2</sub> addition/removal. Whereas, for a given composition, statistical copolymer does not display thermoresponsive behavior, block copolymers present a thermoresponsiveness which depends on the protonation degree of tertiary amine functions. Additionally, CO<sub>2</sub> can be used to reversibly modulate the properties of these materials in aqueous media.

**KEYWORDS:** CO<sub>2</sub>-responsive block copolymers, pH and thermo-responsive block copolymers, RAFT, PNIPAM, tertiary amine moieties

## 1. Introduction

Carbon dioxide (CO<sub>2</sub>) has emerged as an innovative and green trigger for switching the properties (such as solubility, dimension, chain conformation, morphology, shape...) of responsive polymer materials in aqueous media. [1-5] As a key metabolite in living organisms, CO<sub>2</sub> displays excellent biocompatibility and membrane permeability; as an industrial production waste, it is abundant, inexpensive, available and nontoxic. Moreover, in aqueous solution, CO<sub>2</sub> combines with water to form carbonic acid, which lowers the solution pH. The added CO<sub>2</sub> can be easily removed by bubbling inert gases, e.g. nitrogen or argon, or by heating up the solution [6] causing the polymer return back to its original state. Therefore, CO<sub>2</sub>-reponsiveness is a special example of a pH-responsive trigger. Generally, in classical pH-responsive systems, an acid or a base solution is added to trigger the property change, which leads to the accumulation of salt in system. However, such salt build-up can be avoided by using CO<sub>2</sub>. [3,5,7] Thus, developing CO<sub>2</sub>-responsive polymer materials holds great promise for designing switchable vectors in drug delivery, [8,9] for limiting CO<sub>2</sub> emission from industrial process [10-11] and for controllably separating oil/water mixture. [12,13]

Commonly reported CO<sub>2</sub>-responsive polymer materials comprise amidine, [14,15] guanidine, [16] imidazole [17] and amine groups. [18-20] These functions in aqueous media can react with CO<sub>2</sub> to generate charged species. However, the switching between neutral and charged forms upon CO<sub>2</sub> addition/removal is not always fully reversible. [21] Thus, moderately basic tertiary amine-based polymers exhibit reversible behavior, whereas those with a strong or weak basicity cannot be easily recovered to neutral forms or are less sensitive to CO<sub>2</sub> stimulation. Hence polymers with tertiary amines functional groups are readily switched to bicarbonate salt upon CO<sub>2</sub> exposure and return to neutral form after exposure to inert gases.

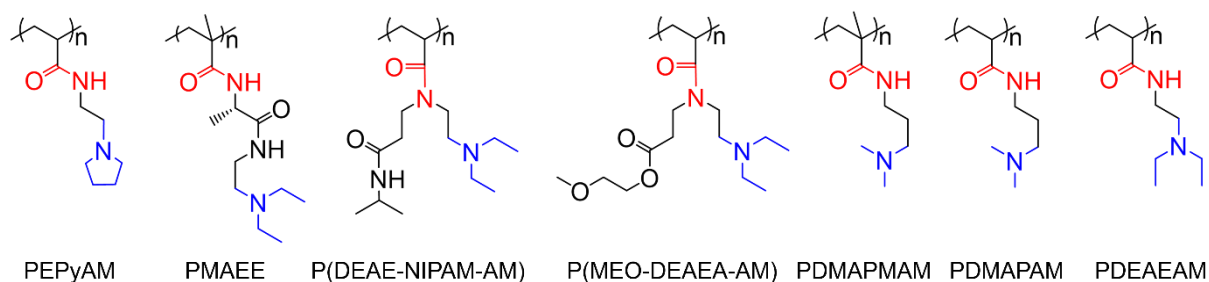
Some amine-based CO<sub>2</sub>-responsive homopolymers, mostly in their uncharged forms, display thermoresponsive behaviors. Among them, poly(*N,N*-(dimethylamino)ethyl methacrylate) (PDMAEMA) (p*K*<sub>a</sub> ~ 7.5) and poly(*N,N*-(diethylamino)ethyl methacrylate) (PDEAEMA) (p*K*<sub>a</sub> ~ 7.4) are the most studied CO<sub>2</sub>-responsive polymers since their corresponding monomers are commercially available. [22, 23]

PDMAEMA and PDEAEMA exhibit a lower critical solution temperature (LCST) within a specific pH window. The number of carbon atoms in alkyl substituent (methyl or ethyl) has a pronounced effect on the value of transition temperature: PDMAEMA is well soluble in water at room temperature whatever the pH value and presents a LCST behavior only at high pH values (above  $pK_a$ ). In contrast, PDEAEMA is soluble and thermoresponsive in its protonated form and is no longer soluble at high pH whatever the temperature. [24] Copolymers based on these two  $CO_2$ -responsive polymers have thus been extensively studied for their ability to form different colloidal structures (nanospheres, gels, micelles, polymersomes...) and to induce reversible morphological transition upon  $CO_2$  exposure from a simple volume change to a sol-to-gel or micelle-to-vesicle transition. [20,25-27]

Poly(*N*-isopropylacrylamide) (PNIPAM) presents, like poly(*N*-vinylcaprolactam) [28,29] or poly(oligo(ethylene glycol) methyl ether (meth)acrylate)s, [30] a thermoresponsive character which is materialized by the presence of a LCST in its phase diagram. This results from a coil-to-globule transition due to the dehydration of polymer chains at temperature around 32 °C. [31-33] The incorporation of hydrophilic or hydrophobic comonomers into PNIPAM allows fine-tuning of the cloud point temperature ( $T_c$ ). [34,35] Therefore, the objective here is to insert into PNIPAM structure  $CO_2$ -responsive moieties, whose hydrophilicity can be modulated by addition/removal of  $CO_2$ , which will make it possible to play on the thermosensitive properties of the considered polymers. Therefore, previous studies described the synthesis of PNIPAM-based  $CO_2$ -responsive copolymers with diblock (PDEAEMA<sub>60</sub>-*b*-PNIPAM<sub>40</sub>) [36], triblock (PNIPAM-*b*-PCL-*b*-PDMAEMA with PCL corresponding to poly( $\epsilon$ -caprolactone)) [37] or more complex structures (PCL-*b*-P(NIPAM-*co*-DMAEMA) with a pyrene termination group).[38]

With the objective to insert such  $CO_2$ -sensitive groups in PNIPAM, the use of monomers based on acrylamide is of special interest compared to the poly(methacrylate) monomers: a higher hydrolytic stability during heating/cooling cycles at different pH values and a radical reactivity close to the

one of PNIPAM. **Scheme 1** illustrates CO<sub>2</sub>-responsive poly((meth)acrylamide) polymers reported in the literature.

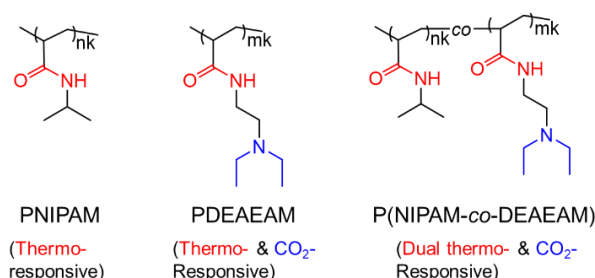


**Scheme 1.** Chemical structures of *N*-substituted poly((meth)acrylamide)s with CO<sub>2</sub>-responsiveness. (PEPyAM: poly(*N*-ethylpyrrolidine acrylamide), [39] PMAEE: poly(*N*-methacryloyl-*L*-alanine 2-(diethylamino) ethylamide), [40] P(DEAE-NIPAM-AM): poly(*N*-(2-(diethylamino) ethyl)-*N*-(3-(isopropylamino)-3-oxopropyl) acrylamide), [41] P(MEO-DEAEA-AM): poly(2-methoxyethyl 3-(*N*-(2-diethylamino ethyl) acrylamido) propanoate), [42] PDMAPMAM: poly((*N,N*-dimethylamino)propyl methyl acrylamide), [43] PDMAPAM: poly((*N,N*-dimethylamino)propyl acrylamide), [44] PDEAEAM: poly((*N,N*-diethylamino)ethyl acrylamide). [45]

Among them, PDEAEAM homopolymer which is thermo- and pH-/CO<sub>2</sub>-responsivis is of special interest. PDEAEAM is thermoresponsive in its unprotonated form and lose this property in its protonated form upon CO<sub>2</sub> bubbling. Bubbling inert gases enable to recover partially its thermoresponsiveness depending on the final protonation degree of the tertiary amine: hence  $T_c$  value decrease from 50.2 to 33.1 °C when the pH increases from 8.5 to 11.[45]. However, literatures involving multi-stimuli responsiveness of PDEAEAM-based copolymers are relatively scarce. Guan and co-workers designed a multi-stimuli responsive star polymer of tetraphenylethene-*graf*-tetra-poly(*N,N*-(diethylamino) ethyl) acrylamide), which displayed a good performance in long-term cell tracing. [46] Kim et al. employed P(DEAEAM-*r*-NIPAM) as responsive surfactant to

modulate shape transition of poly(styrene)-*b*-poly(4-vinylpyridine) particles between football and lens over a subtle change of temperature and pH. [48]

In this work, the objective is to evaluate how copolymer composition and architecture affect the thermoresponsive properties and the aggregation behavior of PDEAEAM-based copolymers (**Scheme 2**). A statistical copolymer P(NIPAM-*stat*-DEAEAM) and a series of block copolymers PNIPAM-*b*-PDEAEAM with variable compositions were synthesized by Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization, their solubilities in water as well as the self-assembly behavior driven by temperature upon CO<sub>2</sub> addition/removal were then explored.



