Small angle X-ray scattering study of poly(N-isopropyl acrylamide) based cryogels near the volume-phase transition temperature

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Abstract. The structural modifications induced by changes in temperature are investigated by Small-Angle X-ray Scattering (SAXS) over a broad range of $q$-values ($3.5 \times 10^{-2} - 12 \text{ nm}^{-1}$) in cryogels based on N-isopropylacrylamide (NIPA) and/or 2-Hydroxyethyl methacrylate-L-Lactide-Dextran (HEMA-LLA-D) macromer. Various copolymeric cryogels of these two monomers are prepared by cryopolymerization yielding macroporous gels (cryogels). For the plain pNIPA cryogel, the SAXS curves obtained at each temperature are well fitted by a sum of four equations describing respectively the scattering resulting from the gel surface (power law), from the solid-like (Guinier equation) and liquid-like (Ornstein-Zernike equation) heterogeneities and from the chain-chain correlation yielding a broad peak (pseudo-Voigt equation) in the high-$q$ domain. The temperature dependence of the parameters obtained from the fit is analyzed and discussed. It is shown that the existence of an isoscattering (or isosbestic) point observed in pNIPA gels and in some copolymers is related to features observed by Differential Scanning Calorimetry and swelling ratio measurements.

1. Introduction
Intelligent polymeric materials which exhibit response to external stimuli such as temperature have received considerable interest in recent years because of their applications in the field of biomaterials, biomedicine and biotechnology [1]. Poly(N-isopropylacrylamide) (pNIPA) gel is a typical example of a temperature sensitive gel as it exhibits a volume phase transition at a critical temperature ($T_c$) of about 34 °C in aqueous media [2]. Below $T_c$, pNIPA hydrogels are swollen, hydrated, and hydrophilic. Above $T_c$, the gels shrink due to the distortion of the hydrophilic/hydrophobic balance in the network.
structure. The rate of response of pNIPA hydrogels is low due to the formation of a dense “skin layer” of the shrunken gel, which prevents the mass transport of water out of the shrinking gel [3]. One possibility to circumvent this problem inherent to macroscopic gels (or macrogels) consists in the synthesis of a macroporous gel [4]. The macropores serve as channels that facilitate convective transport of liquid released during the shrinkage of the gel constituting the thin macropore walls. An efficient way to obtain macroporous gels is the cryotropic gelation technique [5], implying polymerization at a sub-zero temperature, yielding cryogels. These highly porous polymeric materials exhibit a broad variety of porosities and morphologies [6-10] allowing the preparation of macroporous gels with properties tailored for a given application, particularly in the field of biochemistry or bioengineering (e.g., tissue engineering scaffolds [11]).

For many applications, the mechanical properties of pNIPA must be improved. Increasing the degree of cross-linking which increases the elastic modulus of the gel decreases the degree of swelling and accordingly, the amplitude of the volume change at \( T_c \). The most often proposed solution to overcome this drawback is the preparation of copolymer hydrogels. Copolymerization of NIPA with acrylic acid (AA) yields gels that have a higher swelling ratio and a higher critical temperature [12]. The introduction of dextran based derivatives in pNIPA copolymers was shown to improve the mechanical properties, to increase the swelling ratio and also to confer to the copolymer hydrogel a better reversibility [13]. The present investigation is devoted to a series of NIPA based copolymers synthesized for drug release purposes. Previously synthesized L-lactide-dextran (LLA-D) based macromers were associated with NIPA in order to combine the thermosensitive characteristics of NIPA with the high hydrophilicity of dextran (D). The presence of hydrolyzable LLA combined with 2-hydroxyethyl methacrylate (HEMA) allows the degradation of the cross-links in these copolymers and eventually permits the solid gel to transform into a liquid solution.

As information about the internal structure of the gels and its variation as a function of temperature is essential for using gels as functional materials, this field has been widely investigated since the last 30 years [14]. It appears that Small-Angle Scattering techniques [15] constitute experimental methods that are well adapted to investigate the multiscale structure of polymer gels. The usual \( q \)-domain for Small Angle X-ray Scattering (SAXS) or Small Angle Neutron Scattering SANS (10\(^{-2}\) to 1 nm\(^{-1}\)) allowing structural information between roughly 100 and 1 nm in the real space can be extended up to a few \( \mu \)m by Small Angle Light Scattering (SALS) for transparent gels.

