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- Effect of isothermal aging on Poly(N-isopropylacrylamide): Correlation between 1
- chemical structure and phase transition temperature property. 2
- 3
- Camille Mathieu<sup>a,b</sup>, Samar Issa<sup>a\*</sup>, Emmanuel Richaud<sup>b</sup> 4
- <sup>a</sup> Ecole de Biologie Industrielle EBI, UPR EBInnov<sup>®</sup>, 49 Avenue des Genottes CS90009 95895, Cergy-Pontoise, 5 6 France
- 7 <sup>b</sup> Laboratoire Procédés et Ingénierie en Mécanique et Matériaux (PIMM), Arts et Metiers Institute of Technology,
- 8 CNRS, Cnam, Hesam University, 151 Boulevard de l'Hôpital, 75013 Paris, France
- 9 \*Corresponding author: s.issa@hubebi.com
- 10 11

Ecole de Biologie Industrielle - EBI, UPR EBInnov®, 49 Avenue des Genottes CS90009 95895, Cergy-Pontoise, France

### 12 Abstract Poly(N-isopropylacrylamide) (PNIPAm) is a stimuli-responsive polymer with wide

- applications as a scaffold in biomedical applications. This study presents the PNIPAm's 14 15 thermoresponsive behavior during storage and its stability in potential pharmaceutical formulations as a drug delivery excipient. Therefore, the turbidity curves of linear PNIPAm 16 aqueous solutions are investigated before and after its thermal oxidation under air at 120°C and 17 18 130°C. First, PNIPAm aqueous phase separation diagram is determined for unaged polymer and then compared to thermally aged samples at two distinct points (1-10% (w/w)). After isothermal 19 aging, the transition temperature has increased with a shift up to 4°C. The correlation between 20 21 PNIPAm's chemical structure and phase transition temperature properties is illustrated in terms of chemical modifications, macromolecular changes as well as the polar/non-polar groups 22 balance. It is observed that the water affinity and glass transition temperature (Tg) have 23
- increased at the macromolecular level. In addition, chains scissions are highlighted and seem to 24 explain the shift of transition temperature. Chemical changes have revealed the presence of 25
- supplementary polar groups induced by aging. This multiscale study suggests a possible 26
- mechanism for the partial thermal oxidation based on all the observations. 27
- 28

#### **Keywords** 29

- Poly(N-Isopropylacrylamide) (PNIPAm), Isothermal aging, Phase transition, Lower Critical 30
- 31 Solution Temperature (LCST), Chain scissions.
- 32

#### 1. Introduction 33

34 The increasing occurrence of chronic diseases has boosted the biologics market. An increase in R&D investments and technological advancements forecast an average of 5.9% annual growth 35

rate of the global biologics market drug administration [1]. Several approaches to drug delivery 36

through modifications (of the drug, its environment and delivery systems) have been developed 37

to improve the efficiency and release of active pharmaceutical ingredients (API) while 38 minimizing its accumulation outside target [2]. 39

Poly(N-isopropylacrylamide) (PNIPAm) is a widely described model polymer for its 40 thermoresponsive properties as a smart polymer. In 1968, Heskins and Guillet have described 41 the PNIPAm's unique properties in aqueous solution, specific phase diagram and Lower 42 Critical Solution Temperature (LCST) around 25-35°C [3]. Aqueous solutions of linear 43 PNIPAm are commonly studied as water-soluble polymers [4]. Changes in LCST can be related 44 to physicochemical as well as thermodynamic factors explaining the PNIPAm's wide 45 applications in biomaterials [5]. In water, the thermoresponsive behavior is related to the 46 equilibrium between hydrophobic (isopropyl) and hydrophilic (amide) groups associations [6]. 47 At temperatures lower than LCST, amide groups are linked to water molecules through 48 hydrogen (H)-bonds. Upon heating, H-bonds are broken, and hydrophobic interactions 49 dominate, resulting in the collapse of polymeric chains, PNIPAm aqueous solutions showed a 50