**Scheme 2.** Structure of PNIPAM and PDEAEAM homopolymers, as well as P(NIPAM-*co*-DEAEAM) copolymer.

## 2. Materials and methods

### 2.1. Reagents

*N*-isopropylacrylamide monomer (NIPAM, Sigma-Aldrich), 4-cyano-4-((dodecylsulfanylthiocarbonyl)sulfanyl) pentanoic acid (CDTPA, 97%, Sigma-Aldrich) were used as received. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from methanol. *N,N*-(diethylamino)ethyl acrylamide (DEAEAM) was synthesized as described elsewhere [43]. 1,4-Dioxane was purified by passing through alumina column prior to use. All other reagents were used as received unless otherwise specified. Ultrapure water with a resistivity of 18.2 MΩ·cm was obtained using a Purelab Flex 1 apparatus.

## 2.2. Characterization

2.2.1. *Nuclear Magnetic Resonance Spectroscopy (NMR)*.  $^1\text{H}$  NMR spectra were recorded on Bruker Advance spectrometers (300 or 500 MHz) with  $\text{D}_2\text{O}$  as the solvent and 1,3,5-trioxane was used as an internal standard. The theoretical molecular weights ( $M_{n,\text{theo}}$ ) of synthesized (co)polymers were calculated by (eq. 1):

$$M_{n,\text{theo}} = \frac{[\text{M}]_0 \times M_M \times \text{Conv.}}{[\text{CTA}]_0} + M_{\text{CTA}} \quad (\text{eq. 1})$$

Where  $[\text{M}]_0$  and  $[\text{CTA}]_0$  are initial concentrations of monomer and chain transfer chain agent, respectively.  $M_M$  and  $M_{\text{CTA}}$  are molecular weights of monomer and chain transfer agent, respectively. Conv. is the monomer conversion determined by  $^1\text{H}$  NMR spectra.

2.2.2. *Size exclusion chromatography (SEC)*. Molecular weights and molecular weight distributions of all the polymers were determined on a SEC apparatus composed of a MALVERN multi-angle light scattering detector (MALS), a Waters 2140 refractive index (RI) detector and UV detector via using a Styragel HR4E column. Samples were prepared with a concentration of  $10 \text{ mg}\cdot\text{mL}^{-1}$  and filtered through  $0.45 \mu\text{m}$  PTFE filters. DMF (containing 10 mM LiBr) and THF (containing 2 vol.% TEA) were both used as eluents with a flow rate of  $1 \text{ mL}\cdot\text{min}^{-1}$  at  $35 \text{ }^\circ\text{C}$ . The molecular weight was studied from the knowledge of  $\text{dn}/\text{dc}$  value measured in DMF (containing 10 mM LiBr):  $[\text{dn}/\text{dc}]_{\text{PN}} = 0.08598 \text{ mL}\cdot\text{g}^{-1}$  (PN stands for PNIPAM),  $[\text{dn}/\text{dc}]_{\text{PD}} = 0.09715 \text{ mL}\cdot\text{g}^{-1}$  (PD stands for PDEAEAM); in THF with 2 vol.% TEA:  $[\text{dn}/\text{dc}]_{\text{PN}} = 0.1131 \text{ mL}\cdot\text{g}^{-1}$ ,  $[\text{dn}/\text{dc}]_{\text{PD}} = 0.1057 \text{ mL}\cdot\text{g}^{-1}$ ), which was measured by using a DNDC- 2010 differential refractometer (PSS) at 620 nm. For the block copolymers, the refractive index increments ( $\text{dn}/\text{dc}$ ) value were calculated using (eq. 2):



$$dn/dc = w_A \times (dn/dc)_A + w_B \times (dn/dc)_B \quad (\text{eq. 2})$$

2.2.3. *Differential Scanning Calorimetry (DSC)*. Glass transition temperatures ( $T_g$ ) of bulk (co)polymers were measured on heating (heating rate:  $10 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ ) on a Mettler Toledo DSC 1 STARe System Thermal Analysis calorimeter with a Gas Controller GC200.

2.2.4. *Dynamic light scattering (DLS)*. Dynamic light scattering (DLS) measurements were carried out on Zetasizer Nano-ZS (Malvern Instruments, Ltd, UK), with a 4 mW He-Ne laser ( $\lambda = 633 \text{ nm}$ , light scattering measured at  $173^\circ$ ). The correlation function was analyzed via the cumulant method to get the Z-average diameter of the colloids and by the general purpose method (NNLS) to obtain the distribution of diffusion coefficients of the solutes. The apparent equivalent hydrodynamic diameter was then determined using the Stokes–Einstein equation. Standard deviations were evaluated from diameter distribution. Zeta potential values of aggregates were obtained by doppler anemometry technique, and the test process requires an electric field to be applied to the entire sample solution. This process performed on Zetasizer Nano-ZS (Malvern Instruments, Ltd, UK) using folded capillary cells (DTS 1070) at  $25 \text{ }^\circ\text{C}$ .

2.2.5. *Turbidimetry measurements*. The transmittance of (co)polymer solution was recorded on a variable UV-visible spectrophotometer at  $500 \text{ nm}$  under continuous stirring with a heating rate of  $1 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ . The cloud point temperature ( $T_c$ ) was obtained either by the inflection point ( $T_{c,ip}$ ) on transmission curves or by the temperature ( $T_{c,onset}$ ) at which the transmittance of solution begins to decrease.

2.2.6. *Transmission electron microscopy (TEM)*.

TEM observations were performed on a MET hitachi HT7700 instrument at an accelerating voltage of  $80 \text{ kV}$ . Samples were prepared in a dry box with setting temperature of  $25$  or  $60 \text{ }^\circ\text{C}$ . Copolymer solution, copper grids and pipette tips were preheated in the dry box for  $20 \text{ min}$ . A  $6 \text{ }\mu\text{L}$  drop of the copolymer solution was deposited onto the copper grid until the solvent was evaporated, then these samples were

stained with 2 wt.% uranyl acetate solution preheated at the sample temperature. After 2 min, the excess solution was removed by filter paper and samples were dried again. **Note:** to prepare samples in the presence of CO<sub>2</sub> or N<sub>2</sub>, copolymer solution was bubbled with CO<sub>2</sub> or N<sub>2</sub> at room temperature with a flow rate of 10 min·L<sup>-1</sup> for 10 min or 30 min, respectively, and sealed with rubber caps. Then samples at different temperatures were prepared with the similar procedures but a CO<sub>2</sub> or N<sub>2</sub> flow was employed during the drying process.

2.2.7. *Small Angle X-rays scattering (SAXS)* on 1 wt.% PN<sub>5k</sub>-*b*-PD<sub>5k</sub> solution was performed with a XEUSS 2.0 SAXS/WAXS laboratory beamline equipped with a Cu source (E = 8 keV). The 2D SAXS patterns were collected using a pixel detector PILATUS3 1 M from Detrics. The sample to detector distance was 1216.5 mm (beam size 0.8 × 0.8 mm), covering a *q*-range from 0.007 to 0.5 Å<sup>-1</sup>. *q* is the scattering wave vector defined as  $q=(4\pi/\lambda)\sin \vartheta/2$ ,  $\lambda$  is the wavelength ( $\lambda \sim 1.54$  Å) and  $\vartheta$  is scattering angle. The solutions were loaded in borosilicate capillaries of 1.5 mm diameter in thermostatic sample holders. The SAXS curves were measured at 25 and 60 °C.

The model used to describe the curves at 25 °C as star-like assemblies is the one developed for star-polymers formed of gaussian polymer arms: [49]

$$I(q) = \frac{2}{fv^2} \left[ v - 1 + e^{-v} + \frac{f-1}{2} (1 - e^{-v})^2 \right] \quad (\text{eq. 3})$$

$$v = \frac{\langle R_g^2 \rangle q^2 f}{(3f-2)} \quad (\text{eq. 4})$$

with  $\langle R_g^2 \rangle$  is the square of the ensemble average gyration radius of the full assembly and *f* is the number of arms of the self-assembly.

The scattering curve of the sample at 60 °C upon CO<sub>2</sub> treatment was described using the model of (eq. 5) [50] formed by two terms, the first one describes the Porod scattering from clusters, while the second one is a Lorentzian function describing the scattering from polymer chains.

$$I(q) = \frac{A}{q^m} + \frac{B}{1+(\xi q)^n} \quad (\text{eq. 5})$$

A and B are two scale constants, m is the Porod exponent from the scattering of clusters,  $\xi$  and n are the correlation length and power law exponent of polymer chains, respectively. Best fits of the form factor  $P(q)$  for the measured data were derived using the SASView program (<http://www.sasview.org/>).

*2.2.8. pKa measurement.* The pKa value of PD<sub>5k</sub> homopolymer was determined by titration with a Mettler Toledo seven excellence pH meter. Prior to measurement, pH value of the studied solution (1.76·10<sup>-2</sup>wt%) was adjusted to 3 by addition of HCl solution (0.1 mol·L<sup>-1</sup>) to ensure full protonation of tertiary amine group. Then the solution was titrated with NaOH solution (0.1 mol·L<sup>-1</sup>). The value of pKa was determined by the inflection point on pH curve as a function of added volume of NaOH.