The first extensive study of pNIPA gels (macrogels) by SANS near the volume phase transition was published by Shibayama et al. in 1992 [16]. Since that time, several papers reporting SAXS or SANS studies of thermally induced structural modifications in pNIPA gels [17-21] have been published. Because of their fast kinetic response, microgels [22, 23] or core-shell colloidal particles [24, 25] were also extensively investigated. The first paper devoted to the comparison of SAXS curves obtained for macrogels and cryogels appeared recently [26]. It was shown that combining SAXS and Wide-Angle X-ray Scattering (WAXS) by extending the \( q \)-domain generally investigated (0.05 - 1 nm\(^{-1}\)) up to larger \( q \)-values (12 nm\(^{-1}\)) brings new information about the changes in the molecular arrangement below and above the transition temperature.

The aim of the present paper is to show the structural modifications occurring during the stepwise increase of temperature not only at the mesoscale but also, for the first time, at the molecular scale in plain pNIPA and (HEMA-LLA-D) cryogels and in NIPA-co-HEMA-LLA-D copolymers with different NIPA/(HEMA-LLA-D) ratios. The macroscopic structure (macropore size distribution and wall thickness) of these cryogels and its change with temperature have been investigated previously by bi-photonic fluorescence microscopy [27]. The present paper is devoted to the study by SAXS of the internal structure of these walls, at the meso and nanoscale at different temperatures between 18 and 37°C. The observed features will be related to that occurring at the macroscopic scale obtained by Differential Scanning Calorimetry (DSC) and swelling ratio (SR) measurements.
2. Materials and experimental methods

2.1. Cryogel sample preparation

Macroporous hydrogels were prepared by free radical cryopolymerization of the synthetic macromer, HEMA-LLA-D, and NIPA. Copolymers (NIPA-co-HEMA-LLA-D) with different NIPA/(HEMA-LLA-D) ratios (80/20, 60/40 and 40/60 w/w) were prepared. In the following, these copolymer samples will be labeled as coNIPA(80/20), coNIPA(60/40) and coNIPA(40/60) respectively. Synthesis of the macromer was described elsewhere [11]. The polymerization reactions were carried out in tubular-shape glass moulds. NIPA monomer and HEMA-LLA-D macromer were dissolved in water to reach a final concentration of 6% wt/v. The cross-linker, N,N'-methylenebisacrylamide (MBAA), was dissolved in this mixture (6.6 wt% of total amount monomer/macromer) and nitrogen was passed-through the solution for 15 min. N,N,N',N'-Tetramethylethylenediamine (TEMED) (1 wt %) used for reaction activation was added and the solution was cooled in an ice bath for 5 min. The radical initiator, ammonium persulfate (APS) (1 wt %), was added and the reaction mixture was stirred during 1 min. 1 ml of the reaction mixture was injected into the glass mold. The solution was frozen at -20ºC for 1 hour and then at -12ºC during 16 hours. The samples were subsequently thawed at room temperature. In all cases, the yield of the polymerization reactions exceeded 85%. The cryogel matrix contained in each glass mold was washed by passing distilled water to remove any possible unreacted monomer and other ingredients and subsequently dried in air until reaching a constant weight. From a chemical point of view, the copolymers consist of chains of dextran (D) with HEMA-LLA side chains connecting pNIPA chains (figure 1). The synthesis of plain pNIPA cross-linked with MBAA and that of the pHEMA-LLA-D cryogels was described earlier [11, 26].

![Figure 1. Scheme of the chemical structure of the NIPA based copolymers.](image)

2.2. SAXS measurements

SAXS experiments were performed at the European Synchrotron Radiation Facility (ESRF) Grenoble, France, on the French CRG beamline D2AM. The incident energy was set to 16 keV (λ = 0.77 Å). The detector, an indirect illumination CCD camera (Princeton Instruments), with pixel size equal to 50 µm was located at distances of 2 and 0.3 m from the sample. These configurations provided data for wave vectors $q$ ($q = \left(\frac{4\pi}{\lambda}\right)\sin\left(\frac{\theta}{2}\right)$) ranging between $3.5\times10^{-2}$ and 12 nm$^{-1}$. The beam-stop was a small pillar (2 mm diameter lead wire).

The swollen samples were placed in a stainless steel holder closed by two mica windows 1 mm apart. The sample holder was inserted in a small furnace allowing temperature to vary between 10 and 40°C with an accuracy better than 0.2 °C. Heating was provided by a Thermocoax® wire coiled

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[http://www.esrf.eu/](http://www.esrf.eu/)
around the furnace concentrically to a cold water circuit. Temperature control was achieved by means of an Eurotherm® controller and sample temperature was measured by a Pt resistance located close to the cryogel sample. SAXS measurements were performed at different temperatures between 18 and 37°C. The duration of each temperature plateau was 16 minutes. After about 8 minutes, the shape of the curves did not change anymore as equilibrium was reached. Temperature control and data acquisition were automated.

