Polymer Chemistry

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

Journal Name

ARTICLE

The use of electrostatic association for rapid RAFT synthesis of histamine polyelectrolyte in aqueous solutions at and below 25°C

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Tao Huang,[a] Zhigang Cui,[a] Yi Ding,[a] Xinhua Lu[a] and Yuanli Cai*[,a]

Aqueous synthesis of a well-defined polyion or polyelectrolyte *via* reversible degenerative radical polymerisation (RDRP) in dilute solution at or below room temperature is increasingly imperative for emerging biological applications, such as in situ grafting of polyelectrolyte onto heat-sensitive proteins and quantum dots. However, the polymerisation generally suffers from a time-consuming and less effective process because of electrostatic repulsion. This problem can be circumvented by media-adjustable electrostatic association. Herein we report the use of electrostatic association for rapid and quantitative aqueous RAFT of histamine-based ionic monomers in dilute solution under visible light irradiation at and below 25°C. We demonstrate that electrostatic interactions dictate polymerisation rates, including initialization period and chain growth. The use of bulky counter-anions and addition of methanol and sodium chloride can enhance electrostatic association, and thus promote chain-growth rate. More intriguingly, abnormal chain-growth acceleration can be achieved in a cold solution at 8°C, which proceeds faster than at 25°C, because of ion-pairing association as judged by variability of solution conductivity. These external regulations over aqueous RDRP show general implications for rapid and quantitative aqueous synthesis of well-defined polyelectrolyte in dilute solution at and below room temperature.

Introduction

Aqueous synthesis of well-defined polyion or polyelectrolyte in dilute solution at or below room temperature is increasingly imperative in emerging biological fields,[1-4] as a cell-penetrating agent,[5] for bacteria detection/inhibition[6] and for controlled drug/gene delivery and bio-imaging systems, *via* in situ grafting polyelectrolyte onto heat-sensitive proteins/DNAs. To this goal, a challenge that has to be tackled, i.e. electrostatic repulsion interactions,[7-9] which impede or even prevent ionic monomers from chain growth as observed in conventional free radical polymerisation.[10-14] A majority of the current synthesis is the use of reversible degenerative radical polymerisation (RDRP) in absence of additional metal ions, reversible addition-fragmentation chain transfer (RAFT)[15, 16] polymerisation of ionic monomers in the hot solutions at 60-80°C.[17-19] In contrast, room-temperature aqueous synthesis was less exploited because it is rather time-consuming and labile to loss of living character.[17] Basically, it is indispensable to properly understand the variability of electrostatic interactions upon chain growth, for exploration of a new approach for rapid aqueous synthesis of well-defined polyelectrolyte in dilute solution at or below room temperature.

Herein we report the use of electrostatic association for rapid quantitative aqueous RAFT synthesis of polyelectrolyte in dilute solution at and below 25°C. Histamine acrylamide (HA) was synthesized and acidified in situ using different acids (HCl, HBF_4, CF_3SO_3H or TfOH) into cationic monomers (HA-HX, Chart 1). As well known, the ionic polypeptide moieties play crucial roles in hierarchical structures and living functions of proteins, in which histidine units (precursor of histamine) are chargeable peptide motifs that dictate enzymatic hydrolysis,[20] oxidation,[21] and acid-base catalysis.[22] These inspirations gave rise to many biomedical products[23] and the materials for solar cells,[24] CO_2 capture,[25] and selective anion recognition.[26] Zhong and co-workers[27] achieved poly(histamine acrylamide) (PHA) via thiol-mediated radical polymerization in methanol at 60°C for 2 days. Andrew and co-workers[28] obtained PHA by RAFT of an activated ester monomer in acetonitrile at 70°C and then post reaction with histamine. With respect to biological application, it is imperative to explore a new approach for rapid aqueous synthesis of histamine-polymer at or below room temperature.

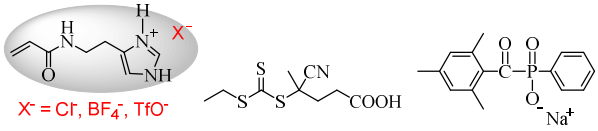

Chart 1 Chemical structures of the ionized histamine acrylamide (HA-HX; HX = HCl, HBF_4, CF_3SO_3H or TfOH) monomers, 4-cyano-4-ethylsulfanylthiocarbonylsulfanylpentanoic acid (CEP) chain transfer agent, and sodium phenyl-2,4,6-trimethylbenzoylphosphinate (SPTP) photo-initiator that were utilized in this article.

[a] *Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Suzhou Key Laboratory of Macromolecular Design & Precision Synthesis, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China. E-mail: ylcai@suda.edu.cn; Tel & Fax: +86-512-65884419*

Electronic Supplementary Information (ESI) available: ^{1}H NMR and UV-vis spectra of HA, HA-HX and reaction solutions; variation of solution conductivity; SEC traces and M_n & PDI variation. See DOI: 10.1039/x0xx00000x

Visible light irradiation was attempted for this synthesis to promote the room-temperature aqueous RAFT polymerisation. Our results have demonstrated that visible light irradiation is sufficient to activate aqueous RAFT and thus induce a ultrafast polymerisation of non-ionic monomer at 25°C.[29] Moreover, the chain growth can be induced and suspended immediately by turning on and off visible light,[30, 31] such that reaction rate can be measured by suspending reaction at predetermined time.[32-34] Fu and co-workers[35, 36] revealed effectiveness of visible light irradiation for activating cobalt-mediated polymerization. Boyer and co-workers[37-39] exploited photo-induced electron transfer-RAFT (PET-RAFT) polymerisation *via* visible light irradiation at room temperature. In contrast, our method is more straightforward in terms of elucidation of the electrostatic association on room-temperature aqueous RAFT synthesis of polyelectrolyte owing to the absence of additional metal ions. In addition, the living character can be maintained by the use of the fast reaction under such aqueous conditions. Different from as-reported aqueous RAFT synthesis,[29-34] the present work focused on the polymerisation in dilute aqueous solution to ensure significant and adjustable electrostatic interactions. The effects of electrostatic repulsion-association on the room-temperature aqueous RAFT of HA-HX monomers were studied by differing counter-anions and concentrations, addition of methanol or sodium chloride. Moreover, reaction in a cold solution at 8°C was studied to explore low-temperature reaction for as-mentioned biological applications. Quantitative synthesis of structure-tuned polyelectrolyte was conducted to illustrate the synthesis efficiency.

