# The pH-responsive behaviour of poly(acrylic acid) in aqueous solution is dependent on molar mass†

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Fluorescence spectroscopy on a series of aqueous solutions of poly(acrylic acid) containing a luminescent label showed that polymers with molar mass, $M_n < 16.5$ kDa did not exhibit a pH responsive conformational change, which is typical of higher molar mass poly(acrylic acid). Below this molar mass, polymers remained in an extended conformation, regardless of pH. Above this molar mass, a pH-dependent conformational change was observed. Diffusion-ordered nuclear magnetic resonance spectroscopy confirmed that low molar mass polymers did not undergo a conformational transition, although large molar mass polymers did exhibit pH-dependent diffusion.

## Introduction

The properties of stimulus responsive materials change in response to perturbations in their environment.[1,2] A class of stimulus responsive materials which has been extensively studied is that of polyelectrolytes in aqueous media. These generally undergo step changes in chain conformation, passing from open, fully solvated, coils to desolvated globular conformations over a small range of pH. This environmentally stimulated conformational change has given rise to the term "smart" materials. At low molar mass, the chain is expected to form an extended rod-like structure when the polymer is charged, which changes to an open coil conformation for chains longer than the persistence length.[3] The uncharged polymer is expected to form a more globular structure for all chain sizes. Here this concept is tested for a model weak polyelectrolyte, poly(acrylic acid) (PAA),[4] which is shown not to undergo this rod-to-coil transition with increasing molar mass but rather displays a transition from a relatively pH-independent conformation over typical ranges of pH at low molar mass to a polymer that exhibits a coil-to-globule transition for greater masses.

PAA is an important industrial polymer, with a particularly important role in dispersants, where smaller molar mass polymers are regularly used, but larger molecules are less effective.[5,6] PAA undergoes a reversible conformational transition (coil-to-globule transition) around pH 5 that is driven by the state of ionization of the carboxylic group,

$$\text{RCOOH} \rightleftharpoons \text{H}^+ + \text{RCOO}^-. \quad (1)$$

At low pH, PAA adopts a compact (but not fully collapsed) globular conformation. However, as the pH is increased, ionization occurs and the polymer expands into a fully solvated open coil conformation.[7] Until now it had always been assumed that the pH response of PAA and similar polymers was independent of degree of polymerization, although a degree of polymerization dependence of conformational transitions is known in other systems,[8] and these generally reflect a greater solubility of smaller polymers.

There are numerous theories considering the size of polyelectrolyte chains[1] but the model used the most is the Flory model, which shows that, in the simplest calculation, polyelectrolytes in their most charged state are expected to form rods. This is obtained by minimization of the Helmholtz free energy for partially charged chains,

$$F = \frac{k_B T R^2}{2bN} + \frac{3}{5}\left(\frac{f^2 N^2 e^2}{4\pi\varepsilon_0 \varepsilon_r R}\right), \quad (2)$$

where $N$ is the number of monomers in the chain; $b$ is a monomer size; $f$, the fraction of monomers that are charged; $R$ is the chain dimension; $\varepsilon_0$, the electric constant; $\varepsilon_r$ is the relative permittivity of the solution; $k_B$ is the Boltzmann constant; and $T$, the absolute temperature. This free energy consists of an entropic (Hookean spring) term and a Coulombic term. The factor 3/5 is to account for the assumption that the charge is uniformly distributed throughout the polymer. As a consequence of this free energy, $R \propto N$. Rod conformations are only associated with the longest chains and significant charging, and formally eqn (2) cannot be used for strongly stretched chains because the Hookean spring term is invalid in this limit.

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Weakly charged polyelectrolytes can generally be expected to take a prolate conformation in which the major axis is extended due to the effect of charge interactions, whilst the two minor axes are defined by the Flory radius, $bN^{1/2}$.[1,9] This is also the case in neutral polymers, where an extended conformation is simply due to the chain ends not being located at the same point.[10] In a more rigorous Flory approach, the Helmholtz free energy for partially ionized polymers is given by[1,9]

$$F = \frac{R^2 k_B T}{b^2 N} + \frac{(efN)^2}{4\pi\varepsilon_0\varepsilon_r R}\ln\left(\frac{R}{b\sqrt{N}}\right). \quad (3)$$

The pH dependence of the swelling is included through the fractional charge, $f$, for values of $R$ sufficiently large that excluded volume can be neglected. The Flory radius, $bN^{1/2}$ defines the chain size over which thermal energy dominates over electrostatic effects; for chains where the Bjerrum length multiplied by the square of the number of ionized monomers is smaller than the Flory radius, excluded volume controls the chain size and the chain adopts a self-avoiding walk. At this size pH dependent effects disappear, except insofar as they control the excluded volume, for example through a transition between hydrophobic and hydrophilic states. The chain size at which excluded volume effects are important is anticipated to be quite small, although it is dependent upon the degree of charging in the chain. For this reason, this simple theoretical model suggests that a coil-to-globule transition would normally be expected for all but the smallest chains.[1]

## Experimental

A series of poly(acrylic acid) (PAA) samples was synthesized with a range of molar masses using controlled reversible addition-fragmentation chain transfer (RAFT) chemistry, using three separate chain transfer agents. These polymers were dissolved in water at a concentration dependent upon the technique being used to interrogate the samples, and acid or base added to change the pH. Buffer was not used in these experiments in order to avoid charges being shielded.