- coil-to-globule transition [3] [7]. For many years, the mechanism of phase separation and 51 52 physical crosslinking has been successfully investigated [4].
- As PNIPAm's LCST is close to the body's temperature, in biomedical applications, this 53 polymer is considered as "smart" stimuli-sensitive excipient for drug delivery systems, as well 54 as an innovative biomaterial in tissue engineering and stem cell therapy [8]. 55
- According to the International Council for Harmonisation (ICH) Topic Q 1 A (R2) [9], the 56 thermal stability testing of drug substances should be examined. Thermal stability studies 57 should take into consideration storage and shipment thermal conditions limitations. 58
- 59 There have been a few reports describing PNIPAm's thermoresponsive behavior during storage
- under different stress conditions at the solid state (oxygen, temperature, water). Gómez-Galván 60
- and H. Mercado-Uribe have studied soft aging on PNIPAm (1% (w/w)) solution both at 24°C 61
- and 37°C and showed a slight shift (only 1°C) of phase separation temperature.[10]. The 62 hypothesis drafted suggests that such polymer could establish more H-bonds with an aging at 63
- 24°C and form increased polymer-polymer bonds at 37°C [10]. 64
- The purpose of this study is to explore the correlation between PNIPAm's chemical stability 65
- under thermal and longtime aging within accelerated aging temperature (120 and 130°C chosen 66
- to provide a sufficient acceleration of aging) to confirm the potential of PNIPAm's scaffolds in 67 drug delivery. We have studied the modification of phase separation followed by a turbidity 68
- 69 method as well as the attempt to better understand the possible mechanisms underlying the
- observed alterations. In addition, to better understand the possible changes due to polymer-70 71 water interaction, we have conducted a multiscale study of aging based on macromolecular
- 72 analyses including chemical analysis, average molar mass by Gel Permeation Chromatography
- (GPC), glass transition temperature by Differential scanning calorimetry (DSC), and study of 73 the water's uptake in polymer by Dynamic Vapor Sorption (DVS). We investigated and 74 75 compared analysis of thermal aging between PNIPAm and P (N-isopropylacrylamide-co-Acrylic Acid (P (NIPAm-co-AAc)) [11], a random copolymer containing acrylic acid 76 comonomers randomly distributed. P(NIPAm-co-AAc) has been used as a very first model 77
- system oxidized PNIPAm, and a more hydrophilic derivative with known applications in drug 78 79 release where the possible formation of carboxylic acids is later discussed.
- 80 81

## 2. Materials and methods

82

#### 83 Chemicals and reagents. 2.1

PNIPAm (ref 535311, Sigma Aldrich, Merck KGaA, Darmstadt, Germany) and P(NIPAm-co-84

- 85 AAc), with an acrylic content equal to 15 mol% (ref 741930-5G, Sigma Aldrich, Merck KGaA,
- Darmstadt, Germany) are used as received. 86
- Water used in this work is purified with an Elix<sup>®</sup> Essential system. 87
- 88

#### 2.2 Aging conditions of PNIPAm. 89

- PNIPAm is ground to fine powder before storage in pillboxes. Unsealed samples are thermally 90
- 91 aged at 120°C and 130°C, until 90 and 120 days, respectively in a ventilated oven (AP60, SCS,
- 92 Froilabo, Collégien, France).
- 93

#### 94 2.3 PNIPAm and P(NIPAm-co-AAc) characterizations.

- 2.3.1 UV-absorbance and transmittance of light (UV-Vis) analysis. 95
- The changes of the PNIPAm's physical state reflected in the demixing curve and the cloud point 96
- temperature ( $T_{co}$ ) of solutions (with weight fractions ranging from 0.1% (w/w) to 40% (w/w)) 97
- are determined based on turbidity analysis with a UV-Spectrometer (UV-Vis (water) Lambda 98
- 35, Perkin Elmer, Waltham, MA, USA) equipped with a heating device. The utilized 99
- wavelength is 651 nm [12], and the heating rate is 0.3°C.min<sup>-1</sup>. The blank used in all 100

measurements is the purified water using Elix<sup>®</sup> Essential system. All samples are corrected by 101 UV Winlab Software (2012, PerkinElmer Inc., Waltham, MA, USA) and Origin Software 102 (2022, academic version, ©OriginLab Corporation). 103

Additionally, the same procedure is conducted on different aged solutions at 1% (w/w) in water 104

at room temperature ( $20 \pm 2$  °C), at 1 nm intervals in the wavelength range from 200 to 600 nm. 105

- The sample denominations (aging conditions and concentration solution for UV analysis) are 106
- detailed in Table 1 and complementary experimental precisions are provided in Table S1. 107
- 108

Table 1. PNIPAm's sample denominations with aging conditions and concentration 109 solution for UV analysis. 110

Samples	Weight Fraction (wt %)	Aging Temperature (°C)	Aging Time (days)
S1	1	-	0
S2	10	-	0
A1		120	30
A2	1		60
A3			90
A4			120
A5	10		30
A6			60
A7			90
A8			120
A9	1	130	10
A10			30
A11			90
A12	10		10
A13			30
A14			90