Experimental

Materials

Acryloyl chloride, histamine di-hydrochloride, deuterium oxide, and deuterchloric acid (DCl) were purchased from Sigma-Aldrich; trifluoromethanesulfonic acid (TfOH), fluoroboric acid (HBF_4), hydrochloric acid (HCl), triethylamine (TEA), dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), methanol, NaOH, NaCl were from Sinopharm. Acryloyl chloride was distilled; methanol was distilled over Mg/I_2. Other agents were used as received. 4-cyano-4-ethylsulfanylthio-carbonylsulfanylpentanoic acid (CEP) [40] chain transfer agent and sodium phenyl-2,4,6-trimethylbenz-oylphosphinate (SPTP)[41] initiator were synthesized according to literature procedures. Deionized water (R > 18.2 MΩ/cm) was used for the synthesis and characterization.

Visible light source

A mercury lamp was used as an initial light source. UV light (λ_{em} < 400 nm) was filtered by JB400 filters. The light intensity was decrease to I_{420nm} = 0.20 mW cm^{-2} (*ca.* 5% sunlight intensity in May in Suzhou), as determined by a UV-A radiometer equipped with a λ=420 nm detector.

Synthesis of non-ionic histamine acrylamide (HA) monomer

Histamine dihydrochloride (25.00 g, 0.136 mol) and NaOH (16.30 g, 0.408 mol) were dissolved in water (200 mL) in a 500 mL flask. Acryloyl chloride (13.54 g in 100 mL DCM, 0.150 mol) were added dropwise under stirring at -8°C overnight. After removal of DCM, the solution was lyophilized in Labconco Free-zone 2.5L freeze-drier. Crude product was extracted using 2-propanol and passed through silica column. Drying in vacuum afforded a white solid product. Yield: 12.62 g, 56%. ^{1}H NMR (in DCl/D_2O, δ, ppm): 8.48, 7.13 (C=C**H**-NHC**H**=NH^+ in imidazoliums, 2H); 6.05, 5.61 (CH_2=C**H**, 3H); 3.46 (CONHCH_2, 2H); 2.85 ($CONHCH_2CH_2$, 2H).

Aqueous RAFT under visible light irradiation at 25°C

Typically, HA monomer (0.62 g, 3.76 mmol) was dissolved in water (4.3 g) in a 10 mL round-bottom flask. CEP chain transfer agent (3.3 mg in 0.56 g methanol, 13 μmol) and SPTP photo-initiator (0.97 mg in 96 mg water, 3.13 μmol) were added in the flask. The solution was diluted to $[HA\text{-}HCl]_0$=0.6 M, adjusted to pH 3.1 using a 5.0 M hydrochloric acid. The flask was immersed into a water bath at 25°C, bubbled with argon gas for 60 min, and irradiated with visible light. Solution samples were taken at predetermined times, and reaction was ceased by exposure to air and adding hydroquinone for ^{1}H NMR and SEC studies.

Quantitative synthesis of a structure-tuned polyelectrolyte

HA monomer (0.508 g, 3.08 mmol) and water (3.1 g) were added in a 25 mL flask. The CEP chain transfer agent (2.7 mg in 0.28 g methanol, 10.3 μmol) and SPTP photo-initiator (0.794 mg in 80 mg water, 2.58 μmol) were added in the flask. The solution was adjusted by addition of water and TfOH (50% in water) under stirring up to complete dissolving and $[HA\text{-}TfOH]_0$ = 0.6 M at pH 3.1. The solution was bubbled with argon gas for 60 min and irradiated at 25°C for 80 min. Thereafter, visible light was turned off, and argon gas-saturated monomer solution (0.485 g HA-TfOH in 2.08 g water, 1.54 mmol) was added in the dark, and the solution was irradiated for 80 min. Finally, the monomer solution (0.485 g HA-TfOH in 2.08 g water, 1.54 mmol) was added after shielding visible light, and solution was irradiated for 80 min. The reaction was ceased by exposure to air and addition of traces of hydroquinone.

Characterization

^{1}H NMR spectroscopy was performed on Inova 400 MHz NMR instrument. *Size exclusion chromatography (SEC)* was carried out on a PL-GPC220 integrated system fitted with refractive index detector and a set of SEC columns (2 × PLGel MIXED-B + 1 × PLGel MIXED-D). The eluent was DMF that contained 10.0 mM LiBr. PMMA standards (Agilent, 7.36-2136.0 kDa) were used for calibration. Calibration and analysis were conducted at a flow rate of 1.0 mL min^{-1} at 80°C. Samples were protected by di-*tert*-butyldicarbonate according to the literature procedures,[42] such that the absorption of ionic motifs onto SEC column materials could be circumvented. *Solution conductivity* was measured on a Bibby Scientific JENWAY 4520 conductivity meter at controlled temperature within ± 0.1°C. *UV-vis spectra* were recorded on SHIMADZU UV-3150 instrument. Acid-base titration was conducted on a Denver UB-7 digital pH meter.

The laser light scattering intensity was measured on a Brookhaven BI-200SM setup at an angle of 90°.

Results and discussion

Conditions for aqueous RAFT of the ionic monomers at 25°C

The non-ionic HA monomer was charged into ionic monomers in water using HCl, HBF_4 and TfOH, in which the electrostatic association interactions can be mediated by the use of different counter-anions. The solution was adjusted to pH 3.1 before irradiation, in which monomer salts were molecularly dissolved (as judged by discernible signals of as-lyophilized monomer salts by ^{1}H NMR) with negligible free acids according to the acid-base titration results (Fig. S1).