### 2.3. Synthesis

The desired PNIPAM and PDEAEAM homopolymers, and a series of PNIPAM-*b*-PDEAEAM block copolymers were synthesized by RAFT polymerization as previously described, [45-46] their corresponding synthetic procedures and characterizations were described in **Supporting Information** (scheme S1-S3 and Figures S1-S10).

**Synthesis of poly((*N*-isopropylacrylamide)-*stat*-(*N,N*-(diethylamino)ethyl acrylamide)) (P(NIPAM<sub>5k</sub>-*stat*-DEAEAM<sub>5k</sub>)).** NIPAM (1.00 g, 8.84 mmol), DEAEAM (1.00 g, 5.87 mmol), AIBN (0.017 g, 0.104 mmol), 4-cyano-4-((dodecylsulfanylthiocarbonyl) sulfanyl) pentanoic acid (CDTPA, 0.084 g, 0.208 mmol), 1,3,5-trioxane (0.053 g, 0.587 mmol, as an internal standard) and 1,4-dioxane (3.23 g) were added

into a Schlenk tube under argon flow, then the tube was immersed in a preheated oil bath at 70 °C. After a given time, the polymerization was stopped by rapidly cooling in liquid nitrogen, the desired statistical copolymer was collected by removing solvent, a subsequent precipitation in cold diethyl ether and drying under vacuum. Overall conversion: 98.5%,  $M_{n,theo} = 9870 \text{ g}\cdot\text{mol}^{-1}$ ,  $M_{n,NMR} = 9460 \text{ g}\cdot\text{mol}^{-1}$ . In DMF (containing 10 mM LiBr):  $M_{n,SEC} = 12060 \text{ g}\cdot\text{mol}^{-1}$ ,  $\bar{D} = 1.25$ . In THF (containing 2 vol.% TEA):  $M_{n,SEC} = 7490 \text{ g}\cdot\text{mol}^{-1}$ ,  $\bar{D} = 1.34$ .  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ )  $\delta_{\text{H}}$  (ppm): 3.92 (s, 1H,  $-\text{CH}(\text{CH}_3)_2$ ), 3.33 (s, 2H,  $-\text{CONH}-\text{CH}_2-$ ), 2.64 (s, 6H,  $-\text{N}(\text{CH}_2)_3$ ), 2.33 - 1.31 (m,  $-\text{CH}_2\text{CH}-\text{CONH}-$ ,  $-\text{CH}_2\text{CH}-\text{CONH}-$ ,  $-\text{C}(\text{CN})-\text{CH}_3-$ ,  $-\text{CH}_2\text{CH}_2\text{COOH}$ ,  $-\text{CH}_2\text{CH}_2\text{COOH}$ ), 1.16 (s, 6H,  $-\text{NHCH}(\text{CH}_3)_2$ ), 1.07 (s, 6H,  $-\text{N}(\text{CH}_2\text{CH}_3)_2$ ), 0.89 (s, 3H,  $-(\text{CH}_2)_{10}-\text{CH}_3$ ).

## 2.4. Gas aeration

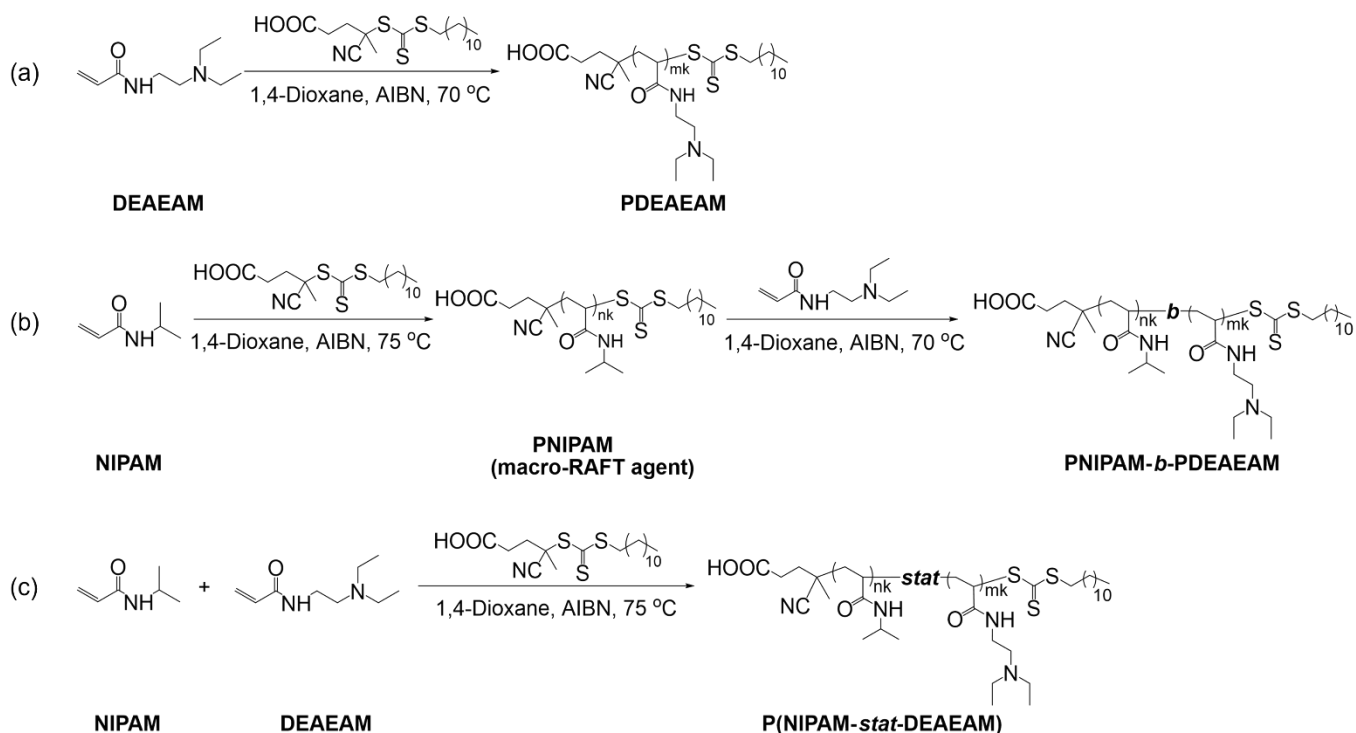
A gas regulator was used to quantitatively control gas amount,  $\text{CO}_2/\text{N}_2$  was introduced at room temperature by inserting a needle in (co)polymer solution with an appropriate flow rate at a given period of time.

## 3. Results and Discussion

### 3.1. Synthesis and characterization of (co)polymers.

PDEAEAM and PNIPAM reference homopolymers, as well as P(NIPAM-*co*-DEAEAM) copolymers with different architectures and compositions were synthesized by RAFT polymerization according to previously described synthetic pathways - using azobisisobutyronitrile (AIBN) as free-radical initiator and 4-cyano-4-((dodecylsulfanylthiocarbonyl) sulfanyl) pentanoic acid (CDTPA) as RAFT agent (**Scheme 3**). [45-46] These copolymers were designed with a targeted molecular weight of  $10000 \text{ g}\cdot\text{mol}^{-1}$ .

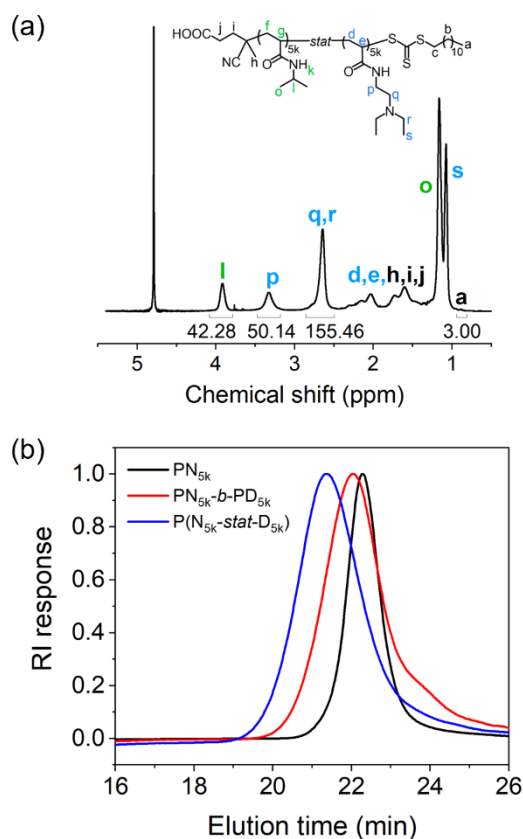
All PDEAEAM<sub>mk</sub> homopolymer, PNIPAM<sub>nk</sub> homopolymer, PNIPAM<sub>nk</sub>-*b*-PDEAEAM<sub>mk</sub> and P(NIPAM<sub>nk</sub>-*stat*-DEAEAM<sub>mk</sub>) copolymers discussed in this work are abbreviated as PD<sub>mk</sub>, PN<sub>nk</sub>, PN<sub>nk</sub>-*b*-PD<sub>mk</sub> and P(N<sub>nk</sub>-*stat*-D<sub>mk</sub>) (mk and nk are molecular weights of PD and PN, respectively).