111

2.3.2 Attenuated total reflectance -Fourier Transform Infrared (ATR-FTIR) spectroscopy 112 113 analysis.

- Spectra of solid polymer samples are collected on a FTIR Frontier (Perkin Elmer, Waltham, 114 MA, USA). All the spectra are recorded in a dry atmosphere at room temperature  $(20 \pm 2^{\circ}C)$ 115 within a range of 4000 – 650 cm<sup>-1</sup> by averaging 32 scans, with a wavenumber resolution of 116 4 cm<sup>-1</sup>. The ATR effect, as well as the atmospheric contributions from carbon dioxide and water 117 vapor, are corrected using the Origin Software (2022, academic version, ©OriginLab 118 Corporation). 119
- 120
- 2.3.3 Gel Permeation Chromatography (GPC) analysis. 121

Weight average molecular weight (or molar mass) (M<sub>w</sub>) and number average molecular weight 122 (or molar mass) (M<sub>n</sub>) estimation are determined by GPC (GPC, Malvern Corporation, Viscotek 123 124 T3000 - T6000, Worcestershire, United Kingdom) using 2 mg.mL<sup>-1</sup> in tetrahydrofuran (THF) at a flow rate of 1 mL.min<sup>-1</sup> and at 35°C. 5 poly(methyl methacrylate) (PMMA) standard samples 125  $(M_w = 4.7.10^3 - 137.10^3 \text{ g.mol}^{-1})$  are used for calibration. OMNISEC Software (2022, Malvern 126 Panalytical Ltd, Cambridge, United Kingdom) is used for M<sub>w</sub> determination. 127

128

2.3.4 Differential Scanning Calorimetry (DSC) macromolecular analysis. 129

130 DSC analysis is extensively performed on polymer/water solutions (from 5% (w/w) up to 50%

(w/w)) and solid polymer's powder. Samples are analyzed with a Q10 (DSC, TA Instruments -131

Waters, Milford, MA, USA) equipped with a Refrigerated Cooling System (RCS) driven by
QSeries Explorer. Results are investigated using TA Instrument Universal Analysis.

134  $T_{cp}$  (°C) (measured at the peak's onset) and enthalpy of transition  $\Delta H_{transition}$  (J per g of solution,

135 J.g<sup>-1</sup>) are studied on 10 - 15 mg of PNIPAm/water solutions at different concentrations. The

system is cooled at 20°C, for 5 minutes and then heated until 45°C at 1°C.min<sup>-1</sup> scanning rate.

137 Several cycles are performed on each sample to investigate the reversibility of phase transition.

Glass transition temperature  $T_g$  (°C) is studied on 5 mg aged and unaged solid powder polymer

from 25°C to 200°C under nitrogen atmosphere (50 mL.min<sup>-1</sup>). Two cooling and heating cycles
are conducted with a scanning rate of 10°C.min<sup>-1</sup>.

141

142 2.3.5 Dynamic Vapor Sorption (DVS) isothermal analysis.

Polymer-water interaction is investigated by DVS (IGAsorp, Hiden Isochema Corporation,
Warrington, United Kingdom) driven with HISorp (v4, 2017) software. Samples are analyzed at
a constant temperature of 40°C. Samples are obtained by a deposit of polymer solutions using
ethanol in a jar (Fig. S1). The relative humidity (RH) is increased from 0% to 90% (in 5% steps).
The stage time between the different steps is 3 hours. No desorption is studied.

148

## 149 **3. Results and Discussion**

150

## 151 **3.1 Phase separation of the linear PNIPAm.**

Fig. 1a displays the typical change of transmittance of a PNIPAm solution as a function of temperature. The curve shows two distinct regions at low and high temperature. At low temperature, PNIPAm is fully soluble in water which corresponds to 100% transmittance, whereas at high temperature, H- bonds are activated resulting in the precipitation of PNIPAm chains with a subsequent drop in transmittance.

157 The frontier between both regions is a clear transition temperature designated as  $T_{cp}$  (the 158 decrease of 50% in normalized transmittance). For example, the fraction S2 of 10% (w/w) in 159 PNIPAm (Fig. 1a) presents a  $T_{cp}$  of 27.3 ± 0.2°C.