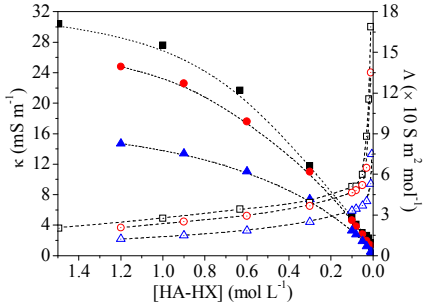

Fig. 1 the solution conductivity (κ, solid) and molar conductivity (Λ, hollow) of HA-HCl (*square*), HA-HBF_4 (*cycle*), and HA-TfOH (*triangle*) as a function of concentrations.

Solution conductivity was measured to evaluate electrostatic interactions since electrostatic association is correlated with the conductivity decrease.[43] As shown in Fig. 1, the κ value depends on monomer concentration and counter-anion, in which the κ values with bulkier counter-anions (BF_4^-, TfO^-) are lower than those with Cl^- at constant concentrations, in good agreement with the electrostatic association capacity in the order of $TfO^- > BF_4^- > Cl^-$.[44-46] Moreover, the ion-paired monomers dissociated into free ions[43] upon diluting to a critical concentration, $[HA\text{-}TfOH]_{crit}$=0.09 M, $[HA\text{-}HBF_4]_{crit}$=0.11 M, $[HA\text{-}HCl]_{crit}$=0.18 M, as judged by abrupt increase of molar conductivities (Λ, κ/[HA-HX]). Ion-pair dissociation increase the electrostatic repulsion, thus hampers the chain growth as observed in traditional free radical polymerization.[11, 12] Accordingly, it is indispensable to elucidate the effects of electrostatic interaction, in order to achieve fast and efficient aqueous RAFT.

Water-soluble SPTP photo-initiator (Chart 1) was synthesized and used herein because of high efficiency in initialization under visible light irradiation in aqueous solution.[47] In addition, the CEP chain transfer agent (CTA) was synthesized according to literature procedure[40] and used to mediate the aqueous RAFT of acrylamide monomer.[17] Otherwise mentioned, minor fractions of methanol (10 wt. %) were added, such that CEP was dissolved in reaction solution and electrostatic association was enhanced before initiation of polymerisation.[48, 49] The reactions proceeded at a target degree of polymerization DP_{target} = 300, i.e. $[HA\text{-}HX]_0/[CEP]_0$=300. A ratio of $[HA\text{-}HX]_0/[SPTP]_0$=1200 was used to ensure sufficient initialization rate under weak visible light irradiation at an I_{420nm} = 0.20 mW cm^{-2}. Both monomer salts and the ionic polymers were molecularly dissolved as judged by ^{1}H NMR and laser light scattering (ESI, Fig. S2). Thus, monomer conversions were precisely determined by ^{1}H NMR.

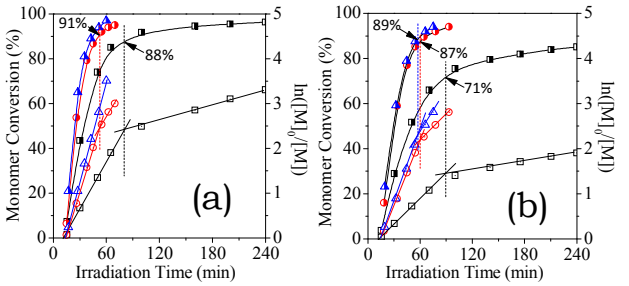

Fig. 2 kinetic plots of RAFT of HA-HCl (*square*), HA-HBF_4 (*cycle*) and HA-TfOH (*triangle*) that started at $[HA\text{-}HX]_0$=0.9 M (**a**) and 0.6 M (**b**).

Electrostatic repulsion induces chain growth slowing-down

As shown in Fig. 2a, high conversions (97%) have been achieved at $[HA\text{-}TfOH]_0$ = 0.9 M on irradiation for 1 h, roughly comparable to HA-HBF_4 but faster than HA-HCl. The reaction rates differed clearly in the dilute solutions at $[HA\text{-}HX]_0$ = 0.60 M (Fig. 2b). Moreover, at $[HA\text{-}HX]_0$=0.9 M, HA-TfOH polymerized in a typical linear kinetic manner to above 97% conversions, but the others showed downward curvature of linear semi-logarithmic kinetic plots after a critical conversion (91% HA-HBF_4, 87% HA-HCl). The downward curvature is more significant in the dilute solutions at $[HA\text{-}HX]_0$=0.60 M (Fig. 2b), as observed at conversions of 90% HA-TfOH, 87% HA-HBF_4, 72% HA-HCl. Critical concentrations of un-reacted monomers were determined: 0.06 M HA-TfOH, 0.08 M HA-HBF_4, and 0.16 M HA-HCl. Considering electrostatic contribution of growing chains,[50] above-assessed un-reacted monomer concentrations are consistent with corresponding critical concentrations of ion-pair dissociation (Fig. 1). Thus, electrostatic repulsion that was induced by ion-pair dissociation gave rise to slowing-down of chain growth. More importantly, electrostatic association of bulky anions can circumvent this chain-growth slowing-down.

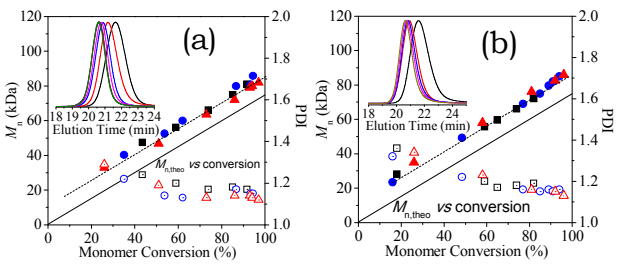

Fig. 3 molecular weight (M_n) and polydispersity (PDI, M_w/M_n) as a function of conversions in reactions at $[HA\text{-}HX]_0$ = 0.90 M **(a)** or 0.60 M **(b)**. *Insert*: the shift of representative SEC traces at $[HA\text{-}TfOH]_0$ = 0.90 M **(a)** and $[HA\text{-}HCl]_0$ = 0.60 M **(b)**, respectively.