The CCD images were processed by means of the software bm2img available on the beamline\(^b\). All intensity curves were corrected by taking into account the flat field, dark current and distortion. Scattering of the same thickness of pure water was subtracted from the total intensity.

3. Results and discussion

3.1. Evolution of the SAXS curves with the NIPA/(HEMA-LLA-D) ratio at room temperature

Figure 2a shows that the SAXS curves obtained for the pNIPA, the p(HEMA-LLA-D) and the coNIPA cryogels are very different from each other. The dashed lines plotted in figure 2b were obtained from the sum \(xI_{\text{pNIPA}} + (100-x)I_{\text{p(HEMA-LLA-D)}}\) with \(x=40\) or \(60\), assuming a mixture of pNIPA and p(HEMA-LLA-D). It appears that these curves do not fit the experimental ones. It follows that the copolymer cryogels do not consist of two independent components. They have their own structure that depends on the concentration of pNIPA and p(HEMA-LLA-D).

![Figure 2. SAXS curves obtained at room temperature (24°C) for pNIPA, p(HEMA-LLA-D) and their copolymers (a) and comparison with the curves calculated (dashed lines) by assuming a mixture of pNIPA and p(HEMA-LLA-D) in the proportions 60/40 and 40/60 (b).](image)

3.2. Evolution of the SAXS curves with temperature

Figure 3 collects the SAXS curves obtained for the cryogels at different temperatures: the plain pNIPA (a), the 60/40 copolymer p(NIPA-co-HEMA-LLA-D) (b) and the p(HEMA-LLA-D) (c). The pNIPA graph reveals, as expected, a strong temperature dependence.

\(^b\) [http://www.esrf.fr/exp_facilities/BM2/BM2.html](http://www.esrf.fr/exp_facilities/BM2/BM2.html)
For pNIPA an isoscattering point is observed at 3.633 nm\(^{-1}\) up to 30°C. This point is no longer observed at 32°C and above, i.e., above the volume phase transition temperature (\(T_c=31.4°C\), see next paragraph). The SAXS curves obtained for pNIPA have been extensively analyzed elsewhere [28]. A summary of the results will be given in the next paragraph.

As shown in figure 3c, the temperature increase has a much weaker effect on the SAXS curves obtained for the p(HEMA-LLA-D) cryogel than for the pNIPA one. In the low-\(q\) domain and above 5 nm\(^{-1}\), the intensity slightly increases with \(T\) but no isoscattering point can be observed. A precise analysis of these curves is by far more difficult than that of pNIPA and would probably require measurements with neutrons on deuterated samples. Meanwhile, it may be noticed that the curves display a change of slope around \(q^*\approx1.11\) nm\(^{-1}\). Below \(q^*\), the absolute value of the slope is slightly larger than 2. Above \(q^*\), it is close to 1. Such a feature could be attributed to the crossover between the scattering by coil (\(q<q^*\)) and a rod (\(q>q^*\)) allowing the determination of the persistence length \(L_p\) of a polymer chain by means of the following relation \(L_p=1.91/q^*\) [29] yielding \(L_p=1.72\) nm. This

\[L_p=1.91/q^*\]

\[L_p=1.72\text{ nm}\]
value agrees with the persistence length for dextran (1.5 - 1.8 nm depending on the molecular weight) reported by White and Deen [30].

As for the 60/40 copolymer cryogel, the intensity decreases with temperature in the 0.1 - 1 nm\(^{-1}\) \(q\)-domain while the contrary is observed for pNIPA (figure 3a) for \(T \leq 30°C\). Also the curves display an isoscattering point over the whole range of temperature but located a larger \(q\)-value (4.442±0.012 nm\(^{-1}\)). The origin and the physical meaning of the isoscattering points will be discussed in a next paragraph.

3.3. Analysis of the SAXS curves obtained for pNIPA at different temperatures

3.3.1. Fit of the experimental data. In the \(q\) domain investigated by a large majority of researchers \((q<1\ \text{nm}^{-1})\), small-angle scattering of neutrons (SANS) or X-rays (SAXS) by neutral gels is assumed to result from the sum of two contributions [16]:

- the scattering from density fluctuations in thermal blobs which exist also in semi-dilute polymer solutions (liquidlike or solutionlike fluctuations) and which is described by the Ornstein-Zernike (OZ) equation

\[
I_{OZ}(q) = \frac{I_{OZ}(0)}{1 + q^2 \xi^2} \tag{1}
\]

where \(\xi\) is the size of the thermal blobs (polymer rich domains) and \(I_{OZ}(0)\) is the value of the O-Z intensity when \(q\rightarrow0\)

- the scattering from solidlike density fluctuation due to the gel cross-linking often described by a Guinier equation

\[
I_G(q) = I_G(0) \exp \left[-\frac{R_G^2 q^2}{3}\right] \tag{2}
\]

where \(R_G\) is the radius of gyration related to the diameter \(a\) by \(a = 2R_G \sqrt{5/3}\) for spherical objects and \(I_G(0)\) is the value of the Guinier intensity when \(q\rightarrow0\).