The demixing curve (Fig. 1b) is plotted with T<sub>cp</sub> against the polymer fraction. At low PNIPAm 160 concentration, cloud points sharply decrease with increased concentration and seem to reach the 161 LCST for 50% (w/w) in PNIPAm at 25°C in accordance with the described values in the 162 literature [4]. Below that specific temperature, no concentration dependence is observed, and 163 164 solutions are all miscible. UV-Vis method shows that the analysis at high weight concentration 165 in PNIPAm is difficult to perform and is not discussed in the present work. PNIPAm solutions are prepared in closed pillbox to avoid any water loss during solubilization and homogenization 166 within 48 hours at room temperature (20°C). To further study the phase separation, DSC 167 measurements are conducted between 5% and 60% (w/w) in PNIPAm. Indeed, phase transition 168 phenomena comes with an endothermic peak corresponding to the transition enthalpy  $\Delta H_{\text{transition}}$ . 169 For example, at 10% (w/w) in PNIPAm S2 has a T<sub>cp</sub> of 29.3± 0.3°C (Fig. 1c). For the solution at 170 50% (w/w) in PNIPAm,  $T_{cp}$  is equal to 27.4°C. It is observed that DSC measurements show 171 higher T<sub>cp</sub> values, around 2.6°C compared to UV-Vis measurements. This difference is already 172 explained in the literature by Halperin et. al. exposing the inhomogeneous transition of 173

174 PNIPAm, and the metastable region correlated with the thermodynamic phase transition [4]. In

a further section, DSC analysis is used as well to explain the interactions between water and

176 polymeric chains.



**Fig. 1.** Comparison of  $T_{cp}$  value of unaged PNIPAm with, (a) 50% transmittance analysis of 10% (w/w) solution, (b) Demixing curves, (c) DSC measurement of a 10% (w/w) solution.

In addition to UV-Vis and DSC analysis, the impact of aging on phase transition is described 180 181 with turbidity curves plotted with two distinct concentrations chosen at 1% (w/w) in PNIPAm 182 (in the decreasing zone of the demixing curve) and 10% (w/w) in PNIPAm (in a more stable 183 zone of the demixing curve) (Fig. 2a, 2b, 2c and 2d). For both concentrations, it is observed that turbidity curves have shifted with aging to higher  $T_{cp}$  up to  $4.4 \pm 0.2$  °C for A11 (1% (w/w) in 184 PNIPAm at 130°C within 90 days). Overall, T<sub>cp</sub> increases with aging time (Fig. S2-S3). 185 Therefore, based on UV-Vis analysis, increased PNIPAm aging (temperature and time) is 186 correlated with a higher turbidity curve shift. 187



Fig. 2. Turbidity curves comparing unaged vs. aged samples, (a) 1% (w/w) solutions: S1
without aging; A1 30-days, A2 60-days, A3 90-days and A4 120-days aging at 120°C, (b) 10%
(w/w) solutions: S2 without aging; A5 30-days, A6 60-days, A7 90-days, and A8 120-days
aging at 120°C, (c) 1% (w/w) solutions: A9 10-days, A10 30-days, A11 90-days aging at 130°C,
(d) 10% (w/w) solutions: A12 10-days, A13 30-days, A14 90-days aging at 130° (Table 1).

### 194 3.2 Chemical structure analysis of PNIPAm and P(NIPAm-co-AAc).

196 Fig. 3a shows the main absorption peaks of an unaged PNIPAm. Broad bands around 3434 cm<sup>-1</sup> and 3294 cm<sup>-1</sup> correspond to -NH and -OH stretching vibrations. The designation of the 197 different amide groups is previously reported [13] where the band assigned to -C=O stretching 198 vibration in combination with -C-N bond is observed at 1638 cm<sup>-1</sup>. Moreover, the combination 199 of both N-H bending and -C-N stretching vibrations are observed at 1533 cm<sup>-1</sup>. The asymmetric 200 stretching vibration bands due to the isopropyl CH groups are observed at the range 2800 - 3000 201 cm<sup>-1</sup> as well as 1350 - 1460 cm<sup>-1</sup> corresponding to CH<sub>2</sub> and CH<sub>3</sub> bending vibrations, respectively. 202 A specific peak appears on the P(NIPAm-co-AAc) spectra at 1714 cm<sup>-1</sup> corresponding to the 203 carboxylic acid (-COOH) stretching vibration [14]. All the results match well with the structure 204 of the polymers as described in the literature. After PNIPAm's soft aging, 90 days (at 120°C) 205 and 10 days (at 130°C) no structural or chemical modifications are observed (Fig. S4). 206 Interestingly after 90-days-aging at 130°C, a slight peak around 1727 cm<sup>-1</sup> appears which could 207 be a product of the partial thermal oxidation and corresponding to the C=O function of a 208 carboxylic acid or ketone stretching vibration peak (Fig. 3b). In summary, chemical analysis 209 with FT-IR spectra shows the emergence of a new peak at 1727 cm<sup>-1</sup> that could correspond to 210 C=O vibrations related to carboxylic acid or ketone functions, as potential products of partial 211 oxidative degradation. 212



**Fig. 3.** (a) FT-IR Spectra of unaged PNIPAm (black) and P(NIPAm-co-AAc): PNIPAm with 15% mol AAc contents (red), (b) FT-IR Spectra of unaged PNIPAm (black), after an aging at 130°C: 10-days (red) and 90-days(blue).