As illustrated in inserts of Fig. 3, in both cases, SEC traces are unimodal and symmetrical, and shift towards high molecular

weights with conversions. The molecular weight (M_n) increased linearly and parallel to theoretical plots ($M_{n,theo}$, solid lines) with conversions, and the polydispersity (PDI) decrease to $M_w/M_n \approx 1.15$. These well-controlled behaviours suggest high fidelity of CEP chain-ends. These molecular weights were overestimated in compared to theoretical values owing to BOC-protection and the use of PMMA calibration standards. The high fidelity of CTA chain-ends were also confirmed by the invariable characteristic band ($\lambda_{max,CEP}$=427 nm) in a reaction solution at $[HA\text{-}HCl]_0$=0.6 M (Fig. S3). These indicate that electrostatic repulsion (rather than loss of CEP chain-ends) slowed down chain growth after peculiar critical conversions.

Table 1 The solution conductivity of initial HA-HX monomers and reaction solutions

Monomer	κ_0 [a] (mS m^{-1})	Conversion [b] (%)	κ_t [a] (mS m^{-1})	$(\kappa_t - \kappa_0)/\kappa_0$
HA-HCl	25.4	>98%	17.1	-33%
HA-HBF_4	22.0	>98%	15.6	-29%
HA-TfOH	13.4	>98%	9.8	-27%

[a] κ_0 and κ_t are initial and reaction solution conductivity of 0.9 M HA-HX at 25°C; [b] the monomer conversions were determined by ^{1}H NMR.

As shown in Table 1, the $(\kappa_t - \kappa_0)/\kappa_0$ values, as indicatives of conductivity variability with polymerisation, follows HA-HCl > HA-HBF_4 > HA-TfOH, suggesting that electrostatic association follows $Cl^- < BF_4^- < TfO^-$ during polymerisation. On one hand, as-formed polymers molecularly dissolved in the solutions. On the other hand, the chain growth led to simultaneously dilution of un-reacted monomers and increase of polymer chain length. These effects promoted the ion-paired monomers to dissociate into free ions and their counter-anions to enrich surrounding ionic growing-chain coils. In contrast, bulky anions associated strongly with cationic monomer ions, which minimized repulsion interactions, thus promoted chain growth. These results are consistent with the observations by Amajjahe et al.,[12] in which conventional free radical polymerisation of vinyl-imidazolium monomer strongly sensitive to counter-anion complexation.

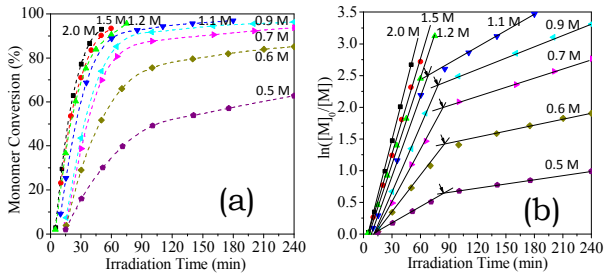

Fig. 4 concentration-dependent kinetic plots of aqueous RAFT of HA-HCl monomer.

Monomer dilution induces the chain growth slowing-down

As discussed above (Fig. 1), chloride anions tended to dissociate into free ions in comparison to BF_4^- and TfO^-. The electrostatic interactions of HA-HCl monomer should thus be highly sensitive to the concentrations and conversions. Clarifying electrostatic interactions of chloride ions with oppositely charged monomer ions on the polymerisation is important because chloride ions are ubiquitous in physiological environment. Accordingly, HA-HCl monomer was used to elucidate the concentration-variable electrostatic interactions on aqueous RAFT polymerisation.

As shown in Fig. 4a, chain growths proceeded rapidly in the solutions over $[HA\text{-}HCl]_0$=2.0-1.2 M (>95% in 1 h) but slowly over $[HA\text{-}HCl]_0$=0.7-0.5 M (4 h of irradiation led to 63% conversion at $[HA\text{-}HCl]_0$ = 0.5 M). As shown in Fig. 4b, the perfect linear kinetic plots were observed till high conversions (>95%) above 1.2 M. While downward curvature of the kinetic plots occurred and become significantly on diluted from 1.10 to 0.50 M (Fig. 4b).

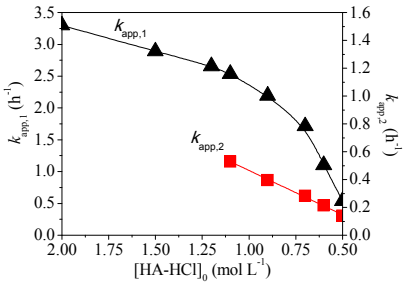

Fig. 5 apparent propagation rate constant ($k_{app,1}$ and $k_{app,2}$) versus initial concentrations.

More specifically, apparent propagation rate constants were determined according to the plot gradients in Fig. 4b. Gradients of fast-reaction parts were named as $k_{app,1}$, and those of slow parts were termed as $k_{app,2}$. As shown in Fig. 5, the $k_{app,1}$ values decreased linearly on dilution from 2.0 M to 1.2 M but sharply from 1.1 to 0.50 M. A downward curvature of semi-logarithmic plots was observed over 1.1-0.5 M, in which the $k_{app,2}$ values decrease linearly with the initial monomer concentrations. Moreover, the un-reacted monomer concentrations at critical conversions are comparable to the critical concentration of ion-pair dissociation (0.09 M, Fig. 1), indicating that electrostatic repulsion impeded chain growth after the critical conversions. Electrostatic repulsion in dilute reaction solutions hampered reaction of monomer radicals with CTA, as judged by relatively long initialisation period.[53, 54] The SEC traces shift toward high molecular weights (Fig. 6a). The M_n values increased linearly at the same variabilities at $[HA\text{-}HCl]_0$ = 0.60, 0.90, and 1.50 M (Fig. 6b). These indicate that the electrostatic repulsion of ionized monomers dictated as-observed propagation slowing-down.