**Scheme 3.** Synthetic routes for preparation of (a) PDEAEAM homopolymer, (b) PNIPAM-*b*-PDEAEAM block copolymer and (c) P(NIPAM-*stat*-DEAEAM) statistical copolymer.

The successful copolymerization of DEAEAM and NIPAM was assessed by  $^1\text{H}$  NMR experiments and size exclusion chromatography (SEC) analyses (**Figures S1 – S10**). Hence, NMR spectra of all copolymers shows both characteristic peaks of the two polymerized monomers as illustrated in the case of P(N<sub>5k</sub>-*stat*-D<sub>5k</sub>) in **Figure 1a**. Moreover, in the case of block copolymers, diffusion-ordered spectroscopy (DOSY) experiments for PN<sub>5k</sub>-*b*-PD<sub>5k</sub> enables to determine a unique diffusion coefficient equal to  $2.8 \text{ m}^2 \cdot \text{s}^{-1}$  ( $2.4 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$  in the case of PN<sub>5k</sub>): the presence of remaining homopolymers is therefore neglectable.[46] Average number molecular weights (M<sub>n</sub>) and dispersity (Đ) were evaluated by size exclusion chromatography (SEC) in DMF or THF. As shown in **Figure 1b**, a clear shift of elution peak of PN<sub>5k</sub>-*b*-PD<sub>5k</sub> and P(N<sub>5k</sub>-*stat*-D<sub>5k</sub>) towards high molecular weight is evidenced compared to macro-RAFT agent PN<sub>5k</sub> in SEC chromatograms. However, in the cases of PD homopolymers and PN-*b*-PD block copolymers the interaction between the nitrogen-containing polymer and SEC columns hampers the precise determination of

molecular weights and induces the presence of a slight shoulder in DMF. [46] As a result, a clear shift of elution peak for block copolymers comprising a high content of PD compared to their macro-RAFT agent PN homopolymers is not always detected [51] whereas this phenomenon is considerably weakened in the case of  $P(N_{5k}\text{-stat-}D_{5k})$ . The main characteristics of synthesized polymers are summarized in **Table 1**.



**Figure 1.** (a)  $^1\text{H}$  NMR spectrum of  $P(N_{5k}\text{-stat-}D_{5k})$  in  $\text{D}_2\text{O}$  and (b) SEC traces of  $\text{PN}_{5k}$ ,  $\text{PN}_{5k}\text{-}b\text{-PD}_{5k}$  and  $P(N_{5k}\text{-stat-}D_{5k})$  in DMF (containing 10 mM LiBr).

**Table 1.** (Co)polymers composition, glass transition temperature and average molecular weights

(Co)polymer	$\text{DP}_N:\text{DP}_D^a$	$T_g^a$	$M_{n,\text{NMR}}^a$	$M_{n,\text{SEC}}^c$	$M_{w,\text{SEC}}^c$	$\mathbf{D}^c$
		( $^{\circ}\text{C}$ )	( $\text{g}\cdot\text{mol}^{-1}$ )	( $\text{g}\cdot\text{mol}^{-1}$ )	( $\text{g}\cdot\text{mol}^{-1}$ )	

PD <sub>5k</sub>	0:26	30	4810	6900 (4210)	8500 (4600)	1.21 (1.09)
PN <sub>5k</sub>	41:0	104	6130	6080 (4720)	6400 (4900)	1.06 (1.03)
PN <sub>2k</sub> - <i>b</i> -PD <sub>8k</sub>	21:34	52	12580	9860 (8940)	13000 (10200)	1.32 (1.14)
PN <sub>5k</sub> - <i>b</i> -PD <sub>5k</sub>	47:27	55	11090	8340 (6860)	9100 (7960)	1.09 (1.16)
PN <sub>8k</sub> - <i>b</i> -PD <sub>2k</sub>	79:8	72	10220	10720 (9120)	11600 (9200)	1.08 (1.01)
P(N <sub>5k</sub> - <i>stat</i> -D <sub>5k</sub> )	42:25	76	9460	12060 (7490)	15100 (10050)	1.25 (1.34)

<sup>a</sup>DP<sub>N</sub>:DP<sub>D</sub> and  $M_{n,NMR}$  calculated from <sup>1</sup>H NMR spectra by comparing integration area of peak **a** corresponding to methyl protons of dodecyl end group at 0.88 ppm and those of signals of **l** and **p** at 3.92 and 3.33 ppm, corresponding to PN (peak **l**) and PD (peak **p**), respectively. <sup>b</sup>T<sub>g</sub> measured from DSC measurements, heating rate: 10 °C·min<sup>-1</sup>, <sup>c</sup>M<sub>n,SEC</sub>, M<sub>w,SEC</sub> and Đ determined from SEC analyses in DMF with 10 mM LiBr or in THF containing 2 vol.% TEA (the value obtained in THF is given in parenthesis).

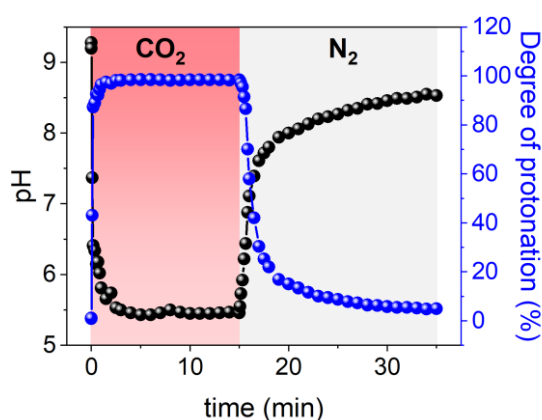
### 3.2. CO<sub>2</sub>-switchable thermoresponsiveness of PD homopolymer.

PD homopolymer, a weak polyelectrolyte, is fully soluble in water whatever the pH at low temperature. The apparent pK<sub>a</sub> value of PD<sub>5k</sub> is 7.25 determined by pH-titration curve (**Figure S11**), and this value is close to the pK<sub>a</sub> values measured for PDEAEMA and PDMAEMA at 7.5 and 7.4 respectively. [22-23] CO<sub>2</sub>-responsiveness of PD<sub>5k</sub> homopolymer was confirmed by recording the pH value during successively bubbling CO<sub>2</sub> and N<sub>2</sub> processes (**Figure 2**). The degree of protonation was calculated by the following equation:

$$\text{Degree of protonation} = \frac{1}{1+10^{pH-pK_a}} \quad (\text{eq. 6})$$

The initial pH value of PD<sub>5k</sub> solution (1 wt.%) obtained by direct dissolution of polymer in ultra-pure water is 9.3 corresponding to a degree of protonation equal to 0.92%. The addition of CO<sub>2</sub> induces a rapid drop of pH down to 5.5 after 2.5 min with no longer further evolution, while the

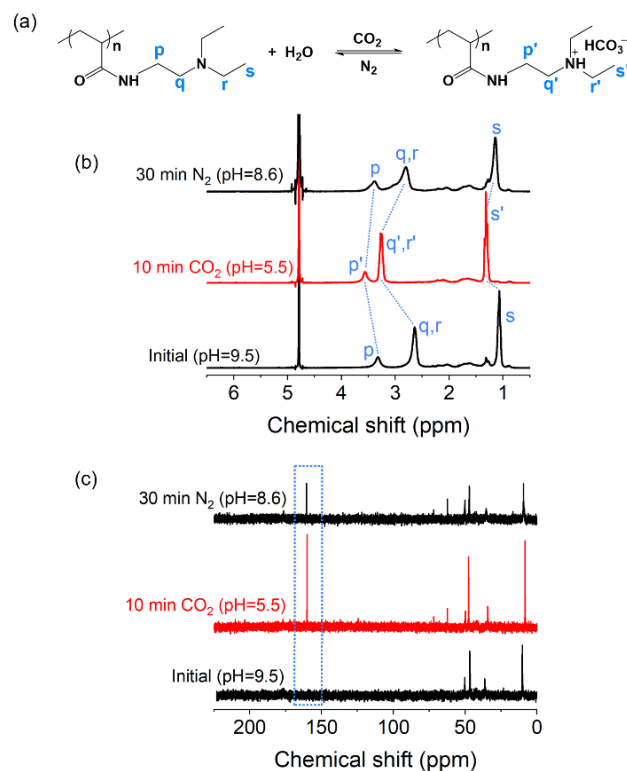
degree of protonation rises to around 99%. When N<sub>2</sub> was bubbled for 20 min, the pH of PD<sub>5k</sub> solution gradually increases to 8.5, while the degree of protonation returns to 5%. Moreover, these variations in pH and/or degree of protonation upon alternately introduction of CO<sub>2</sub>/N<sub>2</sub> is repeated for at least two cycles (**Figure S12**), it turns out that CO<sub>2</sub>-responsiveness is reversible, making CO<sub>2</sub> superior to the classical pH-sensitive system in which an acid or base solution (e.g. HCl or NaOH) is added to tune the reversibility of the system. Consequently, salt (e.g. NaCl) is produced and accumulated after each cycle.



**Figure 2.** pH value and degree of protonation of PD<sub>5k</sub> in water upon alternately bubbling CO<sub>2</sub>/N<sub>2</sub> ([PD<sub>5k</sub>] = 0.1 wt.%, gas flow rate: 2 mL·min<sup>-1</sup>).