In the present case, experiments were extended in the WAXS domain in order to investigate the chain-chain correlation peak recently revealed in cryogels and gels [26]. Without the support of a relevant model, such peaks can be fitted with a pseudo-Voigt equation [31] writing as

\[
I_V(q) = a \left[ \frac{c}{1 + (q - q_1)^2 \xi_1^2} + (1 - c) \exp \left(-\frac{(q - q_1)^2 \xi_1^2}{2\xi_1^2}\right) \right] \tag{3}
\]

where \(a\) corresponds to the maximum of the peak located at \(q_1\) (assumed to correspond to \(d = 2\pi / q_1\) in the real space), \(c\) is the fraction of the lorentzian contribution and \(\xi_1\) is related to the size of the organized domains.

Finally, because the gel objects investigated are the walls (12 µm thick at room temperature [27]) of the macropores, a low-\(q\) intensity turn-up resulting from the scattering of the gel surface is expected and actually observed. Thus, the low-\(q\) part of the intensity curve \(I(q)\) will be described by the following power law

\[
I_p(q) = I_{R_0} q^{-p} \tag{4}
\]

where the exponent \(p\) is related to the surface fractal dimension \(D_s\) by \(D_s = 6 - p\) and \(I_{R_0}\) is the prefactor.

It follows that the equation \(I_{fit}(q)\) used for the fit of the experimental curve \(I(q)\) consists in the sum of four contributions

\[
I_{fit}(q) = I_p(q) + I_G(q) + I_{OZ}(q) + I_V(q) \tag{5}
\]
involving 10 parameters in a non-linear regression. The regression procedure was achieved by means of the Marquardt-Levenberg algorithm provided by SigmaPlot 10.0. Because the data in I(q) extend over more than 4 decades, a weight \( w = 1/ [I(q)]^2 \) was used. The upper \( q \) limit for the regression was fixed at 6.2 nm\(^{-1}\) in order to avoid the contribution of expected scattering features occurring at higher \( q \)-values. The large number of parameters involved in the fit requires a careful analysis of the residuals. The results of this analysis [28] indicate that the fitting equation obtained by the non-linear regression can be considered as describing satisfactorily the experimental data. The comparison between the fitting curve and the experimental points plotted in figure 4 leads to the same conclusion.

The evolution with temperature of the OZ and pseudo-Voigt calculated curves is shown on figures 5a and 5b. Below 32°C (figure 5a), the intensity of the chain-chain correlation peak increases. Above 32°C (figure 5b), the OZ contribution drops significantly while the peak remains nearly unchanged and similar to that obtained for the dry cryogel as already observed previously in other cryogels [26]. Figure 5c shows the sum of the OZ and pseudo-Voigt curves calculated from the fit. These curves display (see insert) the same isoscattering point as the experimental curves plotted in figure 1. This result strengthens the validity of the fitting procedure and proves that the isoscattering point is related

Figure 4. Logarithmic plots of the SAXS curves obtained for the pNIPA cryogel at different temperatures (filled gray circles) and the fitting curves (black continuous line) with their four components (power law, Guinier, Ornstein-Zernike and pseudo-Voigt).
to the structure of the thermal blobs. Actually, such a result is also expected from the curves plotted in figure 4 which show that the power law and the Guinier scattering do not contribute anymore to the intensity in the isoscattering point region.

Figure 5. Evolution of the Ornstein-Zernike (OZ) and the pseudo-Voigt curves calculated by using the parameters obtained from the fit:
(a) between 18 and 32°C
(b) at 32, 34 and 37°C and for the dry cryogel
(c) sum of the O-Z and Voigt curves displaying the same isoscattering point (insert) as the experimental curves.

3.3.2. *Evolution of the fitting parameters with temperature.* Figure 6a shows the variation of the OZ parameters $\xi$ and $I_{OZ}(0)$ defined in equation (1). By means of swelling and calorimetry experiments, Li and Tanaka [32] have shown that pNIPA gels belong to the Ising universality class. Thus, $\xi$ and $I_{OZ}(0)$ are expected to diverge at a critical temperature $T_c$ according to the following equations, near $T_c$:

\[
\xi = A(T_c - T)^{-\nu} \\
I_{OZ}(0) = B(T_c - T)^{-\gamma}
\]

In these conditions, one expects $\nu=0.631$ and $\gamma=1.238$. These exponents are slightly larger than the ones involved in the mean field model (0.5 and 1 for $\nu$ and $\gamma$ respectively). In any case, equations (6)
and (7) imply that $\xi$ scales as $[I_{OZ}(0)]^{\nu/\gamma}$ with $\nu/\gamma$ close to 0.5. This remark allows the determination of the temperature domain in which the fit to equations (6) and (7) is relevant.