213

UV-Vis absorbance solution analysis is another way to explore the chemical modifications due to aging. Fig. 4 shows results for solutions aged at 130°C until 90 days. Two differences are observed in aged samples. The first is a broadening of the 290 nm peak and shift to higher wavelengths. The second observation is the appearance of a peak shoulder at around 360 nm. These two observed differences with aged samples indicate a possible formation of new byproducts due to partial thermal oxidation having a lower charge conjugation.



- **Fig. 4.** UV-Vis Spectra of PNIPAm 1% (w/w) solutions: Without aging (black); A10 30-days (red) and A11 90-days (blue) aging both at 130°C.
- 227

For 120°C-aged samples (Fig. S4), the FTIR analysis does not show the two main differences as clearly as described by UV Spectra of PNIPAm 1% (w/w) solutions for 130°C-aged samples (Fig. 4).

231 Bond dissociation energy (BDE) is defined as the standard-state enthalpy change involved in

the homolytic scission of a covalent bond (kcal.mol<sup>-1</sup>) for the reaction at a specified temperature

and can be estimated according to a machine learning (ML) based approach [15]. For instance,

in the case of PNIPAm, it takes 88.2 kcal of energy to break 1 mol of C–H bonds (Table 2).

- 235
- 236

## Table 2. Bond dissociation enthalpies (BDE) values of the main aged PNIPAm bonds (The corresponding C-H bond is specified with a red arrow).

Chemical group	Am	WWW NH	H H CH-CH-CH-W Am	NH
BDE (kcal.mol <sup>-1</sup> )	88.2	92.6	96.3	100.9

In terms of oxidation mechanism, Newhouse & Baran described the effect of hyperconjugation 240 that influenced the course of C–H oxidation [16]. An easily selective oxidation by small 241 reagents could occur at the most electron rich tertiary positions. Therefore, the bond energy of a 242 C–H bond at smaller values of BDE could suggest that the transfer of H(C) atom to free radicals 243 would be more favored and is influenced by the neighboring substituents. Electron-donating 244 245 group (EDG) such as amide groups (Am) and alkyl groups could increase the C-H oxidation in the case of PNIPAm (Table 2). As hypothesized in Fig. 5, the tertiary hydrogen at the vicinity of 246 amide (BDE = 88.2 Kcal.mol<sup>-1</sup>) is expected to be more easily abstractable, comparatively to the 247 248 C-H held on the polymer's principal chain (BDE = 96.3 Kcal.mol<sup>-1</sup>) [15]. Under the assumption that radical attack is favored on this side, the mechanism proposed in Fig. 5 could occur. 249

250



**Fig. 5.** Scheme for possible mechanism of radical attack oxidation for PNIPAm (PH=Polymer-H which represents a non-oxidized polymer unit).

253

Two possible pathways for decomposition of the resulting PNIPAm's hydroperoxide derivative (Fig. 5) could be suggested in Fig.6 as follows:

- in Fig. 6a, a chain scission is observed, together with the formation of a primary alkyl radical.

257 The oxidation of this latter will give an aldehyde easily oxidized into carboxylic acid.

- In Fig. 6b, a ketone is formed, together with the release of an isopropyl acrylamide radical

(that can possibly decompose into ketone + isopropylamide) [17].



Fig. 6. Two possible pathways for PNIPAm's decomposition after oxidation, (a) Formation ofcarboxylic acid, (b) Formation of ketone with isopropylamide.

A first argument in favor of the mechanism in Fig. 6a is correlated with the shape of an amide band observed by UV-Vis analysis that should remain unchanged. Meanwhile, a shoulder corresponding to the carboxylic acid function should appear in aged sample. This seems consistent with Fig. 3 observations where UV-Vis spectra demonstrate a peak shoulder around 360 nm that could be linked to a new specie with a lower charge conjugation.

Apart from the difference in stable products formed by both mechanisms given in Fig. 6, changes in polarity (and later hydrophobicity) and macromolecular architecture are expected. The mechanism in Fig. 6a induced chain scissions and the appearance of carboxylic acids, on the contrary to Fig. 6b where the polymer's architecture seems to be preserved. The consequence could explain the changes in transition temperature as further discussed with complementary analysis in the following sections.