To further assess the interaction between CO<sub>2</sub> and PD<sub>5k</sub> homopolymer, we conducted <sup>1</sup>H and <sup>13</sup>C NMR spectra in D<sub>2</sub>O at 25 °C before and after bubbling CO<sub>2</sub> and N<sub>2</sub>. As shown in **Figure 3b**, before exposure to CO<sub>2</sub>, PD<sub>5k</sub> homopolymer presents three prominent signals at 3.32, 2.64 and 1.07 ppm which are attributed to -CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>H<sub>3</sub>)<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>/-CH<sub>2</sub>CH<sub>2</sub>N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub> and -CH<sub>3</sub> groups, respectively. Upon 10 min bubbling CO<sub>2</sub>, PD<sub>5k</sub> is protonated, which shifts corresponding signals downfield to 3.56, 3.27 and 1.32 ppm, respectively. Meanwhile, the signal at 160 ppm in <sup>13</sup>C NMR spectrum after CO<sub>2</sub> treatment reveals the formation of ammonium bicarbonate (see **Figures 3a** and **3c**).



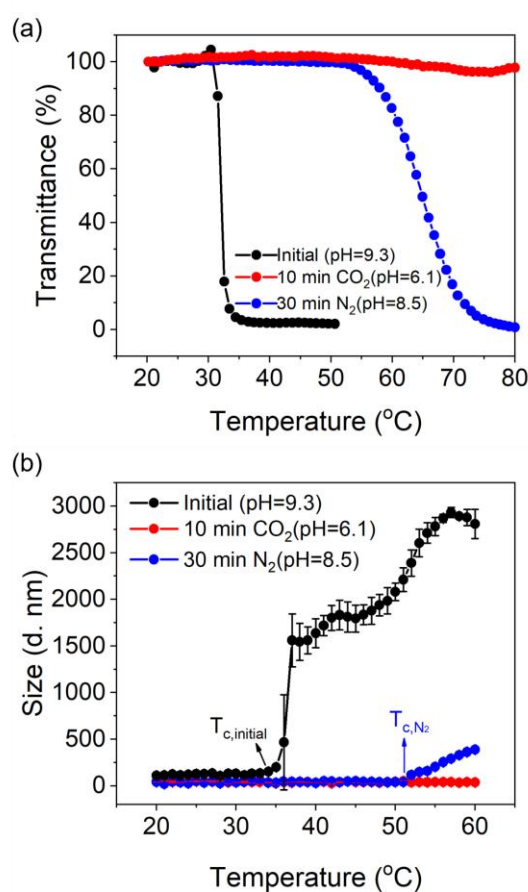


**Figure 3.** (a) Chemical reaction of PD homopolymer with  $\text{CO}_2$  in water. (b)  $^1\text{H}$  and (c)  $^{13}\text{C}$  NMR spectra of PD $_{5k}$  in  $\text{D}_2\text{O}$  recorded at 25 °C upon alternate exposure to  $\text{CO}_2/\text{N}_2$  ( $[\text{PD}_{5k}] = 1 \text{ wt.}\%$ , gas flow rate:  $10 \text{ mL}\cdot\text{min}^{-1}$ ).

As expected in the case of tertiary amine, no formation of carbamate is evidenced here. [18,19,21] After 30 min bubbling  $\text{N}_2$ , chemical shifts of these peaks return to 3.39, 2.80 and 1.14 ppm, respectively. This incomplete recovery to the initial position might be ascribed to the existence of residual bicarbonate salt corresponding to a residual protonation degree equal to 5% as mentioned above.

Coexistence of acrylamide and tertiary amine groups within PD $_{5k}$  structure endows the homopolymer with dual thermo- and pH-responsive properties. Thermoresponsiveness and the intensity of response are determined by the degree of protonation of diethylamino groups tuned by system pH. Considering  $\text{CO}_2$ -induced protonation-deprotonation transition of diethylamino groups and its further effect on solubility of PD chains, turbidimetry measurements were therefore

conducted to study the thermoresponsive properties of PD<sub>5k</sub> solution before and after bubbling CO<sub>2</sub> or N<sub>2</sub> (**Figure 4a**). Before exposure to CO<sub>2</sub> (pH = 9.3), diethylamino groups in PD<sub>5k</sub> are not protonated. Thus, PD<sub>5k</sub> is thermoresponsive and displays a LCST behavior with a measured T<sub>c</sub> around 32 °C. After bubbling CO<sub>2</sub>, the majority of diethylamino groups become protonated, transmittance remains constant around 100% as PD<sub>5k</sub> solution was heated from 20 to 80 °C, indicating that PD<sub>5k</sub> is no longer thermoresponsive. Upon N<sub>2</sub> treatment, LCST behavior of PD<sub>5k</sub> is recovered with a higher T<sub>c</sub> (65 °C) compared to the value in initial solution, this phenomenon is ascribed to the remaining presence of ammonium bicarbonate. [39,44]



**Figure 4.** Plots of (a) Transmittance and (b) Number-distributed size versus temperature for PD<sub>5k</sub> aqueous solution before and after bubbling CO<sub>2</sub> or N<sub>2</sub> ([PD<sub>5k</sub>] = 1 wt.%, gas flow rate: 10 mL·min<sup>-1</sup>).

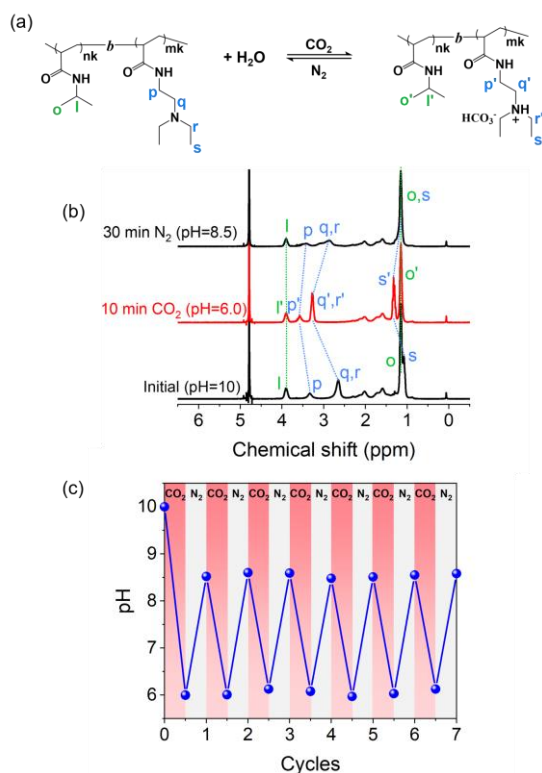
CO<sub>2</sub>-switchable thermoresponsiveness of PD<sub>5k</sub> was further investigated by DLS measurements. At 20 °C, PD<sub>5k</sub> chains in the absence of CO<sub>2</sub> are highly soluble in water, an increase of temperature induces the collapse of PD<sub>5k</sub> chains. As a result, a drastic change in hydrodynamic size takes place as shown in **Figure 4b**: the hydrodynamic diameter of PD<sub>5k</sub> solution starts to increase at 34 °C, then undergoes a rapid rise and reaches 1560 nm at 37 °C. The formation of large colloidal structure is not observed after bubbling CO<sub>2</sub> for 10 min. Upon 30 min bubbling N<sub>2</sub>, most of charged tertiary amine groups in PD<sub>5k</sub> return to deprotonated state, thus, thermoresponsive properties of PD<sub>5k</sub> are recovered with a shift of T<sub>c</sub> towards higher temperatures (51 °C) as reported in Zhang's work. [45] The further repeated addition of CO<sub>2</sub>/N<sub>2</sub> allows to change the protonation state of tertiary amine functions in a complete reversible way without modification of aggregation properties.[20] In comparison, performing such an addition by the alternative addition of HCl and NaOH led to the formation of NaCl. Its presence results in a progressive shift of measured T<sub>c</sub> and a modification of aggregation process measured through turbidimetry measurements (**Figure S13**)

The above investigation on PD homopolymer reveals a strong dependence of thermoresponsive properties upon the protonation degree of polymer chains. In the following we aim at evaluating how the insertion of PD within PN structures affect the thermoresponsiveness of obtained copolymers and how this thermoresponsiveness can be tuned by CO<sub>2</sub> addition/removal. For this, copolymers with a constant molecular weight (10k) but different compositions or architectures (statistical and block) will be compared.