### Benzyl-1*H*-pyrrole-1-carbodithioate (RAFT agent 1) synthesis

Pyrrole (5.00 g, 74.5 mmol) was added dropwise to a rapidly stirring suspension of sodium hydride (1.79 g, 74.5 mmol) in DMSO (80 ml) over 20 min. The solution was then stirred at room temperature for 30 min. The solution was cooled to 5 °C using an ice-water bath before carbon disulfide (5.67 g, 74.5 mmol) was added dropwise. The resultant orange brown solution was stirred at room temperature for 30 min, and then benzyl bromide (12.69 g, 74.5 mmol) was added dropwise. The solution was stirred overnight at room temperature. The mixture was extracted using water (80 ml) and diethyl ether (80 ml), and then the aqueous layer was washed with ether (160 ml) until the entire organic product was extracted. The organic layer was dried over MgSO$_4$ and filtered. The solvent was removed from the mixture by rotary evaporation. The product was purified by flash column chromatography on silica using hexane as the solvent. The RAFT chain transfer agent (CTA) fraction was collected and the solvent removed by rotary evaporation to give 2.4 g (35.1%) of a yellow oil. $^1$H NMR characterization showed the product to contain the following protons: $\delta = 4.65$ (s, CH$_2$), 6.38 (m, H), 7.40 (m, aromatic H), and 7.75 (m, aromatic H).

### 2-Cyano-2-propyl benzodithioate (RAFT agent 2)

2-Cyano-2-propyl-benzodithioate was purchased from Sigma Aldrich (>97% HPLC) and used with no additional purification.

### 2-{[(Butylsulfanyl)carbonothioyl]sulfanyl}propanoic acid (RAFT agent 3) synthesis

Butanethiol (43.00 g, 0.47 mol) and 20 ml acetone was added dropwise to a 90 ml sodium hydroxide solution (16.0 g, 0.40 mol) at 15 °C. The solution was stirred for 30 min, then cooled to 5 °C, at which temperature 30 ml carbon disulfide was added before being left for a further 30 min. 2-Bromopropionic acid (66.0 g, 0.43 mol) was added, followed by another 30 ml NaOH solution and 25 ml deionized water. This was left to stir for 24 h at room temperature before the orange mixture was extracted using 50 ml concentrated HCl and filtered to extract yellow solid. This was repeatedly washed with cold water, resuspended in a stirring solution, and finally recrystallized in hexane to yield 107.3 g (0.45 mol) of product (a 95% yield). Electrospray ionization time-of-flight mass spectroscopy showed a MH$^+$ peak at 239 Da. $^1$H Nuclear magnetic resonance (NMR) characterization showed the product to contain the following protons: $\delta = 3.45$ (t, SCH$_2$C), 1.65 (m, CCH$_2$C), 1.45 (m, CCH$_2$C), and 0.90 (t, CH$_3$C). $^{13}$C NMR also showed the presence of the following carbon atoms: $\delta = 205.61$ (s, SCSS), 36.23 (s, SCH$_2$C), 30.18 (m, CCH$_2$C), 29.84 (m, CCH$_2$C), 21.97 (s, CCH$_2$C), and 13.22 (s, CH$_3$C).

### PAA synthesis

RAFT polymerization[11–14] was used to synthesize a series of poly(acrylic acid) samples with a range in (number average) molar mass from 6.6 to 63.2 kDa. Most of the samples were copolymerized so that approximately 1% of the monomers contained an acenaphthylene (ACE) unit. Monomers were purified by distillation under reduced pressure. Acrylic acid (5 g), 4,4′-azobis(4-cyanovaleric acid) and ACE were dissolved in 1,4-dioxane and placed in 50 ml ampoules with a quantity of chain transfer agent (**1**, **2**, or **3**), as necessary. The polymerization mixtures were degassed *via* repeated freeze–pump–thawing and heated at 60 °C for 24 h. The polymer was precipitated from dioxane into diethyl ether three times before being freeze-dried from water to leave a white solid. $^1$H NMR characterization was carried out in D$_2$O ($^1$H: 2.35 (m, CH) and 1.75 (m, CH$_2$); $^{13}$C: 41 (CH) and 26 (CH$_2$)) using a Bruker AMX-2 400 spectrometer operating at 400 MHz ($^1$H) or 100 MHz ($^{13}$C). ACE and one of the three RAFT CTAs were added prior to polymerization depending on requirements. The presence or absence of ACE was confirmed by UV/Vis analysis using a Specord S-600 spectrophotometer (see ESI†), and potentiometric titration was used to show that the presence of ACE had no discernible effect on the ionization

of the polymer. Furthermore, the use of three distinct RAFT agents allows us to dismiss any possibility that the polymer end group might affect the results presented in this work.