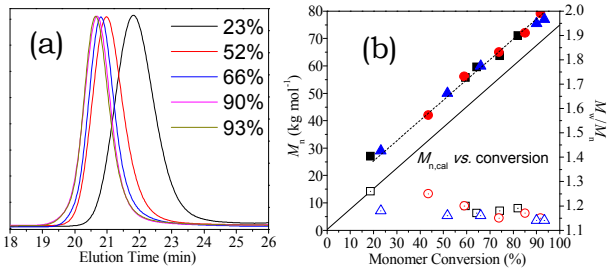

Fig. 6 (a) the shift of SEC trace with polymerisation at a $[HA\text{-}HCl]_0$ = 1.50 M (see Fig. 4); **(b)** variation of molecular weight (M_n) and polydispersity (M_w/M_n) in the reactions at $[HA\text{-}HCl]_0$ = 0.60 (*black*), 0.90 (*red*), and 1.50 M (*blue*), respectively.

Media-sensitive electrostatic association promotes chain growth

Addition of non-ionic organic substance decreases the solution polarity, and thus strengthens electrostatic association of the monomer cations with Cl^- in water.[51, 52] Indeed, polymerisation proceeded rapidly and in a well-controlled manner (Fig. S4) in 30:70 methanol/water at a $[HA\text{-}HCl]_0$ = 0.6 M (Fig. 7a). 93% conversions were achieved on irradiation for 95 min, which was more rapid than in 10:90 methanol/water (just 75% conversions in 100 min), albeit initialization period[53, 54] (25 min) was longer than the latter (15 min). The critical conversion (at 89%) was also higher than the latter (at 71%). The enhanced electrostatic association was confirmed by solution conductivity (18.8 mS/m) lower than the latter (21.8 mS/m). Therefore, addition of non-ionic organic substance can increase electrostatic association, and thus increase the chain-growth rate.

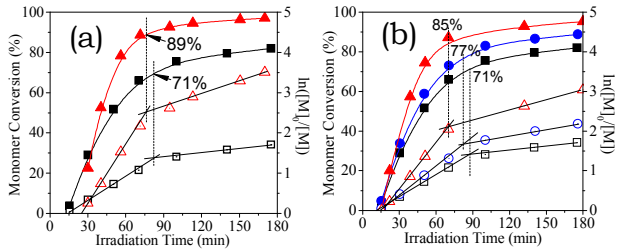

Fig. 7 Kinetic plots of polymerization of HA-HCl in dilute solutions at a $[HA\text{-}HCl]_0$=0.6 M on addition of **(a)** 10% (*black*) and 30% (*red*) methanol, and **(b)** 0 M (*black*), 0.5 M (*blue*) and 1.5 M (*red*) of sodium chloride.

Similarly, addition of strong electrolyte, e.g. sodium chloride, can strengthen electrostatic association.[13, 14, 55-57] Indeed, the polymerisation rates increased with NaCl concentration (Fig. 7b), in which $k_{app,1}$ and $k_{app,2}$ increased with salt concentrations. The critical conversion increased from 70 to 77 and 85% upon increase of NaCl to 0.5 and 1.5 M, respectively. Different from the prolonged initialization period due to addition of methanol, initialization periods maintained roughly constant, suggesting that the monomer radical addition to CEP[53, 54] was less sensitive to ionic strength than the media polarity. These results suggest that the aqueous RAFT of ionic monomers can be regulated on demand simply by adjusting electrostatic association through adjusting the solution media before starting polymerisation.

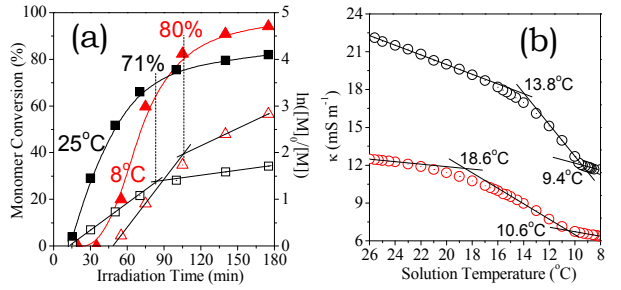

Fig. 8 (a) Kinetic plots of aqueous RAFT at $[HA\text{-}HCl]_0$=0.6 M and 25°C (*black*) and 8°C (*red*); (b) the solution conductivity variability (*black:* the monomer, *red:* the polyelectrolyte at DP=300 and PDI =1.13) with temperatures.

Cooling induces abnormal chain-growth acceleration

Our recent results[30] have demonstrated that visible light can activate efficient aqueous RAFT of nonionic monomer, and thus polymerisation rates maintained constant on cooling from 25°C to 7°C. This unique behaviour facilitates biological applications because proteins, DNAs and RNAs are generally heat-sensitive.[2] This requirement encouraged us to explore aqueous synthesis of the well-defined polyelectrolyte below room temperature. HA-HCl monomer was used because Cl^- ions are ubiquitous in physiological environment and whose electrostatic association is sensitive to temperature.[48, 58]

As shown in Fig. 8a, initialization period prolonged from 11 to 48 min upon cooling the reaction solution from 25 to 8°C. These results indicate that the small-molecule reaction of monomer radicals with CEP[53, 54] was slowed down by cooling. More intriguingly, chain growth proceeded more rapidly at 8°C than at 25°C. 175 min of irradiation gave rise to 94% conversion at 8°C but just 82% at 25°C, despite that the former suffered from significantly long initialization period before the chain growth. More precisely, apparent propagation rate constants at 8°C are roughly two-fold higher than at 25°C, $k_{app,1}$=2.06 h^{-1} and $k_{app,2}$= 0.66 h^{-1} at 8°C, $k_{app,1}$=1.19 h^{-1} and $k_{app,2}$=0.24 h^{-1} at 25°C. The former critical conversion (80% at 8°C) was also higher than the latter (71% at 25°C). ^{1}H NMR and SEC results confirmed the well-controlled RAFT process at 8°C (Fig. S5). Thus, the aqueous RAFT polymerisation shows cooling-induced abnormal acceleration in chain-growth reaction that is unseen in current RDRP family.