### 3.3. Reversible CO<sub>2</sub>/N<sub>2</sub>-response of PN-*b*-PD block copolymers.

*Solubility properties.* CO<sub>2</sub>-responsiveness of PN-*b*-PD was firstly analysed by <sup>1</sup>H NMR at room temperature. The interaction sites between CO<sub>2</sub> and PN<sub>5k</sub>-*b*-PD<sub>5k</sub> are evidenced in **Figure 5b**. Chemical shifts of -CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>CH<sub>2</sub>- and -CH<sub>2</sub>CH<sub>3</sub>, as well as -CH<sub>3</sub> in PD<sub>5k</sub> block upon 10 min bubbling CO<sub>2</sub> move from 3.33, 2.65 and 1.08 ppm to 3.58, 3.28 and 1.33 ppm, then return to 3.43,

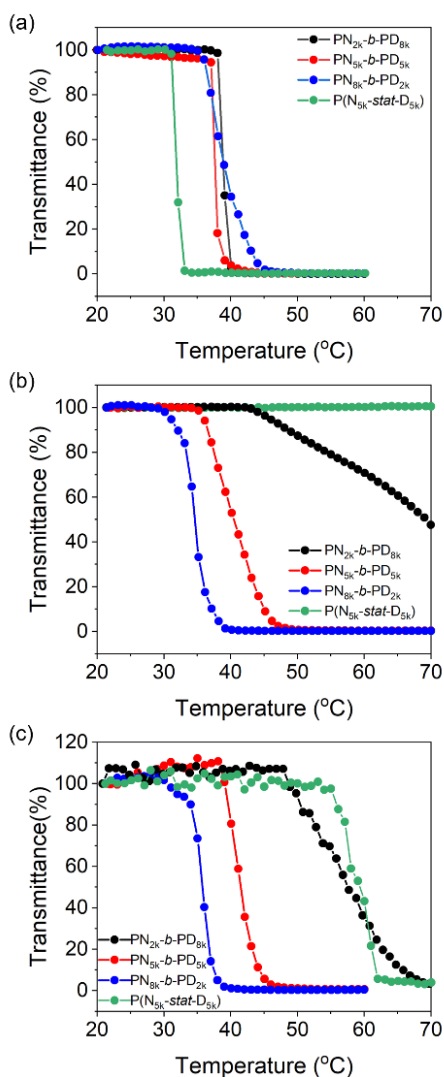
2.87 and 1.15 ppm respectively after 30 min N<sub>2</sub> aeration. Moreover, no shifts of signals at 3.89 and 1.15 ppm corresponding to -CH(CH<sub>3</sub>)<sub>2</sub> and -CH(CH<sub>3</sub>)<sub>2</sub> in PN<sub>5k</sub> block are observed during the whole process of gas treatment. Similar phenomena were also detected in the cases of PN<sub>2k</sub>-*b*-PD<sub>8k</sub> and PN<sub>8k</sub>-*b*-PD<sub>2k</sub> (see **Figure S14**).



**Figure 5.** (a) Chemical reaction of PN-*b*-PD with CO<sub>2</sub> in water. (b) <sup>1</sup>H NMR spectra of PN<sub>5k</sub>-*b*-PD<sub>5k</sub> in D<sub>2</sub>O recorded at 25 °C before and after exposure to CO<sub>2</sub> or N<sub>2</sub>. (c) pH changes of PN<sub>5k</sub>-*b*-PD<sub>5k</sub> system measured at 25 °C upon seven cycles of alternately bubbling CO<sub>2</sub>/N<sub>2</sub>. ([PN<sub>5k</sub>-*b*-PD<sub>5k</sub>] = 1 wt.%, gas flow rate: 10 mL·min<sup>-1</sup>, bubbling CO<sub>2</sub> and N<sub>2</sub> for 10 and 30 min, respectively)

During bubbling CO<sub>2</sub>/N<sub>2</sub> process, the initial pH value around 10, then decreases to 6 upon CO<sub>2</sub> aeration and returns to 8.5 after 30 min N<sub>2</sub> treatment. In the case of PN<sub>5k</sub>-*b*-PD<sub>5k</sub> solution, regardless of the initial pH at 10, its pH value switches between 6.0 and 8.5 after cyclically purging CO<sub>2</sub> (for 10 min) and N<sub>2</sub> (for 30 min) respectively (**Figure 5c**). In the case of PN<sub>2k</sub>-*b*-PD<sub>8k</sub> and PN<sub>8k</sub>-*b*-PD<sub>2k</sub>, a pH window equal to 6.0 - 8.4 and 5.4 - 8.4 respectively upon alternating CO<sub>2</sub>/N<sub>2</sub> were measured

(**Figure S15**). These results suggest that CO<sub>2</sub>-switchable pH change of PN-*b*-PD solution is fully reversible. [5-7] A similar effect could be obtained by successive addition of acid and base but in this case the repeated cycles would lead to a progressive increase of the ionic strength of the solutions and to an alteration of the thermal properties of the polymers.



**Figure 6.** Plots of transmittance *versus* temperature for block and statistical copolymer aqueous solutions (a) before, (b) after bubbling CO<sub>2</sub> and (c) after bubbling N<sub>2</sub> ([P(N-*co*-D)] = 1 wt.%, heating rate: 1 °C·min<sup>-1</sup>, gas flow rate: 10 mL·min<sup>-1</sup>, bubbling CO<sub>2</sub> and N<sub>2</sub> for 10 and 30 min, respectively).

CO<sub>2</sub>-switchable thermoresponsiveness of P(N-*co*-D) system was further studied by turbidimetry measurements (**Table 2**). Initially, without CO<sub>2</sub> stimulation, PN and PD blocks are both thermoresponsive with a common T<sub>c</sub> equal to 32 °C. From the plot of transmittance vs temperature, only one phase transition temperature was detected whatever copolymer composition or architecture. As shown in **Figure 6a**, the transmittances of all PN-*b*-PD block copolymer solutions start to decrease around 37 °C upon heating and they all undergo a soluble-to-insoluble transition in a relatively narrow temperature range of 38 ± 1 °C, suggests that copolymer composition has a slight effect on T<sub>c</sub> for block copolymers. This value, higher than the one measured for PN and PD homopolymers, may be related to a cooperative contribution from both blocks triggering thermal phase transition, which has also been reported in the cases of poly(*N*-isopropylacrylamide)-*block*-poly(*N*-vinylcaprolactam) (PNIPAM-*b*-PVCL) and P(NIPAM-*stat*-VCL). [52-53] Interestingly, T<sub>c</sub> value for the statistical copolymer P(N<sub>5k</sub>-*stat*-D<sub>5k</sub>) solution is lower than the one measured for the block copolymer PN<sub>5k</sub>-*b*-PD<sub>5k</sub> having a with similar composition, i.e. 31 and 37°C respectively. The copolymer architecture has therefore a strong influence on the cloud point values: the statistical insertion of tertiary amine groups disturbs the interaction between the acrylamide moieties and water, resulting in a lowering of measured T<sub>c</sub>.

After exposure to CO<sub>2</sub>, the pH of polymer solution decreases around 6. Only PN chains remain thermoresponsive, while PD protonated moieties promotes the solubility of whole copolymer in water. As observed in **Figure 6b**, the onset points of transmittance in the cases of PN<sub>2k</sub>-*b*-PD<sub>8k</sub>, PN<sub>5k</sub>-*b*-PD<sub>5k</sub> and PN<sub>8k</sub>-*b*-PD<sub>2k</sub> shift to 43, 35 and 30 °C respectively. The insertion of a charged hydrophilic block induces an increase of measured T<sub>c</sub> compared to PN homopolymer. Additionally, in the case of PN<sub>2k</sub>-*b*-PD<sub>8k</sub> slightly turbid solutions were obtained upon T<sub>c</sub>. This suggests that, during the aggregation process, smaller colloidal structures are formed than for PN<sub>8k</sub>-*b*-PD<sub>2k</sub> and PN<sub>5k</sub>-*b*-PD<sub>5k</sub>. Moreover, the effect of architecture appears to be of primary importance. Indeed, PN<sub>5k</sub>-*b*-PD<sub>5k</sub> remains thermoresponsive, whereas statistical copolymer P(N<sub>5k</sub>-*stat*-D<sub>5k</sub>) loses

thermoresponsiveness in the same studied temperature range. After bubbling N<sub>2</sub> (**Figure 6c**), all systems regain their heat-sensitive properties (pH ~ 8.5). However, due to the presence of residual ammonium bicarbonate (protonation degree around 5%), none of the systems can entirely recover to the initial values.

These results indicate that copolymer composition and architecture play a crucial role in CO<sub>2</sub>-tunable phase transition temperature. Moreover, the transition temperature of these copolymers can also be tuned by changing the level of protonation of PD moiety.

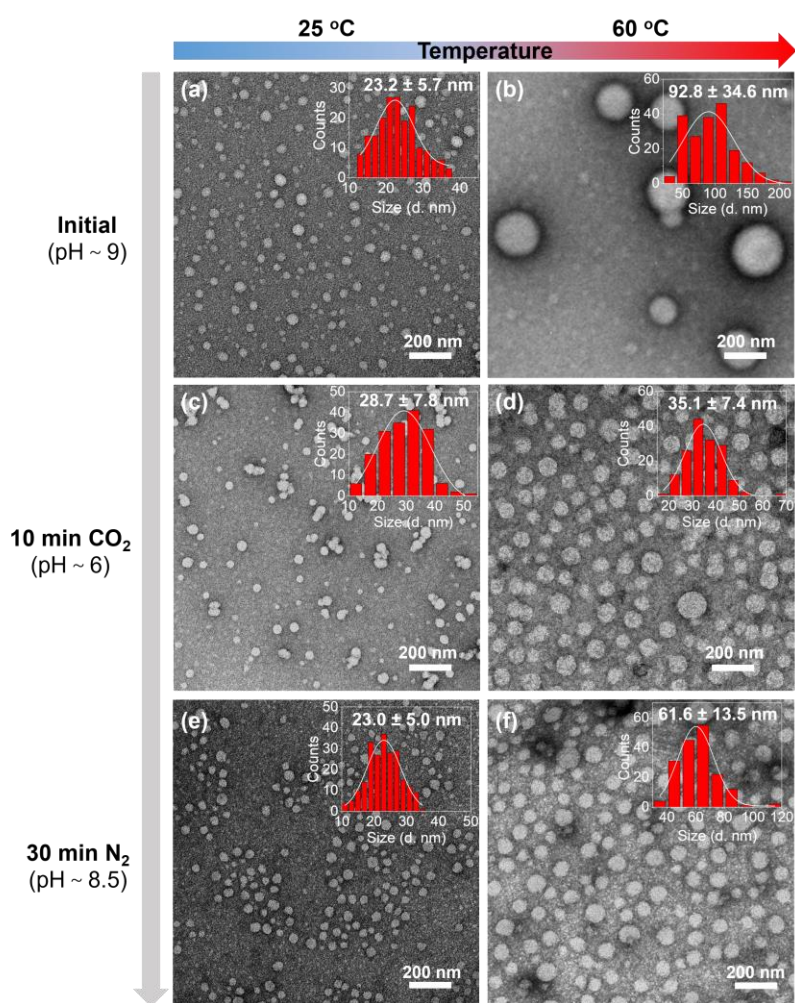
**Table 2.** Measurements of T<sub>c</sub> (°C) for (co)polymers from turbidimetry measurements.