### Molar mass determination

Gel permeation chromatography (GPC) was carried out on a tetrahydrofuran-based system. Samples were prepared *via* a methylation reaction with trimethylsilyldiazomethane then dissolved in tetrahydrofuran (solvent filtered by a 0.45 μm pore). The eluent was passed through 3 × PLgel 10 μm mixed-B LS columns at 1.00 ml min$^{-1}$ using a Kinesis 307 Gilson Pump. Samples were added *via* an Anachem 234 auto injector and the refractive index (RI) signal was recorded using an Erma Inc. ERC-7512 RI detector. The system was calibrated using poly(methyl methacrylate) standards.

### Fluorescence measurements

Conformational changes in polymers can be observed using luminescent labelling techniques, by attaching an aromatic label and observing the lifetime and anisotropic response of the excited state.[4,13] Excited state lifetime and time resolved anisotropy measurements (TRAMS) were recorded using an Edinburgh Instruments 199 fluorescence spectrometer at $\lambda_{ex}$ = 295 nm and $\lambda_{em}$ = 350 nm on dilute solutions of PAA, dissolved in ultrapure water at 1 mg ml$^{-1}$. The aromatic label (ACE) can be covalently bound to the PAA backbone, giving a direct measure of the polymer segmental motion. Excited state lifetime measurements are calculated assuming that the fluorescent label, or probe, is dispersed in a homogeneous environment, so that the decay of the excited state accurately reflects the sensitivity of the species to the solvent and the conformation of the polymer. Assuming this to be the case, the fluorescence intensity, $I(t)$, can be modelled by a dual exponential decay with time constants $\tau_1$ and $\tau_2$,

$$I(t) = A \exp\left(\frac{-t}{\tau_1}\right) + B \exp\left(\frac{-t}{\tau_2}\right). \qquad (4)$$

TRAMS offer an advantage over fluorescence excited state lifetime studies, in that they can be used to study the rotation of the fluorophore. If the fluorophore is covalently bound to the polymer backbone, as for the experiments reported here, then direct measurements of the speed of the polymer segmental motion are possible.[15,16] Parallel and perpendicular polarized intensities, $I_\parallel$ and $I_\perp$ respectively, are used to generate the decay of anisotropy,[15]

$$r(t) = \frac{I_\parallel(t) - I_\perp(t)}{I_\parallel(t) + 2I_\perp(t)}. \qquad (5)$$

The anisotropy $r(t)$ will decay *via* molecular motion within the excited state lifetime $\tau_f$. Assuming that this occurs *via* a simple relaxation mechanism and that the fluorophore is in a homogeneous state, it can be modelled using an exponential decay with time constant $\tau_c$.[13]

Polymers obtained by RAFT are susceptible to fluorescence quenching,[17] but the PAA used here exhibited no luminescence, as determined by steady state measurements (recorded using a Fluoromax-4 spectrofluorometer in ultra-pure water) at these excitation and emission wavelengths, even with the large amounts of chain transfer agent used in the RAFT synthesis.

### DOSY NMR

Between 5 and 20 mg of PAA were dissolved in 80 μl D$_2$O. The pH was adjusted by the addition of HCl and NaOH. Sodium carbonate was used to regulate the pH where necessary. Diffusion-ordered NMR spectroscopy (DOSY, sometimes referred to as pulsed-field gradient or spin-echo NMR) experiments were performed on a Bruker Avance III 400 MHz spectrometer at 25 ± 1 °C, with a temperature stabilization time of at least 5 minutes. The samples were not spun. The gradient strength was increased linearly from 2% to 95% over 16 steps. The maximum gradient strength used was 0.535 T m$^{-1}$. Bipolar rectangular gradients were used with a duration of 2 ms and the gradient recovery delay was 200 μs. Diffusion times were between 0.5 and 1.0 s. The spectrum was phased and baseline corrected before 2D processing *via* Topspin 2.1.6 software (Bruker).