274

# **3.3 PNIPAm's water uptake study in correlation with hydrophilic/hydrophobic affinity.**

To better understand the aging effect on linear PNIPAm and water molecules, a study of vaporsorption is conducted. Sorption isotherms are presented in Fig. 7.

279



Fig. 7. Sorption isotherms at 40°C of linear PNIPAm comparing unaged (black) vs. 90-days
(blue) and 120-days (purple) aged samples at 120°C.

Unaged PNIPAm samples present a small concavity with a maximum at 15.8% water volume fraction. In comparison, both aged samples present higher concavity with a maximum at 20.2% water volume fraction for 90-days aged samples at 120°C and 20.7% water volume fraction for 120-days aged samples at 120°C. Fig. 7. shows that aged samples seem more hydrophilic than unaged ones.

287 The major well-known theories in polymer sorption are Henry's law [18], Flory-Huggins [19], and Zimm-Lundberg clustering [20] theory explanations (Fig. S5-6). Henry's Law is 288 immediately excluded because sorption curves are not linear. The Mean Cluster Size (MCS) 289 290 calculated on unaged PNIPAm are lower than 1.0 (MCS<1.0) until 0.25 in water activity and then the MCS increases until 1.6 at 0.9 water activity. There is no water clustering under 0.25 291 water activity and small formation of clusters above it. For a 120-days aged samples at 120°C, 292 the formation of clusters decreases to 0.6 water activity and then rapidly increases until 1.8 at 293 0.9 water activity (Fig. S5). When MCS's values are smaller than 2, water clustering is limited. 294 Therefore, clusters exist but can be considered as negligible here during sorption. Moreover, a 295 fitting with Flory-Huggins theory [19] is attempted with a unique interaction parameter 296 297 PNIPAm/water ( $\chi_{12}$ ) calculated on a range of water activity without any cluster (Fig. S6). A deviation is observed at high water activity (Fig. S6) maybe due to the very low presence of 298 clusters [20] previously discussed as well as a  $\chi_{12}$  variable during water sorption measurements 299 300 (Table S4). Despite the uncertainty about the exact model for polymer-water interaction, PNIPAm-water affinity seems to increase at least at 40°C which means that  $\chi_{12}$  slightly 301 302 decreases. 303

# 304 3.4 PNIPAm and P(NIPAm-co-AAc) thermodynamic analysis to correlate water and 305 polymeric chains interactions.

306

To better characterize the polymer-water affinity and complete the analysis, DSC measurements is used. Based on thermodynamic analysis (Fig. 1c), the energy balance (intra and intermolecular forces) is described with mixing enthalpy ( $\Delta H_m$  unit in J) (Equation 1) which could be an interesting way to understand the polymer/water interaction parameter  $\chi_{12}$ .

311



**Fig. 8.** Enthalpy (red) and  $T_{cp}$  (black) variations: unaged PNIPAm (  $\blacktriangle$  and  $\bullet$ ), PNIPAm A13

313 30-days aged at 130°C ( $\Delta$  and  $\odot$ ) and unaged P(NIPAm-co-AAc) ( $\Delta$  and  $\circ$ ).

Fig. 8 shows the endothermic peak during heating at 1°C.min<sup>-1</sup> with the mean value of the 3 314 analyzed samples (Table S5).  $T_{cp}$  and transition enthalpy ( $\Delta H_{transition}$ ) (J per g of solution) are 315 plotted against weight polymer fraction. For instance, regarding unaged PNIPAm 316 homopolymer, the changes of  $\Delta H_{\text{transition}}$  with polymer ratio follow the same trend as described 317 previously [21] [22]. The results in Fig. 8 show the conducted comparisons between unaged 318 PNIPAm and P(NIPAm-co-AAc) which contains 15% acrylic acid comonomers randomly 319 distributed. The T<sub>cp</sub> value of unaged P(NIPAm-co-AAc) is slightly higher comparatively to the 320 T<sub>cp</sub> value of unaged PNIPAm's homopolymer. This value is consistent with Graillot *et al.* who 321 described that LCST increases when comparing a homopolymer to a hydrophilic comonomer 322 323 due to a stronger interaction with water [21].