(Co)polymer	Initial		After bubbling CO <sub>2</sub>		After bubbling N <sub>2</sub>	
	(pH ~ 10)		(pH ~ 6)		(pH ~ 8.5)	
	T <sub>c,onset</sub> <sup>a</sup>	T <sub>c,ip</sub> <sup>b</sup>	T <sub>c,onset</sub>	T <sub>c,ip</sub>	T <sub>c,onset</sub>	T <sub>c,ip</sub>
PD <sub>5k</sub>	30 <sup>d</sup>	32 <sup>d</sup>	/ <sup>c</sup>	/ <sup>c</sup>	54	65
PN <sub>2k</sub> - <i>b</i> -PD <sub>8k</sub>	38	39	43	/ <sup>c</sup>	48	/ <sup>c</sup>
PN <sub>5k</sub> - <i>b</i> -PD <sub>5k</sub>	37	38	35	40	38	41
PN <sub>8k</sub> - <i>b</i> -PD <sub>2k</sub>	35	37	30	34	30	36
P(N <sub>5k</sub> - <i>stat</i> -D <sub>5k</sub> )	31	32	/ <sup>c</sup>	/ <sup>c</sup>	56	60

<sup>a</sup>T<sub>c,onset</sub> determined from temperature where transmittance of copolymer solution starts to decrease.

<sup>b</sup>T<sub>c,ip</sub> determined from the inflection point of transmittance curve. <sup>c</sup>Not observed in the studied temperature range of 20 - 70 °C. <sup>d</sup>Initial pH value of PD<sub>5k</sub> solution was 9.3. Turbidimetry measurements were performed with a heating rate of 1 °C·min<sup>-1</sup>. Gas flow rate was 10 mL·min<sup>-1</sup>, 10 and 30 min for bubbling CO<sub>2</sub> and N<sub>2</sub> respectively.

*Aggregation properties.* The modifications of the behaviour in solution of these polymers induced by changes in temperature are then studied for the different compositions of the synthesized polymers. TEM, DLS and SAXS measurements were carried out to investigate the structure and the size of aggregates.

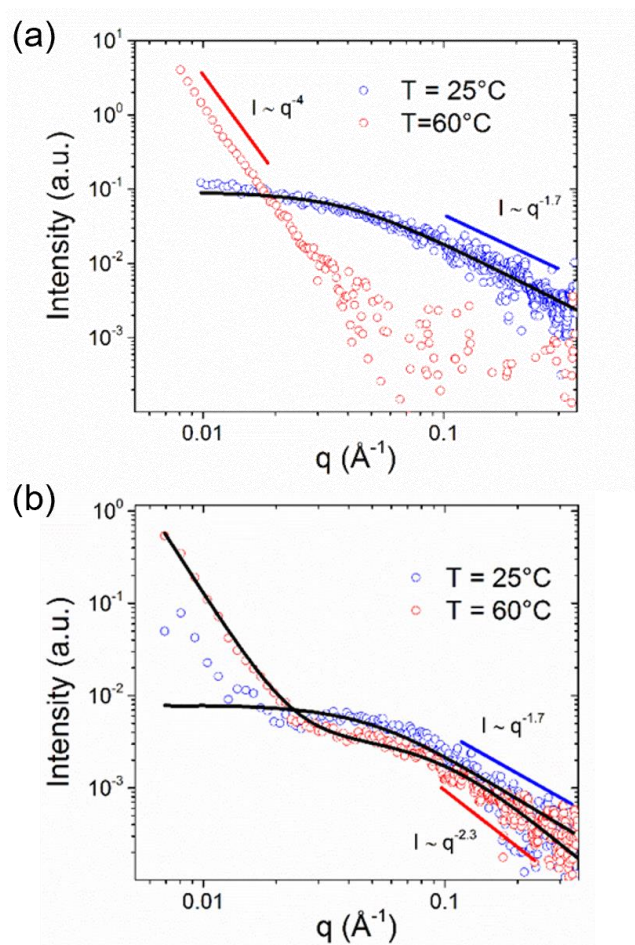


**Figure 7.** TEM images of  $\text{PN}_{5\text{k}}\text{-}b\text{-}\text{PD}_{5\text{k}}$  aggregates formed before, after bubbling  $\text{CO}_2$  and after bubbling  $\text{N}_2$  at 25 and 60 °C ( $[\text{PN}_{5\text{k}}\text{-}b\text{-}\text{PD}_{5\text{k}}] = 0.1 \text{ wt.}\%$ , gas flow rate:  $10 \text{ mL}\cdot\text{min}^{-1}$ , 10 and 30 min for bubbling  $\text{CO}_2$  and  $\text{N}_2$  respectively. All samples were stained with 2 wt.% uranyl acetate before TEM observation).



At low pH the polymer P(N<sub>5k</sub>-stat-D<sub>5k</sub>) remains fully soluble and no aggregates can be evidenced. At pH 9.6, light scattering measurement evidences the formation of the large aggregates that quickly precipitates (**Figure S16**). Therefore, the studies on self-assemblies' properties were only conducted on block copolymers.

At pH ~ 10 and 25°C ( $T < T_c$ ), PN<sub>5k</sub>-*b*-PD<sub>5k</sub> forms spherical structure with an average diameter of  $23.2 \pm 5.7$  nm as evidenced by TEM images (**Figure 7a**). SAXS curves indicate the presence of nano-objects with a radius of gyration ( $R_g$ ) equal to  $3.5 \pm 1$  nm (**Figure 8a**). Interestingly the Guinier region is followed by a power-law trend  $I(q) \sim q^{-\alpha}$ , with  $\alpha = 1.6$  indicating swollen chains in good solvent.[50] These radii of gyration have already been reported for assemblies of amphiphilic PNIPAM described as hairy micelles.[53] A model for star-like polymers fits well our data: by fixing  $R_g$  value, the number of arms is found equal to 1.5, indicating the coexistence of star-like nano-objects and free polymer chains. The hydrodynamic radius i.e.  $24 \pm 5$  nm (**Figure S17**) is in accordance with what found in the literature.[55] When temperature is increased to 60 °C (pH 10), SAXS profile completely changes and large structures are formed in solution (**Figure 8a**). Indeed, no Guinier region is observed, the signature of the polymeric nature is lost at high- $q$  as the polymer is dehydrated and densely packed and only an extended Porod region with a slope of -4 is present : at a temperature above  $T_c$ , both PN and PD chains collapse and become insoluble in water. Spherical aggregates with an average diameter equal to  $92.8 \pm 34.6$  nm and a hydrodynamic diameter equal to  $170 \pm 40$  nm are thus detected by TEM and DLS measurements respectively (**Figure 7b** and **Figure S17**).



**Figure 8.** Small-angle X-ray scattering (SAXS) patterns recorded for 1 wt.% aqueous dispersion of PN<sub>5k</sub>-*b*-PD<sub>5k</sub> at 25 and 60 °C (a) before (pH ~ 10) and (b) after bubbling CO<sub>2</sub> (pH ~ 6). CO<sub>2</sub> flow rate: 10 mL·min<sup>-1</sup>, bubbling time: 10 min).