### Hydrogen bond calculations

In order to assess the energies of hydrogen bonding between the carboxylic group with itself and with water, small molecule interactions were considered numerically.[18] These hydrogen bond energies were calculated using Gaussian 09 (d1). Density functional theory calculations were performed using the M05-2X functional and the 6-311++g** basis set. Solvent (H$_2$O) corrections were applied to monomer energies in the gas phase using single point calculations with the polarizable continuum model. These bond calculations include recognition that a dimer contains two bonds, which mutually reinforce each other, therefore increasing the hydrogen bond energy. The acid–water system may in principle contain two bonds, but the interaction considered is only that between the hydroxyl component of the carboxylic group and the oxygen in the water because this is substantially stronger than the carbonyl (acceptor) on the acid and the hydrogen (donor) on the water, which would not be stable in the gas phase.

## Results

Excited state lifetimes of P(AA-*co*-ACE) in dilute aqueous solutions typically decrease from 35 to 25 ns as the pH increases,[19] as a result of statistical uncoiling of the polymer backbone. However, as shown in Fig. 1, analysis of low molar mass polymer excited state lifetimes did not show the increase in lifetime at low pH, so that the lifetime of the fluorescent label remained at ∼25 ns.

Conversely larger molar mass polymers acted in a manner anticipated for PAA, with a significant increase in lifetime observed at low pH. Thus, these data show that the pH-dependent coil-to-globule transition in PAA is critically dependent on molar mass. This finding was further corroborated by analysis of the equivalent TRAMS data (Fig. 2). It has been shown previously that correlation times of dilute P(AA-*co*-ACE) decrease as the
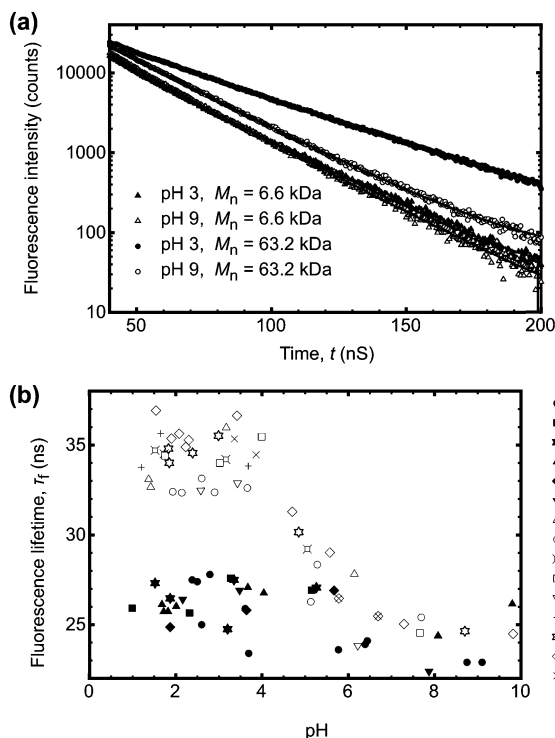
**Fig. 1** (a) Fluorescence decay for a large and a small molar mass PAA sample at pH 3 and pH 9. The large molar mass sample (63.2 kDa) at pH 3 had a longer lifetime than that revealed in the other three experiments, indicating that it alone had a more collapsed conformation. The solid lines are fits to the data. (b) Complete lifetime data as a function of pH, where $\tau_f$ is the average lifetime obtained from the fits to eqn (4). Open symbols are for molar masses of PAA, where $M_n > 16.5$ kDa, which exhibited a pH-dependent coil-to-globule transition. Closed symbols represent polymers that did not show a pH-dependent $\tau_f$. Errors are not shown, but would have been obscured by the symbols. Number average molar masses, $M_n$, are stated in the legend.
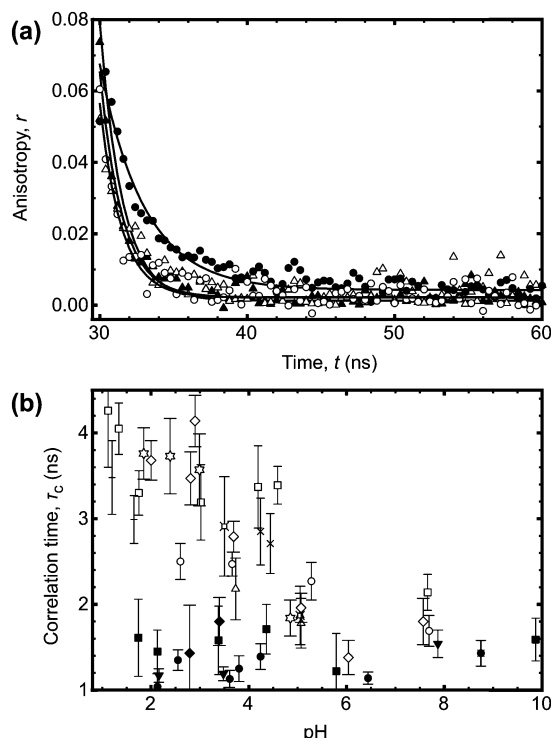