Figure 6. (a) Variation of the correlation length $\xi$ (left axis) and the intensity at $q=0$, $I_{OZ}(0)$ (right axis) with temperature. The solid lines result from the fit with equations (6) and (7). The dashed lines drawn through the points obtained at 18 and 21°C extrapolate to 13.3°C (arrow). (b) Logarithmic plot of the variation of the parameter $\xi$ with $I_{OZ}(0)$. The solid line results from the linear regression of the data measured at 26, 28 and 30°C. The slope of the dashed line joining the points at 18 and 21°C is close to 1; (c) Evolution of the position $q_1$ (left axis) and the intensity $a$ (right axis) of the high-$q$ peak resulting from the fit with the pseudo-Voigt equation (equation 3). The solid lines result from a linear regression of the data (closed symbols); (d) Variation of the pseudo-Voigt intensity $a$ (equation 3) with the blob size $\xi$ (equation 1).

As shown in figure 6b, this scaling law is verified only for three temperatures (26, 28 and 30°C, may be four if the point at 24°C is taken into account) yielding an exponent equal to 0.586 which is
quite small. Nevertheless, a non-linear regression with equations (6) and (7) performed over the three point yields (figure 6a) (i), the same Tc value (31.35 and 31.36 in equations (6) and (7), respectively) and (ii), critical exponents ν=0.712 and γ=1.22. Including the point at 24°C for the non-linear regression yields a significant difference between the values of Tc obtained from the fit by equation (6) (32.30°C) and equation (7) (31.90°C), and unrealistic exponents (ν=1.015 and γ=1.523). Further, the critical temperature (31.4°C) deduced from Differential Scanning Calorimetry (DSC) measurements (see paragraph 3.3) and resulting from the “melting” of the water cages around the isopropyl groups [33] agrees perfectly with that determined by the non-linear regression performed between 26 and 30°C. Thus, despite the fact that the number of data points is very low, the result confirms the validity of an Ising model for describing the transition in the present pNIPA cryogel as it was for the gels investigated by Shibayama et al. [16], yet the value of ν is slightly larger than the theoretical one (0.631). It could be observed in figure 6a that the lines drawn through the points corresponding to 18 and 21°C for ξ and Iq(0) extrapolate to the same temperature (13.3°C). Also, the slope of this line in the logarithmic plot of ξ versus Iq(0) (Figure 6b) is very close to 1. These features do not prove but suggests a linear increase of ξ and Iq(0) in the low temperature domain and incites one to further investigate this question in the future.

Equation (1) indicates that the intensity Iq(0) scale as q−2 for qξ>1, in the asymptotic regime. In fact, this asymptotic regime is not visible because of the high-q extra scattering resulting from an inter-chain correlation revealed by the broad peak fitted with the pseudo-Voigt equation (3). The variation of the peak position q1 and the peak height a as a function of temperature are plotted on figure 6c. This figure shows that the peak height a increases linearly with temperature up to 24°C and remains nearly constant above 32°C. Between 24 and 32°C, the rate of increase becomes larger. It appears (figure 6d) that a scales as the blob size ξ with an exponent equal to 0.26. The physical meaning of the value of this exponent is not obvious. Meanwhile, the scaling law suggests that the increase of a corresponds to the increase of the number of scatterers related to the increase of the size ξ of the thermal blobs. Conversely, the peak position q1 displays a weak monotonous linear increase (between 5.18 and 5.64 nm) with temperature up to 34°C. The values of q1 are very similar to that obtained for the different cryogels investigated earlier [26] below and above the critical temperature but slightly larger than the value (5.7 nm) reported for a weakly cross-linked pNIPA gel at 40°C [20]. The increase of q1 (figure 8) corresponds, in the real space, to a decrease of the distance d between the dehydrated (i.e., without “water cages” around the isopropyl terminal groups in the side-chain) polymer chains. d can be estimated by means of the Bragg equation \( d = \frac{2\pi}{\lambda q} \) from 1.21 to 1.12 nm. As discussed earlier [26], such lengths are smaller or close to twice that of the side chain. It follows that the peak may actually originate from a local packing by means of hydrophobic association of isopropyl groups of two neighbor chains and/or hydrogen bonds between amide groups [33]. Thus, it may be assumed that the scattering object from which this peak originates are the collapsed hydrophobic clusters (or nanopockets) revealed by Ahmed et al. [34] by means of UV Resonance Raman experiments, which are present in the thermal blobs.