After exposure to CO<sub>2</sub> during 10 min, the pH of solution drops down to 6.0 and only PN group remains thermoresponsive while PD is in its protonated form. At 25 °C, spherical aggregates are still evidenced by TEM images (**Figure 7c**) with slightly larger diameter i.e.  $28.7 \pm 7.8$  nm than the one measured without CO<sub>2</sub> treatment ( $D_h = 23 \pm 5$  nm). Electrostatic repulsion among the positive charged PD chains might induce the expansion of aggregates. SAXS curves show the main features already observed at pH 10, a  $R_g$  of few nanometres ( $2.7 \pm 0.8$  nm) is slightly higher than what expected for free polymer chains in solution, and a high  $q$  trend of chains swollen by the solvent (**Figure 8b**). A fit using a form factor of star-polymers indicates a low aggregation number (1.5)

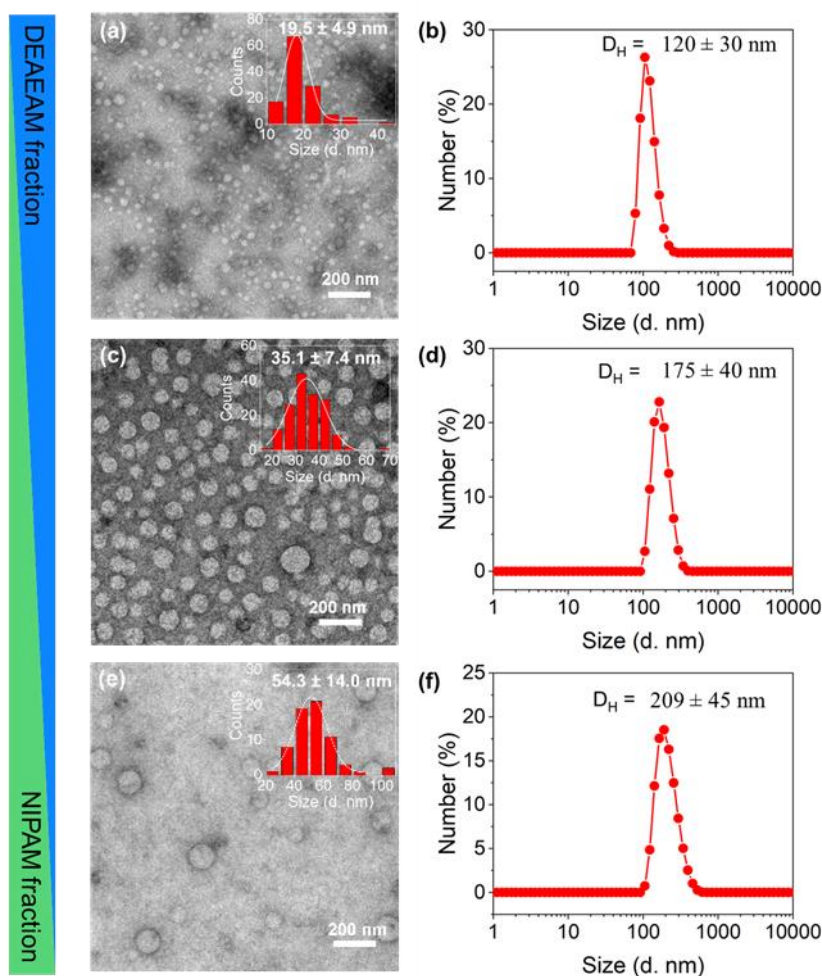
and coexistence of small star-like micelles and free polymer chains. On heating to 60 °C, the sample gets turbid due to the presence of large spherical aggregates with a diameter equal to  $35.1 \pm 7.4$  nm determined from TEM measurements (**Figure 7d**), which is significantly smaller than the ones measured prior to the exposure to CO<sub>2</sub>. Indeed, PN chains collapse upon increasing temperature, whereas protonated PD chains remain hydrated and help to stabilize smaller colloidal structures in solution. These assemblies exhibit larger size than what measured by SAXS technology. We use a model for clusters of polymer chains developed by Hammouda [50] and find a correlation length for the polymer of  $1 \pm 0.3$  nm and a power-law exponent  $2.3 \pm 0.1$  indicating polymers are in semi-dilute solutions. Interestingly, the polymeric features of the assemblies formed above LCST are not completely lost for this sample, which is not the case for the sample without CO<sub>2</sub> stimulation.

Upon 30 min bubbling N<sub>2</sub>, the system pH returns to 8.5, thermoresponsiveness of PD block is recovered. At 25 °C, the diameter of spheres is almost recovered to the initial size through TEM or DLS observations ( $23.0 \pm 5.0$  nm, **Figure 7e**). At 60 °C, PD shrinks along with the collapse of PN block and spheres with an average diameter of  $61.6 \pm 13.5$  nm in dried state are detected (**Figure 7f**).

The aggregates obtained show no significant morphological change for a chosen pH and temperature condition after a cooling/heating cycle or after CO<sub>2</sub>/N<sub>2</sub> bubbling cycle (**Figure S18 and S19**).

Similar trends have been observed concerning the behavior of PN<sub>2k</sub>-*b*-PD<sub>8k</sub> and PN<sub>8k</sub>-*b*-PD<sub>2k</sub> in solution (**Figures S20 and S21**). The diameter of the aggregates in presence of CO<sub>2</sub> at 60 °C seems to decrease significantly with an increase of PD content (**Figure 9**), this could be related to the ability of the protonated chains of PD to stabilize colloids by electrostatic effect: an increase of their proportion favors the stabilization of colloids with smaller size. The evolution of the hydrodynamic diameters shows the same tendency as those observed in the electron microscopy images (**Figure 9**). As expected the values of the hydrodynamic diameters are significantly higher than the

measurements obtained in a dried state in TEM. Moreover, these high values suggest the presence of polydisperse aggregates in solution.

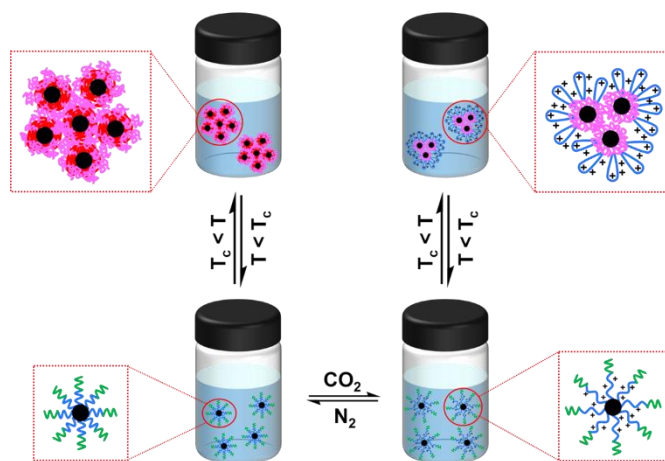


**Figure 9.** TEM images and number-distributed hydrodynamic size of (a, b)  $\text{PN}_{2k}\text{-}b\text{-}\text{PD}_{8k}$ , (c, d)  $\text{PN}_{5k}\text{-}b\text{-}\text{PD}_{5k}$  and (e, f)  $\text{PN}_{8k}\text{-}b\text{-}\text{PD}_{2k}$  aggregates formed at 60 °C after exposure to  $\text{CO}_2$  ( $[\text{PN-}b\text{-}\text{PD}] = 0.1 \text{ wt.}\%$ ,  $\text{CO}_2$  flow rate:  $10 \text{ mL}\cdot\text{min}^{-1}$ , bubbling time: 10 min).

Based on the above results, a mechanism of the formation of different colloids observed at different studied temperatures with/without  $\text{CO}_2$  stimulation can be proposed as follows (**Scheme 4**). In initial solution (pH  $\sim 9$ ), both PN and PD blocks are thermoresponsive. At 25 °C ( $T < T_c$ ), block copolymer PN-*b*-PD forms spherical micelles with a core of hydrophobic  $-\text{C}_{12}\text{H}_{25}$  group and

a corona of PN and PD blocks. This structure is similar to the ones previously observed in the case of PNIPAM-based surfactant with a similar hydrophobic alkyl chain as the terminal group.[55]

At 60 °C, both PN and PD blocks dehydrate and collapse, the formed dense and hydrophobic structures are prone to aggregate in aqueous solution (**Scheme 4 left**). However, the remaining carboxylate terminal function might explain the relative colloidal stability of such structures. Upon CO<sub>2</sub> treatment (pH ~ 6), PD block is protonated, the electrostatic repulsion among charged PD chains causes an expansion of corona region, and induces a slight swelling of spherical nanoparticles (**Scheme 4 bottom**). When temperature increases above T<sub>c</sub>, PN chains shrink whereas charged PD block remains hydrated. Therefore, the copolymer forms aggregates, in which -C<sub>12</sub>H<sub>25</sub> group and dehydrated PN forms the core, while charged PD block forms the corona. In agreement with the proposed mechanism, an increase in zeta potential from +20 to +50 mv is measured when the temperature increase from 20 to 60°C. The observed size is compatible with the possible association of several small aggregates (**Scheme 4 right**).



**Scheme 4.** Schematic illustration of self-organization of PN-*b*-PD in water triggered by temperature and CO<sub>2</sub>.

#### 4. Conclusions

In this article, the insertion of DEAEAM into PNIPAM structure by Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization was studied and makes it possible to obtain a series of copolymers with various compositions and architectures. CO<sub>2</sub> addition/removal induces a reversible protonation/deprotonation transition of DEAEAM units, which results in tunable thermoresponsive properties of the synthesized polymers. For a given composition, the architecture of the copolymer (statistical, block) critically affects the cloud point temperature and the structure of colloids obtained in solution. The statistical copolymer is no longer thermoresponsive upon CO<sub>2</sub> exposure, whereas block one with a similar composition keeps its responsiveness, which depends on the protonation degree. Additionally, these results illustrate how it is possible through chemical engineering and the use of CO<sub>2</sub> as an innovative and ecological trigger to easily and reversibly modulate the properties (solubility, self-assembly behavior) of these smart materials in aqueous media. [4,5,56]

## **Author Contributions**

N. Lauth-de Viguierie and J.-D. Marty conceived the idea and supervised this project. F. Yin performed the experiments and wrote the first draft of the paper. B. Lonetti contributed to the SAXS measurement and analyses. All authors participated in evaluating the results and commented on the manuscript.

### **Conflicts of interest**

There are no conflicts to declare.

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