**Fig. 2** (a) Impulse reconvolution fits to the anisotropy function for the same samples (and legend) as shown in Fig. 1a. As in Fig. 1a, the large molar mass sample ($M_n = 63.2$ kDa) showed pH responsive behaviour, with a longer correlation time at pH 3 than the other three samples. The solid lines are fits to the data. (b) Complete correlation time data as a function of pH. The symbols are the same as those in the legend in Fig. 1b.

compacted polymer chain opens into the solvated conformation.[20] When protonated the correlation time of low molar mass P(AA-*co*-ACE) remained fixed across the entire pH range, whilst for high molar mass polymers this increased from ~2 to ~4 ns at pH < 5. The lifetime data showed a step change in behaviour as $M_n$ was increased from $M_n = 16.3$ to 16.9 kDa. Analysis of the anisotropy data was performed *via* impulse reconvolution which removes the effects of the excitation pulse allowing derivation of fast relaxation processes.[13,15]

Both lifetime and anisotropy data suggest the change in behaviour occurs at between 16 and 17 kDa. The results cannot be derived from quenching of the fluorescent ACE group by the (dithioate) end groups, because increased quenching with increasing concentration of the end groups would produce a more gradual change in behaviour than the very narrow molar mass range observed here. Furthermore, it is known that conformational changes in low molar mass acenaphthylene-labelled chains have been observed using fluorescence experiments, albeit for different polymers.[21,22] Thus, the experiments described in the present work show a direct effect of molar mass on the pH responsive behaviour of PAA. These PAAs are

not generally uniform and the dispersities for those polymers in the region of the transition are: 1.2 (15.9 kDa), 1.6 (16.3 kDa), 1.2 (16.9 kDa), and 1.8 (17.7 kDa). The transition in $\tau_c$ is gradual, as would be expected from disperse samples. The particularly sharp transition in $\tau_f$ is probably due, at least in part, to the dominance of long lifetimes in the data. For example, the integrated area under the pH 3 curve in Fig. 1a for $M_n = 63.2$ kDa is some 30% larger than that for the pH 9 data.

In order to unequivocally eliminate unanticipated problems in the fluorescence experiments, 2D DOSY NMR was employed to examine the conformational change of the poly(acrylic acid) backbone over a wide range of pH. Previously, DOSY NMR has been employed to determine the hydrodynamic radius of polymer samples[23] and the diffusion constants of a stimuli responsive polymer (such as PAA) should vary with the conformation of the polymer backbone. An experiment was performed to vary the pH of solutions of seven polymers in $D_2O$. The larger polymers were observed to undergo clear transitions in diffusion constant between pH 4 and 6 (Fig. 3), whilst smaller polymers exhibited no change in size or diffusion constant. These DOSY experiments therefore provide an important independent means of verifying the transition behaviour of the polymer as observed by fluorescence lifetime and TRAMS. In principle it should be possible to obtain the hydrodynamic radius from the diffusion coefficient using the Stokes–Einstein equation, but in this case,
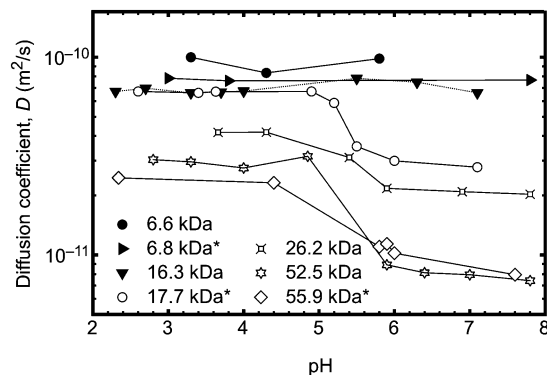
Fig. 3 Diffusion coefficients determined from DOSY measurements of six PAA samples at different pH. Samples marked with an asterisk (*) did not contain an ACE label. Solid lines are guides to the eye.

the solutions of high molar mass polymers were large enough so that the chains were overlapping; DOSY NMR is not sensitive enough with these polymers to provide single molecule results such as those obtained from the fluorescence experiments. Typically single polymer DOSY NMR is restricted to concentrations less than $\sim 1$ g L$^{-1}$,[24] which is much less than the smallest concentration used here. Nevertheless, the experiments confirm the existence of a molar mass-dependent pH-induced conformational transition.

The direct effect of the ACE label on the pH responsive behaviour of PAA is best determined by potentiometric titration. Sample titration data are shown in Fig. 4a. The p$K_a$ obtained from the titration data varied in a narrow range between 4.52 and 4.55, in good agreement with earlier work.[25–27] The curves have the same behaviour, and so it can be confirmed that, regardless of the synthetic method, the PAA has the same pH dependence. That the curves are similar for all of the RAFT agents used indicates that their relative hydrophobicities do not significantly change the behaviour of the polymer.