The weak variation of \( q_1 \) (or d) with temperature could be attributed to tiny changes in the conformation of the pNIPA chains resulting from the change of hydrogen bonding of water at the C=O or N–H sites. The strong increase of a (concordant with the increase of ξ) between 26 and 30°C could be related to the abrupt spectral change in the amide (AmI) bands observed between 30 and 40°C in the pNIPA nanoparticles investigated in [34], for which the transition temperature is 33°C. Figures 5, 6 and 8 show that collapsed clusters are present at lower temperatures, between 18 and 24°C. This feature is consistent with the ones reported in [34]. It is also consistent with the conclusions drawn about the peak intensity in a gel as compared to the cryogel at room temperature [26]. Meanwhile, it is difficult to explain why the values of ξ obtained at 18°C (0.543 nm) and 21°C (0.886 nm) are smaller than the corresponding value of d (1.21 and 1.19, respectively) deduced from the peak position \( q_1 \). For the dry sample, \( q_1 \) (≈5.56 nm) is slightly smaller than in the fully collapsed cryogel at 37°C.
Accordingly, the inter-chain distance would be slightly larger in the dry sample (1.13 or 1.03 nm) than in the collapsed aqueous cryogel (1.11 or 1.02 nm). The analysis of the UV Raman resonance spectra of the dry pNIPA led Ahmed et al. [34] to suggest the existence of interchain hydrogen bonding between pendant amides. Conversely, in the collapsed hydrogel, amides seem to be hydrogen bonded to water and not directly to each others. At this point, and because of the very small differences in the values of $d$, it is difficult to go further into this discussion.

The evolution with temperature of the other parameters extracted from the fit is thoroughly analyzed elsewhere [28].

### 3.3.3. Origin of the isoscattering point in pNIPA

The analysis of the OZ and pseudo-Voigt equation, that of the corresponding parameters ($I_{OZ}(0)$, $\xi$ and $a$), and their correlation render the origin of the isoscattering point more straightforward. In the $q$-range investigated ($q>2$ nm$^{-1}$), the variation of the scattered intensity $I(q,T)$ is equal to $I_{OZ}(q,T)+I_{c}(q,T)$. Both functions contain temperature dependent parameters for which the temperature dependence is described by equations that remain the same over a given range of temperature. It follows that $I(q,T)$ is expressed by a linear combination of two functions, in which case, an isoscattering point $q_{iso}$ is expected [35]. The isoscattering point does no longer exist above $T_c$ because the temperature dependence of the parameters in $I_{OZ}(q,T)$ changes. In the present case, $q_{iso}$ equals 3.633 nm$^{-1}$. The physical meaning of this particular $q$-value is still unclear. Additionally, it must be noted that this isoscattering point does not indicate that the system transforms from one state to an other one as reported in many situations [31, 36-39]. Here, the isoscattering point results from the fact that the number of nanopockets giving rise to the high-$q$ peak is directly related to the size of the thermal blob (diverging at $T_c$=31.4°C). In the domain of temperature ranging between 32 and 37°C, i.e., above the temperature of the transition occurring at the molecular level and characterized by the divergence of the O-Z parameters, it is still necessary to include an O-Z equation to fit the experimental curves (figure 4).

Meanwhile, the intensity in quite small and the values of $\xi$ vary between 1 and 2.5 nm (figure 6a). This feature makes sense because the system is still a gel. However, the gel network is different from what it was below $T_c$ and the isoscattering point vanishes accordingly.

### 3.3.4. Analysis of the isoscattering points in the NIPA based copolymer cryogels

Figure 7 shows linear plots of the SAXS curves measured at different temperatures for the cryogels investigated. As already seen in figure 3, there is no isoscattering point for p(HEMA-LLA-D). For coNIPA-40/60 containing the lowest amount of NIPA, there is a $q$-value below which the curves are merging into a single curve, as a result of the significant contribution of the temperature independent scattering of HEMA-LLA-D. Strictly speaking, this point is not an isoscattering point. Meanwhile, it may be concluded that all coNIPA cryogels display an isoscattering point as does the pNIPA cryogel. It follows that as for pNIPA, the SAXS curves can be described by a linear combination of equations. The shift in $q$ and in intensity could result from the temperature independent contribution coming from HEMA-LLA-D which increases the overall level of the scattered intensity as its concentration increases in the copolymer. It follows also that the effect of the increase of temperature is the same in the coNIPA gels as in the pNIPA gel: the increase of $\xi$ and $I_{OZ}(0)$ is related to that of the peak intensity $a$ up to the critical temperature $T_c$=31.4°C. Examination of the curves obtained for coNIPA-80/20 (figure 7b, insert) containing a large amount of NIPA indicates that, as for pNIPA, the isoscattering point vanishes above 30°C. Thus this sample should, at least, partly collapse above a critical temperature located between 30 and 32°C. On the contrary, for coNIPA-60/40 (figure 7a), the isoscattering point exists over the whole range of temperature investigated. It follows that the critical temperature, if any, is located above 37°C. The same comment applies probably to coNIPA-40/60 (figure 7b).