To elucidate peculiar cooling-induced aqueous chain-growth acceleration, as-obtained resultant solution (>98% conversion) was dialyzed using a membrane at MWCO=1.0 kDa, and then lyophilized. ^{1}H NMR and SEC revealed a DP of 300 and a PDI of 1.13 of as-purified polyelectrolyte. This polyelectrolyte and HA-HCl monomer were dissolved in methanol/water (10:90). The solutions were adjusted to $[HA\text{-}HCl]_0$ = 0.6 M and pH 3.1. Both conductivity (κ) values were inspected upon cooling solutions from 26°C to 8°C.

As shown in Fig. 8b, the κ value decreased with temperatures. More importantly, a sharp decrease was observed at a critical temperature, owing to the ion-pairing.[43] The decrease of κ value become moderate upon cooling to another critical temperature, indicating that an equilibrium of ion-pairing. As expected, the polyelectrolyte exhibits the κ values lower than the monomer since electrostatic association is stronger than corresponding ionic monomer. These observations are consistent with less free chloride anions in positively charged polyelectrolyte solution than in the corresponding monomer solution.[46] In both cases, chloride anions maintained in the equilibrated ion-pairing state at 8°C as judged by lower than low critical temperatures at 9.4°C and 10.6°C, respectively, but in essentially free-ion state at 25°C as judged by higher than high critical temperatures at 13.8°C and 18.6°C, respectively. Polymerisations beyond critical zones thus shows inverse electrostatic regulation over chain growth.

On one hand, the ion-pairing in cold solution at 8°C improved electrostatic association. It inevitably increased

accessibility of growing-chain radicals to the ion-paired monomers, and thus promoted the chain growth. On the other hand, this aqueous polymerisation was dictated by visible light irradiation.[30] Both effects led to cooling-induced chain-growth acceleration. To our awareness, the cooling-induced polymerisation acceleration is unprecedented in the current RDRP family, although it was thoroughly surveyed in hot aqueous solutions.[15, 17, 59, 60] More importantly, cooling-promoted rapid synthesis holds potentials for low-temperature demanded applications.

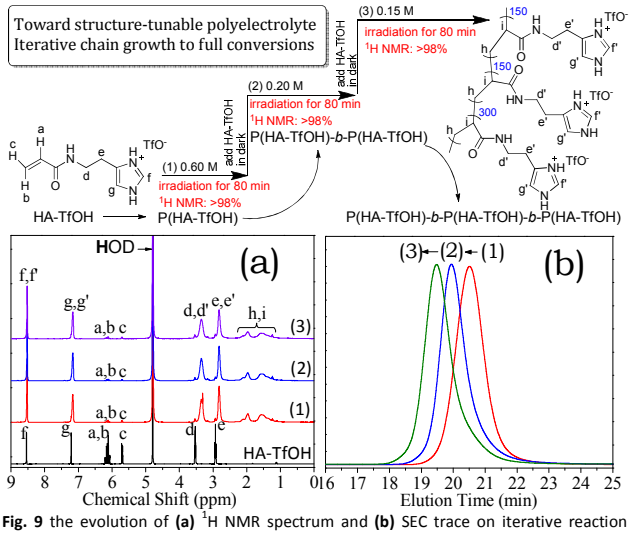

Fig. 9 the evolution of **(a)** ^{1}H NMR spectrum and **(b)** SEC trace on iterative reaction under irradiation for 80 min (spectrum 1), followed by twice monomer addition and irradiated for 80 min (spectra 2, 3). *Top*: schematic illustration of one-pot synthesis.

Quantitative synthesis of a structure-tuned polyelectrolyte

As discussed above (Fig. 2), aqueous RAFT of ionized monomer containing bulky counter-anions under visible light irradiation can reach high conversions due to the electrostatic association. Thus, iterative synthesis *via* RAFT exploited by Perrier,[61] Cu(0)-mediated radical polymerization exploited by Whittaker[62] and Haddleton,[63] and PET-RAFT by Boyer,[64] can be explored for the rapid quantitative synthesis of structure-tuned polyelectrolyte in dilute solution at 25°C. HA-TfOH monomer was chosen due to strong electrostatic association. A quantitative synthesis proceeded *via* subsequent addition of monomer after the former was completely consumed, and visible light irradiation gave successive chain extension.

As shown in Fig. 9a, >97% conversions have been achieved on step-wise irradiation after addition of new HA-TfOH monomer, in which the reactions started up subsequently at $[HA\text{-}TfOH]_0$ = 0.60 M, 0.20 M, 0.15 M. SEC traces of as-generated polymers (Fig. 9b) are unimodal and symmetrical, and shift towards high molecular weights. SEC results revealed a series of well-defined polyelectrolytes with the varied MW from a P(HA-TfOH) at M_n= 80.0 kDa and PDI=1.15, to a P(HA-TfOH)-*b*-P(HA-TfOH) at M_n= 107.9 kDa and PDI=1.19), finally to a P(HA-TfOH)-*b*-P(HA-TfOH)-*b*-P(HA-TfOH) at M_n=149.8 kDa and PDI=1.21.

In short, simply by means of electrostatic association through adjusting solution media, rapid quantitative aqueous synthesis of well-defined polyelectrolyte was achieved in dilute solution at 25°C. More intriguingly, aqueous RAFT in cold solution at 8°C gave rise to abnormal chain-growth acceleration. This photo-mediated polymerization meets perfectly the requirements for the rapid and quantitative aqueous synthesis of well-defined polyelectrolyte, which could have potential use for modification of proteins,[65, 66] and also the controlled drug and gene delivery systems.[67-70]

Conclusions

This article reported the use of electrostatic association for fast aqueous RAFT of histamine-based cationic monomers (HA-HX) in dilute aqueous solution at and below 25°C. To this end, HA was charged using different acids to give counter-anion variable HA-HX monomers. Polymerisation proceeded under visible light irradiation at 25°C or 8°C. Kinetic characters were studied by the use of different counter-anions (Cl^-, BF_4^-, TfO^-), addition of methanol and sodium chloride, and reaction in cold solution at 8°C. Electrostatic interactions in reaction solutions were studied by the solution conductivity analysis. The versatile feature was illustrated using a rapid and quantitative aqueous synthesis of a structure-adjustable polyelectrolyte.