## Discussion

The conformation of the PAA chain at low pH is not a collapsed structure and remains soluble in water. The collapse of PAA at low pH is similar but much less pronounced than that of poly(methacrylic acid) (PMAA) in that it is solely due to the change in (i.e. loss of) ionization of the acid functional group. PMAA, by contrast, undergoes a transition known as hypercoiling in which it collapses into a tight globule due to the influence of the methyl backbone, which may be observed by a comparison with TRAMS of ACE-labelled PMAA, for which $\tau_c$ covers a greater range (55 ns > $\tau_c$ > 5 ns).[15] Lifetime measurements are insensitive to the hypercoiling effect, and ACE-labelled PMAA exhibits similar values[28] of $\tau_f$ to those described above.

As described in the introduction, the coil-to-globule is anticipated to occur for high molar mass polymers, and this assumption can be tested. However, to determine the point at which the coil-to-globule transition occurs, the fractional number of monomers charged in the chain, $f$, is needed.
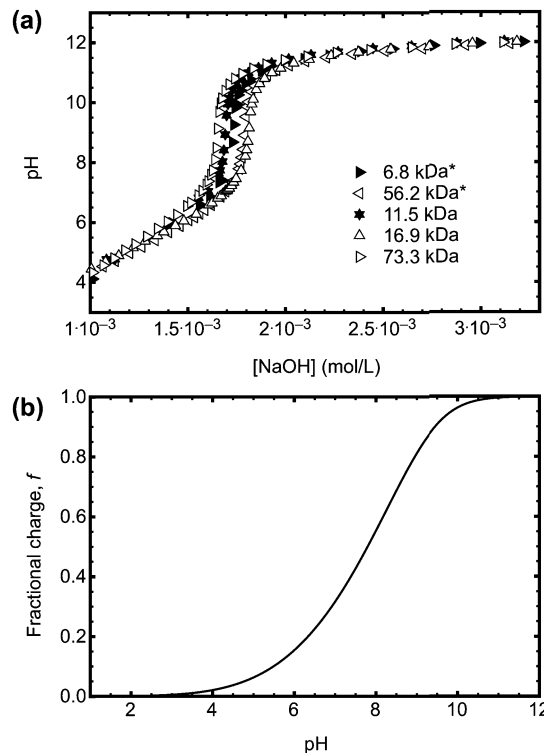


Fig. 4 (a) Potentiometric titration data for a series of PAA samples (initially dissolved in water at a concentration of 1 mg ml$^{-1}$) with and without (*) ACE label. The data shown here are for PAA synthesized using CTA **1** (6.8, 11.5, and 56.2 kDa), **2** (73.3 kDa), and **3** (16.9 kDa). The labelled polymers used different RAFT CTAs. (b) Fractional charge calculated from eqn (6).

The pH dependence of $f$ can be obtained by titration, and an approximate relation for dilute poly(acrylic acid) can be taken from previous work,[26]

$$\mathrm{pH} = \mathrm{p}K_a + 4.10 f^{1/3} - \log\left(\frac{1-f}{f}\right), \qquad (6)$$

where p$K_a$ is the acid dissociation constant extrapolated to $f \to 0$. (The data used are for the most dilute solution, with a concentration of 0.00087 Eq L$^{-1}$. In this work p$K_a$ = 4.26.) The titration data presented in Fig. 4 are also fully consistent with eqn (6); it suffices simply to use p$K_a$ = 4.54 ± 0.01, as determined from the titration data. The results of this calculation (with p$K_a$ = 4.54) are shown in Fig. 4b. A numerical solution to eqn (6) reveals that at pH 7, $f$ = 0.32.

The Bjerrum length multiplied by the square of the number of ionized monomers is equated to the Flory radius at the start of the coil-to-globule transition,[1]

$$\left(\frac{(feN)^2}{4\pi\varepsilon_0\varepsilon_r k_B T}\right) = b\sqrt{N}. \qquad (7)$$

Taking $b$ = 0.64 nm from force spectroscopy measurements,[29] $T$ = 298 K, $\varepsilon_r$ = 80, and using eqn (7), a degree of polymerization, $N$ = 4.3 is obtained for the point at which the conformation becomes prolate. It is necessary to acknowledge that the Flory model can only be approximate, and that it would be preferable,

for example, to equate the electrostatic length to, for example, a self-avoiding walk. Nevertheless, the value of $N$ above which a coil-to-globule transition is experimentally observed ($N \approx 230$) is well over an order of magnitude greater than the value predicted by this simple theory.