The pertinence of the conclusions deduced from the isoscattering points will be analyzed by means of two other independent methods: DSC and swelling ratio (SR) measurements presented in the next paragraph.
4. Relation between information obtained by SAXS and that obtained by other methods

4.1. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry measurements were achieved by means of MICRO-DSC III (Setaram). The heating rate was 0.1 °C/min for all samples except for pNIPA (0.5 °C/min). The cryogel samples were placed in a closed cell. The reference cell was filled with water in order to enhance the signal. The DSC curves obtained for all samples are collected on figure 8a. The curves are shifted along the ordinate axis by addition/subtraction of a constant in order to bring together the baselines on the high temperature side. This figure shows major differences between the DSC curves.

The DSC curves obtained for pNIPA and for coNIPA-80/20 are plotted in figure 8b. Both curves exhibit an endothermic peak characterized by the same extrapolated temperature (31.4°C). This endothermic peak corresponds to the dissociation of the hydrophobic interaction [40]. In other words, it results from the "melting" of the water cages around the isopropyl groups [33]. As already mentioned, this temperature agrees perfectly with that deduced from the divergence of the Orstein-Zernike parameters $\xi$ and $I_{OZ}(0)$ in subsection 3.3.2. Because the temperatures are the same, it may be concluded that the mechanism is the same in both cryogels: coNIPA-80/20 should collapse, at least partly, at 31.4°C. This feature also agrees with the existence of the isoscattering point over the whole temperature range (figure 7b).

For the copolymer containing less NIPA (coNIPA-60/40 and coNIPA-40/60) the DSC curves (figure 8c) reveal the absence of the endothermic peak at 31.4°C i.e., no melting and therefore, no collapse of the gel. This feature also agrees with the existence of the isoscattering point over the whole temperature range.

Figure 7. SAXS curves measured at 18, 21, 24, 26, 28, 30, 32, 34 and 37°C for pNIPA and coNIPA-60/40 (a) and coNIPA-80/20, coNIPA-40/60 and p(HEMA-LLA-D) (b). For p(HEMA-LLA-D) the temperatures corresponding to the curves are 18, 24, 28 32 and 37°C (from bottom to top at high $q$).
range of temperatures investigated for coNIPA-60/40 (figure 7a). Shibayama et al. [40] reported a shift of the endothermic peak toward a higher temperature for NIPA based copolymers containing acrylic acid or dimethylacrylamide. The intensity of the endothermic peak decreased with the decrease of the NIPA fraction and eventually, the peak vanished. The situation seems different for the copolymers investigated here. Thus, it is likely that the absence of the NIPA endothermic peak below 40°C and the evidence of an isoscattering point up to 37°C does not suggest an elevation of the collapse temperature above these limits. This question needs however to be further investigated. Also, the role played by the comonomer and, particularly dextran, needs to be addressed in the future.

4.2. Swelling ratio and macroporous texture
The macroscopic evidence of the physico-chemical phase transition is revealed by significant decrease of the sample volume (the volume phase transition) related to the decrease of the degree of swelling or swelling ratio defined by $SR = \frac{M_{\text{swollen}} - M_{\text{dry}}}{M_{\text{dry}}}$ where $M_{\text{swollen}}$ is the weight of the sample swollen in water and $M_{\text{dry}}$ is the weight of the dry cryogel. The variation of $SR$ with temperature is

![Figure 8. DSC curves measured for the different cryogels at 0.1 °C/min (for pNIPA, the heating rate was 0.5°C/min). The curves are shifted by addition of the constant needed to bring together the baselines on the high temperature side. The heat flow, expressed in mW is not normalized.](image-url)
plotted on figure 9a in linear coordinates. In figure 9b, a logarithmic ordinate axis is used in order to compare the shape of the curves.