The results demonstrated that the electrostatic interactions dictate effectiveness of aqueous RAFT synthesis. For instance, the electrostatic repulsion was promoted during chain growth due to dissociation of monomer ion pairs into free ions, which slowed down chain-growth reaction. On the contrary, increase of electrostatic association, by the use of bulky counter-anions, addition of methanol and sodium chloride, promoted chain-growth rate. More intriguingly, an abnormal chain-growth acceleration was achieved in a cold solution at 8°C, which proceeded faster than at 25°C due to ion-pairing association. These external regulations were highly efficient to dictate the aqueous RAFT. Therefore, well-defined and structure-tuned polyelectrolyte could be synthesized on demand even in dilute aqueous solution at or below room temperature, by means of electrostatic association *via* adjusting the solution media. This approach meets requirements for the direct aqueous synthesis of well-defined polyelectrolyte and thus holds potential for the controlled drug/gene delivery and bio-imaging systems.[67-70]

Acknowledgements

This research work was supported by National Natural Science Foundation of China (21274097, 21474069, and 21334004) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Notes and references

1. H. Cabral, N. Nishiyama and K. Kataoka, *Acc. Chem. Res.*, 2011, **44**, 999-1008.

2. X. Li, L. Wang, G. Chen, D. M. Haddleton and H. Chen, *Chem. Commun.*, 2014, **50**, 6506-6508.
3. S. Chuanoi, Y. Anraku, M. Hori, A. Kishimura and K. Kataoka, *Biomacromolecules*, 2014, **15**, 2389-2397.
4. K. Hayakawa, S. Uchida, T. Ogata, S. Tanaka, K. Kataoka and K. Itaka, *J. Controlled Release*, 2015, **197**, 1-9.
5. X. Hu, G. Liu, Y. Li, X. Wang and S. Liu, *J. Am. Chem. Soc.*, 2015, **137**, 362-368.
6. Y. Li, H. Yu, Y. Qian, J. Hu and S. Liu, *Adv. Mater.*, 2014, **26**, 6734-6741.
7. S. Forster and M. Schmidt, *Adv. Polym. Sci.*, 1995, **120**, 51-133.
8. J. Bohrisch, C. D. Eisenbach, W. Jaeger, H. Mori, A. H. E. Muller, M. Rehahn, C. Schaller, S. Traser and P. Wittmeyer, *Adv. Polym. Sci.*, 2004, **165**, 1-41.
9. S. Forster, V. Abetz and A. H. E. Muller, *Adv. Polym. Sci.*, 2004, **166**, 173-210.
10. H. Ohno, *Electrochimica Acta*, 2001, **46**, 1407-1411.
11. S. Beuermann, M. Buback, P. Hesse, S. Kukučková and I. Lacık, *Macromol. Symp.*, 2007, **248**, 23-32.
12. S. Amajjahe and H. Ritter, *Macromolecules*, 2008, **41**, 716-718.
13. K. S. Anseth, R. A. Scott and N. A. Peppas, *Macromolecules*, 1996, **29**, 8308-8312.
14. R. Losada and C. Wandrey, *Macromol. Rapid Commun.*, 2008, **29**, 252-257.
15. J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559-5562.
16. G. Moad, E. Rizzardo and S. H. Thang, *Acc. Chem. Res.*, 2008, **41**, 1133-1142.
17. A. B. Lowe and C. L. McCormick, *Prog. Polym. Sci.*, 2007, **32**, 283-351.
18. W. Jaeger, J. Bohrisch and A. Laschewsky, *Prog. Polym. Sci.*, 2010, **35**, 511-577.
19. M. H. Allen, Jr., S. T. Hemp, A. E. Smith and T. E. Long, *Macromolecules*, 2012, **45**, 3669-3676.
20. X. Zhou, T.-F. Chou, B. E. Aubol, C. J. Park, R. Wolfenden, J. Adams and C. R. Wagner, *Biochemistry*, 2013, **52**, 3588-3600.
21. S. Nagano, M. Tanaka, K. Ishimori, Y. Watanabe and I. Morishima, *Biochemistry*, 1996, **35**, 14251-14258.
22. S. Mendel, A. Arndt and T. D. H. Bugg, *Biochemistry*, 2004, **43**, 13390-13396.
23. L. Zhang, X.-M. Peng, G. L. V. Damu, R.-X. Geng and C.-H. Zhou, *Med. Res. Rev.*, 2014, **34**, 340-437.
24. S. Li, L. Qiu, C. Shi, X. Chen and F. Yan, *Adv. Mater.*, 2014, **26**, 1266-1271.
25. J. E. Bara, D. E. Camper, D. L. Gin and R. D. Noble, *Acc. Chem. Res.*, 2010, **43**, 152-159.
26. Z. Xu, S. K. Kim and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 1457-1466.
27. S. Luo, R. Cheng, F. Meng, T. G. Park and Z. Zhong, *J. Poly. Sci., Part A-Polym. Chem.*, 2011, **49**, 3366-3373.
28. J. Y. Quek, P. J. Roth, R. A. Evans, T. P. Davis and A. B. Lowe, *J. Poly. Sci., Part A-Polym. Chem.*, 2013, **51**, 394-404.
29. Y. Shi, H. Gao, L. Lu and Y. Cai, *Chem. Commun.*, 2009, 1368-1370.
30. Y. Shi, G. Liu, H. Gao, L. Lu and Y. Cai, *Macromolecules*, 2009, **42**, 3917-3926.
31. G. Liu, H. Shi, Y. Cui, J. Tong, Y. Zhao, D. Wang and Y. Cai, *Polym. Chem.*, 2013, **4**, 1176-1182.
32. J. Tong, Y. Shi, G. Liu, T. Huang, N. Xu, Z. Zhu and Y. Cai, *Macromol. Rapid Commun.*, 2013, **34**, 1827-1832.
33. Y. Jiang, N. Xu, J. Han, Q. Yu, L. Guo, P. Gao, X. Lu and Y. Cai, *Polym. Chem.*, 2015, **6**, 4955-4965.
34. H. Shi, K. Zhou, Q. Yu, Z. Cui, Y. Jiang, X. Lu and Y. Cai, *Polym. Chem.*, 2015, DOI: 10.1039/c5py01092f.
35. Y. Zhao, M. Yu, S. Zhang, Y. Liu and X. Fu, *Macromolecules*, 2014, **47**, 6238-6245.
36. Y. Zhao, S. Zhang, Z. Wu, X. Liu, X. Zhao, C.-H. Peng and X. Fu, *Macromolecules*, 2015, **48**, 5132-5139.
37. J. Xu, K. Jung, A. Atme, S. Shanmugam and C. Boyer, *J. Am. Chem. Soc.*, 2014, **136**, 5508-5519.
38. S. Shanmugam and C. Boyer, *J. Am. Chem. Soc.*, 2015, **137**, 9988-9999.
39. S. Shanmugam, J. Xu and C. Boyer, *J. Am. Chem. Soc.*, 2015, **137**, 9174-9185.
40. A. J. Convertine, D. S. W. Benoit, C. L. Duvall, A. S. Hoffman and P. S. Stayton, *J. Controlled Release*, 2009, **133**, 221-229.
41. B. Bronstert, A. Henne, A. Hesse, M. Jacobi and G. Wallbillich, Patent US 4719297, 1988.
42. W. Liu, A. B. Greytak, J. Lee, C. R. Wong, J. Park, L. F. Marshall, W. Jiang, P. N. Curtin, A. Y. Ting, D. G. Nocera, D. Fukumura, R. K. Jain and M. G. Bawendi, *J. Am. Chem. Soc.*, 2009, **132**, 472-483.
43. Y. Marcus and G. Hefter, *Chem. Rev.*, 2006, **106**, 4585-4621.
44. C.-L. Wong, A. N. Soriano and M.-H. Li, *Fluid Phase Equil.*, 2008, **271**, 43-52.
45. N. V. Plechkova and K. R. Seddon, *Chem. Soc. Rev.*, 2008, **37**, 123-150.
46. J. Yuan and M. Antonietti, *Polymer*, 2011, **52**, 1469-1482.
47. B. D. Fairbanks, M. P. Schwartz, C. N. Bowman and K. S. Anseth, *Biomaterials*, 2009, **30**, 6702-6707.
48. W. J. Li, Z. F. Zhang, B. X. Han, S. Q. Hu, Y. Xie and G. Y. Yang, *J. Phys. Chem. B*, 2007, **111**, 6452-6456.
49. W. Liu, L. Cheng, Y. Zhang, H. Wang and M. Yu, *J. Mol. Liq.*, 2008, **140**, 68-72.
50. P. Jia, Q. Yang, Y. Gong and J. Zhao, *J. Chem. Phys.*, 2012, **136**, 084904.
51. H. T. Xu, D. C. Zhao, P. Xu, F. Q. Liu and G. Gao, *J. Chem. Eng. Data*, 2005, **50**, 133-135.
52. F. Hallberg, I. Furo and P. Stilbs, *J. Am. Chem. Soc*, 2009, **131**, 13900-13901.
53. J. B. McLeary, J. M. McKenzie, M. P. Tonge, R. D. Sanderson and B. Klumperman, *Chem. Commun.*, 2004, 1950-1951.
54. J. B. McLeary, F. M. Calitz, J. M. McKenzie, M. P. Tonge, R. D. Sanderson and B. Klumperman, *Macromolecules*, 2004, **37**, 2383-2394.
55. C. L. McCormick and L. C. Salazar, *J. Macromol. Sci.-Pure Appl. Chem.*, 1992, **29**, 193-205.
56. V. F. Kurenkov, I. N. Nadezhdin, O. A. Antonovich and F. I. Lobanov, *Russ. J. Appl.Chem. Commun.*, 2004, **77**, 804-808.
57. A. Dobrynin and M. Rubinstein, *Prog. Polym. Sci.*, 2005, **30**, 1049-1118.
58. O. Zech, A. Stoppa, R. Buchner and W. Kunz, *J. Chem. Eng. Data*, 2010, **55**, 1774-1778.