The inability to explain the pH-induced swelling of a model polyacid from a simple consideration of Coulombic effects means that another approach is required. The swelling of a polymer chain under different solvent conditions was first presented by Ptitsyn, Kron, and Eizner,[30] in which a simple Flory model for the chain was used. Here the energy of polymer–polymer contacts will be considered as a means to investigate why small chains do not collapse but larger chains do. The swelling, $\alpha$, of the polymer chain (i.e. the ratio of the radii of gyration of the perturbed to the $\Theta$ state) is obtained from the minimization of the Helmholtz free energy of a polymer chain, given by

$$F = \left(\frac{3k_B T}{2}\right)\left(\alpha^2 - 2\ln\alpha\right) - \frac{3U\sqrt{N}}{8\pi\alpha^3} - Nk_B T \ln\left(1 - \frac{3}{8\pi\alpha^3\sqrt{N}}\right), \quad (8)$$

where the first term represents the elastic contribution including terms for swollen ($\alpha^2$) and compressed ($\ln\alpha^2$) chains, and the second and third terms represent contact energies. Here $U$ is the energy gain of monomer–monomer contacts with respect to contacts between monomers and solvent molecules. The swelling term, $\alpha$, is found by minimizing eqn (8) to give

$$\alpha^5 - \alpha^3 - \frac{3\sqrt{N}}{8\pi\left(\alpha^3 - 3/(8\pi\sqrt{N})\right)} = \frac{3\sqrt{N}}{8\pi}\left(1 - \frac{U}{k_B T}\right). \quad (9)$$

Depending on the format of the free energy, slightly different forms for the swelling can be derived.[31–34] The result of eqn (9) is that the swelling of polymers is a non-trivial function of the degree of polymerization. In Fig. 5a, the swelling of the chain is plotted as a function of $N$ for different values of $U$. The swelling increases monotonically with $N$, contradicting the experimental results, which show that, at low pH, there is a discontinuity (and concomitant decrease) in the swelling, with chains larger than 16.5 kDa having a collapsed conformation. Fig. 5b shows the swelling as a function of interaction energy.

For hydrogen bonding to take place, the energy of the carboxylic acid dimer must be less than that of the acid with water. The hydrogen bond between a carboxylic acid dimer (of energy $-37.6$ kJ mol$^{-1}$) in solution is thus $U_{cc} = -15.4 \ k_B T$. A dimer and a hydrogen bond between two water molecules ($-13.3$ kJ mol$^{-1}$ or $-5.4 \ k_B T$) can be compared with two different carboxylic groups hydrogen bonding with water ($2U_{cw} = -11.8 \ k_B T$), which leads to a change in energy on the formation of a dimer of $U = -9.1 \ k_B T$. There are significant approximations here, because, for example, the possibility that the dimer is formed with only one hydrogen bond has been ignored, which would leave one on each carboxylic group free to react with water. Furthermore, only one hydrogen bond between water molecules is assumed, and this is probably not the case in the real systems.
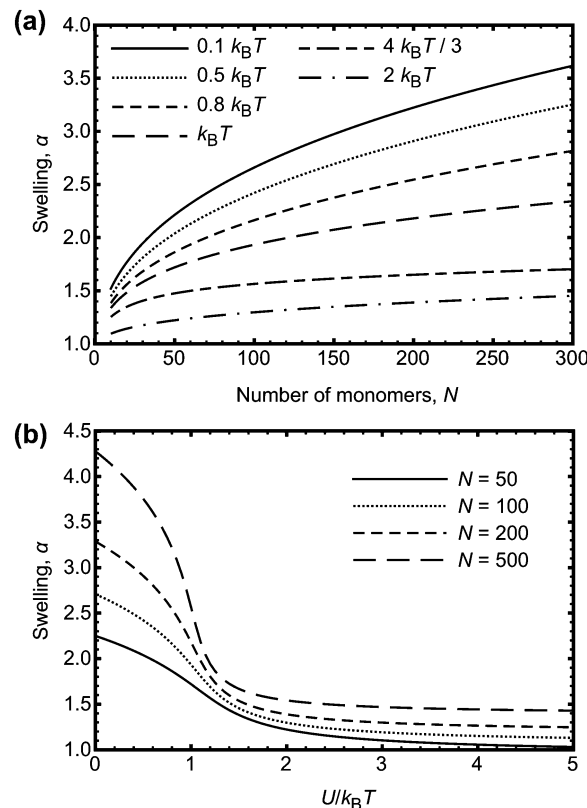


Fig. 5 (a) Swelling, $\alpha$, of a polymer chain as a function of degree of polymerization, $N$, for different interaction energies, $U$. (b) Swelling, $\alpha$, of a polymer chain as a function of $U/k_B T$, for different $N$. The results are numerical solutions to eqn (9). The experimentally observed behaviour where the swelling decreases with increasing $N$ is not predicted from these calculations.