Similarly, the next analysis reports on the aged PNIPAm samples.  $T_{cp}$  variations, as previously presented, increase with aging (Fig. 2). Therefore, regarding aged PNIPAm, the  $\Delta H_{transition}$ decreases during aging. According to our interpretation, the  $\Delta H_{transition}$  given in Fig. 8 is the opposite of the  $\Delta H_m$  as given by Equation 1:

328

$$\Delta H_{\rm m} = RT \,\chi_{12} n_1 \,\Phi_2 = -\Delta H_{\rm transition} \tag{1}$$

329

330 Where R is the ideal gas constant (8.314 J.mol<sup>-1</sup>.K<sup>-1</sup>), n<sub>1</sub> the number of molecules in a specific 331 mesh and  $\Phi_2$  the volumetric fraction of polymer.  $\chi_{12}$  is the PNIPAm-water interaction 332 parameter. In our opinion, the decrease in the  $\Delta H_{transition}$  (as for  $\Delta H_m$ ) is correlated with the  $\chi_{12}$ 333 changes. In the very simple case of mixing, where interactions are dominated by Van der 334 Waals forces,  $\chi_{12}$  follows the Equation 2:

335

$$\chi_{12} = \frac{z}{kT} \left[ \varepsilon_{12} - \frac{\varepsilon_{11} + \varepsilon_{22}}{2} \right]$$
(2)

336

337 Where  $\varepsilon_{ij}$  represents the interaction energy between individual molecules of species *i* and *j*, 338 while z represents the coordination number of the species *j* around the species *i*. Namely  $\varepsilon_{11}$ , 339  $\varepsilon_{12}$ ,  $\varepsilon_{22}$  correspond to water-water pair, water-polymer pair, and polymer-polymer respectively. 340 For instance, much more complex models are specifically designed for the case of PNIPAm in 341 presence of water, where H-bonds play a key role, nevertheless their use goes out of the frame 342 of the present work [22]. 343 Interestingly, it seems clear that the  $\Delta H_{transition}$  of unaged PNIPAm is higher than the  $\Delta H_{transition}$ 

of aged PNIPAm and both  $\Delta H_{\text{transition}}$  are positive ( $\Delta H_{\text{transition}}$  of unaged PNIPAm is higher than

345  $\Delta H_{\text{transition}}$  of aged PNIPAm both > 0). At this point, if expressed in terms of mixing, the

- PNIPAm-water interaction parameter  $\chi_{12}$  for unaged PNIPAm is less than the  $\chi_{12}$  for aged
- PNIPAm, and both are negative ( $\chi_{12}$  of the unaged PNIPAm <  $\chi_{12}$  of the aged PNIPAm < 0). A
- possible explanation comes from the relative changes of the interaction energies when  $\varepsilon_{12}$  is compared to  $\varepsilon_{22}$ .
- Schematically, despite the increase in the polymer hydrophily ( $\varepsilon_{12}$  decrease, in line with
- 351 DVS), would be compensated by  $\varepsilon_{22}$  decrease (in line with T<sub>g</sub> increase). The whole reasoning
- would require modifying the model as proposed by Zhi *et al*. [23] by adding the contribution
- of oxidized PNIPAM sites, a fascinating challenge still to be investigated.
- A first experimental validation of these reasoning results comes from the analysis of
- 355 P(NIPAm-co-AAc) samples. This copolymer holds carboxylic groups responsible of a better
- 356 interaction with water. Its DSC analysis reveals indeed a lower  $\Delta H_{transition}$ .
- The aging effect will be further explained in the following section by means of molar mass changes.
- 359
- 21 22

#### 360 3.5 Thermal aging impact on PNIPAm's molar mass M<sub>w</sub>.

361

Along with aging, based on GPC measurements, retention times have shifted to higher values 362 (Fig. 9a and 9b), which is consistent with chain scissions, and decrease in M<sub>w</sub>. These 363 observations, coupled with the analysis according to the Saito theory (Equations 3 and 4) (S: 364 chain scission and X: cross-linking) [24], could demonstrate that aging has generated chain 365 366 scissions:

367

$$\frac{1}{M_n} - \frac{1}{M_{n0}} = S - X \tag{3}$$

$$\frac{1}{M_W} - \frac{1}{M_{W0}} = \frac{S}{2} - 2X \tag{4}$$

368

Where M<sub>n0</sub> and M<sub>w0</sub> are respectively the initial number and weight average molar mass (kg 369 370 mol<sup>-1</sup>).

For instance, after 30 days at 120°C, the M<sub>w</sub> decreases by 19.9%. A1 and A5 samples show an 371 increase in phase transition of  $0.3 \pm 0.2$  °C and  $1.9 \pm 0.2$  °C, respectively. The estimation with 372 Saito theory [24] gives an approximation of S = 2.51  $10^{-5}$  mol.kg<sup>-1</sup> S against X = 2.28  $10^{-5}$ 373 374 <sup>6</sup>mol.kg<sup>-1</sup> for this aging. In other words, chain scissions clearly predominate (Fig. S7). Consistently, Furyk et al. show that turbidity curves are overly sensitive to the M<sub>w</sub> and the group 375 interactions with water [25]. Shorter chains polymers are formed during aging which could 376 explain the increase in T<sub>cp</sub> reported in Fig. 2. Interestingly, the extensive review by Halperin *et* 377 *al.* shows that the  $T_{cp}$  of 10% (w/w) solution shifted to an increase of +5 ± 0.2°C °C when  $M_w$ 378 decreases from 19 to 9 kg.mol<sup>-1</sup> [4]. These observations align with the current experimental data. 379 In other words, the T<sub>cp</sub> shift presented in Fig. 2 results from molar mass changes (induced by 380 381 chain scissions) rather than from polarity induced by oxidized groups (formation of carbonyl groups) even if both phenomena have the same molecular origin. 382