59. S. Yusa, Y. Shimada, Y. Mitsukami, T. Yamamoto and Y. Morishima, *Macromolecules*, 2003, **36**, 4208-4215.
60. M. Barsbay, O. Gueven, T. P. Davis, C. Barner-Kowollik and L. Barner, *Polymer*, 2009, **50**, 973-982.
61. G. Gody, T. Maschmeyer, P. B. Zetterlund and S. Perrier, *Nat. Commun.*, 2013, **4**.
62. C. Boyer, A. H. Soeriyadi, P. B. Zetterlund and M. R. Whittaker, *Macromolecules*, 2011, **44**, 8028-8033.
63. Q. Zhang, P. Wilson, Z. Li, R. McHale, J. Godfrey, A. Anastasaki, C. Waldron and D. M. Haddleton, *J. Am. Chem. Soc.*, 2013, **135**, 7355-7363.
64. J. Xu, K. Jung and C. Boyer, *Macromolecules*, 2014, **47**, 4217-4229.
65. C. Boyer, V. Bulmus, J. Liu, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *J. Am. Chem. Soc.*, 2007, **129**, 7145-7154.
66. J. Xu, K. Jung, N. A. Corrigan and C. Boyer, *Chem. Sci.*, 2014, **5**, 3568-3575.
67. J. Liu, V. Bulmus, D. L. Herlambang, C. Barner-Kowollik, M. H. Stenzel and T. P. Davis, *Angew. Chem. Int. Ed.*, 2007, **46**, 3099-3103.
68. J. K. Pokorski, K. Breitenkamp, L. O. Liepold, S. Qazi and M. G. Finn, *J. Am. Chem. Soc.*, 2011, **133**, 9242-9245.
69. P. De, M. Li, S. R. Gondi and B. S. Sumerlin, *J. Am. Chem. Soc.*, 2008, **130**, 11288-11289.
70. J. D. Wallat, K. A. Rose and J. K. Pokorski, *Polym. Chem.*, 2014, **5**, 1545-1558.

For Table of Contents Entry Use Only

Electrostatic association for rapid and quantitative synthesis of well-defined polyelectrolyte in dilute aqueous solutions at and below 25°C.