If the interaction energy $U$ were to be a function of $N$, the result obtained from eqn (9) could perhaps be used to explain the experimental data. One possibility is that an effective energy does increase with degree of polymerization because of hydrogen bonds being located on neighbouring monomers. This is more likely on longer chains than on smaller ones, because there are fewer polymer–polymer contacts on smaller chains. Adjacent monomers are more likely to be involved in hydrogen bonding if the free energy benefit is greater than that for forming hydrogen bonds at random points on the chain. It is not obvious that this is the case; adjacent hydrogen bonds can stabilize crystalline structures, such as for polyurethanes,[35] but there are also examples of non-co-operative systems, with hydrogen bonds randomly distributed along the chain.[36,37] The effect of neighbouring monomers participating in hydrogen bonding can be considered in the context of Fig. 5b, if it is stated that the larger chains have an increased $U$ to account for a consideration of neighbouring interactions as one contact. Nevertheless, the hydrogen bond energy is too large to change the swelling of the PAA; multiple neighbouring bonds each with an energy gain of $-9.1 \ k_B T$ will not significantly affect the swelling.

There are other examples of mass dependent transitions; for example, it has also been known for many years that there is a molar mass effect in the temperature-induced shape transition

of poly(ethylene glycol) in water.[38–41] Furthermore, results from fluorescence experiments have hinted that PAA itself exhibits interesting mass-dependent behaviour.[42] Viscosity measurements have also tentatively indicated a different behaviour for low molar mass PAA.[43]

Ultimately, hydrogen bonds are strong, and the presence of such bonding between non-adjacent monomers is likely to cause chain collapse. The models presented above treat interactions as transient contacts but this cannot be the case for hydrogen bonds. A chain with one long-lived monomer–monomer contact is less likely to be able to swell fully. It is also likely that smaller chains will have fewer such contacts. Consider a single chain of size $bN^\nu$ in dilute solution. The probability of a monomer in that chain contacting another monomer in the same chain is proportional to the square of the concentration of that chain,

$$\phi^2 \propto \left( \frac{b^3 N}{4\pi b^3 N^{3\nu}/3} \right)^2 \propto N^{2-6\nu}. \qquad (10)$$

The number of contacts within an individual chain is proportional to the volume of the chain, which makes the number of contacts proportional to $N^{2-3\nu}$. For the swelling of chains whose behaviour is approximated by a random or self-avoiding walk ($0.5 \leq \nu \leq 0.6$), the number of contacts is a very sensitive function of $\nu$. As long as the average number of hydrogen bonds on a chain remains less than one, the chain can remain fully swollen. Of course, hydrogen bonds will eventually form on chains even when the average number is less than one, and the number of such bonds will increase if the hydrogen bonds are essentially permanent. However, the lifetime of a bond is likely to scale as $\tau \exp(U/k_B T)$, where $\tau$ is of the order of ps. For $U/k_B T = 9$, this is less than 1 μs. These bonds are long-lived, but cannot be treated as permanent. Any full discussion of the structure of such polymers will need to account for the long lifetime of hydrogen bonds.

Some conformational transitions are molar mass and concentration dependent. For example, poly($N$-isopropyl acrylamide) (PNIPAm) has a well-known temperature-dependent conformational transition, with a lower critical solution temperature of 32 °C.[44] However, for low molar mass PNIPAm at low concentrations, this transition has been observed to shift to higher temperatures,[45] indicating the existence of an activation energy associated with the transition. This effect is not present at greater concentrations.[46] Given a molar mass dependence of PNIPAm it is worth considering the same for PAA. Here, it was necessary that the fluorescence measurements were performed in very dilute solution in order to limit the effect of self-quenching,[4,47,48] but the DOSY-NMR experiments were performed at concentrations of typically two orders of magnitude greater. The concentrations used for the DOSY-NMR diffusion measurements are listed in Table S4 of the ESI.†

## Conclusion

A complete set of poly(acrylic acid) chains was synthesized by RAFT polymerization and fluorescence experiments were used to demonstrate that a pH-induced conformational transition only occurred for chains of a molar mass greater than 16.5 kg mol$^{-1}$; smaller chains remained solvated. At high pH the correlation time (from TRAMS experiments) and fluorescence lifetime were independent of chain size but at low pH a transition to longer times was only observed for the larger chains. DOSY NMR experiments were also performed to support the conclusions drawn from the fluorescence experiments. Here, the diffusion coefficients of poly(acrylic acid) were measured as a function of pH. For small chains, the diffusion coefficient measured was independent of pH, but for larger chains the diffusion coefficient decreased above a pH of 5. The large molar mass of the polymer above which the conformational transition was observed is particularly surprising. It is possible that the explanation for this phenomenon involves the critical role played by hydrogen bonding in poly(acrylic acid). Small chains are less likely to engage in hydrogen bonding, and it may be that above a critical molar mass, the likelihood of hydrogen bonding in acid solution is sufficient for the chain to undergo a conformational transition from the swollen state at high pH.

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