383



Fig. 9. GPC chromatograms of PNIPAm without aging (black) and aged at: (a) 120°C during 384 30-days (red) and during 90-days (blue), (b) at 130°C 30-days (red) and during 90-days (blue) 385 (■PMMA calibration). 386

387

389

#### 3.6 Thermal aging effect on linear PNIPAm's glass transition temperature (T<sub>o</sub>). 388

The impact of thermal aging on linear polymers with chain scissions goes along with lower T<sub>g</sub>. 390 391 A link between  $M_n$  and  $T_g$  is described by the Fox-Flory relationship (Equation 5) [26]:

$$T_{g=i T_{g_*} - \frac{k_{FF}}{M_n}i}$$
(5)

393 Where  $k_{FF}$  (°C. kg.mol<sup>-1</sup>) is the Fox-Flory constant correlated with stiffness of polymer chain,

M<sub>n</sub> the number average molar mass (kg.mol<sup>-1</sup>) and  $T_{g_{\infty}}(^{\circ}C)$  the glass transition temperature for a polymer with an infinite molar mass.

396

**Table 3. Variation of the PNIPAm T**<sub>g</sub> with aging.

Aging Temperature (°C)	Aging Time (days)	Τ <sub>g</sub> (°C)
-	0	$137.8 \pm 0.4$
120	30	$140.2\pm0.3$
	90	$142.8 \pm 0.2$
130	30	$140.9 \pm 0.3$
	90	$147.5 \pm 0.3$

398

In the current research,  $T_g$  gradually increases along with aging (Table 3 and Fig. S8) while  $M_n$ unambiguously decreases. Indeed, both parameters of Fox-Flory relationship ( $T_{g\infty}$  and  $k_{FF}$ ) are tightly correlated [27]. The formation of acrylic acid groups with aging would increase the chain rigidity ( $k_{FF}$ ), promote more intra and inter bonding between groups and increase  $T_{g\infty}$ . In other

words, if the increase in  $T_{g\infty}$  is the predominant effect, the  $T_g$  value will increase despite the decrease in  $M_n$ .

To confirm this hypothesis, a P(NIPAm-co-AAc) copolymer is investigated as a model representing an aged sample. The P(NIPAm-co-AAc)'s glass transition ( $T_g$ ) is approximately 10°C higher than the unaged PNIPAm's  $T_g$ .

This increase is consistent with the theory related to a partial oxidation induced by aging and observable in the P(NIPAm-co-AAc) presenting 15% acrylic acid groups.

410

## 411 **4. Conclusion**

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This research is the first phase of exploration of PNIPAm's stability under accelerated 413 414 isothermal aging conditions at 120°C and 130°C. After accelerated aging and data analysis, two 415 possible oxidative mechanisms are proposed, with more favorable paths of decomposition and formation of carboxylic acid. In all cases, the measurements for aged PNIPAm samples are 416 417 compared with a P(NIPAm-co-AAc) showing similarities to aged PNIPAm. The formation of shorter chains after chain scissions during aging can be the main explanation for the T<sub>cp</sub> shift to 418 higher values. The formation of carboxylic acid species may have also contributed in the T<sub>cp</sub> and 419 T<sub>g</sub> increase with better interactions between shorter groups. At lower aging temperature, 420 oxidative mechanisms are slow and PNIPAm seems stable with almost no T<sub>cp</sub>, T<sub>g</sub> shifts (less 421 than  $1 \pm 0.2$  °C °C and  $2 \pm 0.2$  °C respectively) and no visible structural modifications. 422

To conclude, this multiscale study is based on the accelerated aging of PNIPAm a biocompatible [28], thermosensitive polymer with unique and versatile properties. The final purpose is to suggest mechanisms of oxidation and confirm that PNIPAm's model would be stable for the pharmaceutical formulation as a drug delivery excipient. The undergoing next step is the study of the drug delivery based on PNIPAm absorption/release of active pharmaceutical ingredients (API).

429

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