For the pNIPA cryogel, the swelling ratio begins to decrease significantly at 26°C. This temperature corresponds to the beginning of the phase transition domain reported in section 3.3.3. It appears that the curve $SR(T)$ displays an inflexion point at the critical temperature $T_c=31.4°C$ [28]. Clearly, the $SR$ curves obtained for the copolymers and for p(HEMA-LLA-D) do not show the same feature as pNIPA. Meanwhile all copolymer cryogels show a diminution of their swelling ratio as the temperature increases. Figure 9b suggests that the relative diminution of $SR$ is the largest for coNIPA-80/20. The decrease of $SR$ seems to become smoother as the amount of NIPA in the copolymer decreases from 80 to 40. For coNIPA-80/20, the volume transition expected from the SAXS and the DSC measurements does not appear clearly in the $SR(T)$ curves. Meanwhile, this cryogel shows a slightly larger decrease above $T_c$ which is not observed for the other copolymer cryogels and which could agree with the SAXS and DSC features.

Figure 9 also reveals a noticeable feature: the significant increase of $SR$ as the amount of NIPA decreases, i.e., as the amount of the HEMA-LLA-D macromer increases. This feature could be explained by the fact that dextran is more hydrophilic than NIPA. However, the degree of swelling of p(HEMA-LLA-D) is only slightly larger than that of pNIPA. Thus it may be concluded that the particular chemical structure of the copolymer gels renders the system more hydrophilic than each of its comonomer alone. The temperature stability of the swollen samples could proceed from a similar mechanism.

The origin of the nearly continuous decrease of $SR$ in the copolymer cryogels and in p(HEMA-LLA-D) is still difficult to explain by structural changes occurring at the meso/nanoscale. However, it must be noted that the $SR$ curves shown in figure 9 characterize macroporous gels; thus they take into account not only the water in the swollen gel but also the water filling the macroporosity.

The macroscopic structure (macropore size distribution and wall thickness) of these cryogels have been investigated by bi-photonic fluorescence microscopy [27]. It was shown that the macropore size distribution goes progressively from a relatively narrow distribution peaking at 37 µm in the pNIPA cryogel to a very broad distribution extending up to 200 µm in the p(HEMA-LLA-D) cryogel when the
NIPA/(HEMA-LLA-D) decreases from 80/20 to 60/40 and to 40/60. Also the wall thickness in the copolymer cryogels was smaller (6±2 µm) than in the pNIPA cryogel (12±2 µm at 23°C) and nearly independent on temperature. Thus, as a first approximation, the macroporous texture of the different cryogels agrees with their swelling ratio.

For pNIPA, the wall thickness decreases from 12±2 µm at 23°C to 10±2 µm at 28°C and to about 4 µm at 34°C. The mean pore size varies between 37.2, 43.7 and 33.6 nm accordingly. It must be noted that the larger mean pore size at 28°C than at 24°C results from a significant decrease of the contribution of the smaller pores yielding a non-symmetrical distribution. The diminution of the number of small pores also participates to the deswelling of the macroporous gels. In order to check this features on the copolymer cryogels, bi-photonic fluorescence microscopy measurements at intermediate temperatures will be performed in the near future.

5. Conclusions
The aim of the work described in this paper was to show the structural modifications occurring during the stepwise increase of temperature not only at the mesoscale but also, for the first time, at the molecular scale in plain pNIPA and p(HEMA-LLA-D) cryogels and in NIPA-co-HEMA-LLA-D cryogels with different NIPA/(HEMA-LLA-D) ratios. In order to analyze and explain the results obtained for the copolymer cryogels it was necessary first to thoughtfully analyze that obtained for plain pNIPA. To this end, the experimental curves were fitted by means of a sum of four equations in order to take into account the scattering of each component of the system.

The analysis of the parameters yielding structural information at the meso and nano-scale were compared with the macroscopic volume change and the thermodynamic effects over the same range of temperature. It is shown that the critical temperature $T_c=31.4^\circ$C determined from the divergence of the Ornstein Zernike equation is the same as that of the endothermic effect measured by DSC resulting from the "melting" of the water cages surrounding the side chains (dissociation of the hydrophobic solvation). The evolution of the OZ parameters $\xi$ and $I_{OZ}(0)$ with temperature confirms the validity of an Ising model for describing the transition in this pNIPA cryogel. The scaling law between $\xi$ and the intensity $a$ of the chain-chain correlation peak shown here for the first time explains the origin of the isoscattering point observed up to $T_c$. The SAXS curves obtained for the copolymer cryogels do not result from a simple combination of curves obtained for each polymer. Thus, fitting these curves was not possible. Meanwhile, each bunch of isothermal SAXS curves shows also an isoscattering point which allows the comparison with plain pNIPA. Particularly, the analysis of the isoscattering points indicates the absence of a gel collapse which agrees with DSC and swelling ratio measurements.

Finally, the SAXS experiments presented here bring a very new series of information about the structural changes induced by temperature in pNIPA gels and, therefrom, in a new series of NIPA based copolymer cryogels. It is also worth mentioning that the information deduced from the analysis of the SAXS data agrees quite well with the ones obtained by other independent methods.

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