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Aqueous SARA ATRP using Inorganic Sulfites†

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Abstract

Aqueous supplemental activator and reducing agent atom transfer radical polymerization (SARA ATRP) using inorganic sulfites was successfully carried out for the first time. Under optimized conditions, a well-controlled poly[oligo(ethylene oxide) methyl ether acrylate] (POEOA) was obtained with <30 ppm of soluble copper catalyst using tris(2-pyridylmethyl)amine (TPMA) ligand in the presence of an excess of halide salts (e.g. NaCl). Inorganic sulfites (e.g. Na₂S₂O₄) were continuously fed into the reaction mixture. The mechanistic studies proved that these salts can activate alkyl halides directly and regenerate the activator complex. The effects of the feeding rate of the SARA agent (inorganic sulfites), ligand and its concentration, halide salt and its concentration, sulfite used, and copper concentration, were systematically studied to afford fast polymerizations rates while maintaining the control over polymerization. The kinetic data showed linear first-order kinetics, linear evolution of molecular weights with conversion, and polymers with narrow molecular weight distributions (~ 1.2) during polymerization even at relatively high monomer conversions ($\sim 80\%$). “One-pot” chain extension and “one-pot” block copolymerization experiments proved the high chain-end functionality. The polymerization could be directly regulated by starting or stopping the continuous feeding of the SARA agent. Under biologically relevant conditions, the aqueous SARA ATRP using inorganic sulfites was used to synthesize a well-defined protein-polymer hybrid by grafting of P(OEOA₄₈₀) from BSA-O-[iBB_r]₃₀.

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Introduction

Reversible deactivation radical polymerization (RDRP) techniques offer control over structure that is similar to ionic living polymerizations,¹ but with all the advantages associated to radical based polymerizations.^{2,3} Nitroxide-mediated polymerization (NMP),⁴⁻⁶ atom transfer radical polymerization (ATRP),⁷⁻¹² and reversible addition–fragmentation chain transfer polymerization (RAFT)¹³⁻¹⁵ are the preferred RDRP methods in the scientific community. ATRP is among the most efficient and versatile RDRP processes. It provides access to a myriad of (co)polymers with precisely controlled architecture, including stars, brushes, and nanogels, as well as block, gradient, and statistical copolymers with specific functionalities.^{9,16-20} Several variations of the normal ATRP method have been developed aiming to reduce the catalyst levels, such as: activators regenerated by electron transfer (ARGET) ATRP²¹⁻²⁴; initiator for continuous activator regeneration (ICAR) ATRP^{25,26}; supplemental activator and reducing agent (SARA) ATRP²⁷⁻³⁴; electrochemically mediated ATRP (eATRP)³⁵⁻³⁸; and photochemically mediated ATRP.³⁹⁻⁴⁴

Recently our research team has discovered a new class of efficient SARA agents for ATRP, sulfite salts: sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$), sodium dithionite ($\text{N}_2\text{S}_2\text{O}_4$) and sodium bisulfite (NaHSO_3).⁴⁵ These salts can directly activate alkyl halides and also reduce *in situ* Cu(II) to Cu(I) species.⁴⁵⁻⁴⁹ In general, sulfites are efficient, inexpensive, safe, and environmentally friendly “co-catalysts” for the synthesis of well-defined polymers both in organic solvents (e.g. DMSO)⁴⁵ and in more eco-friendly solvents like ionic liquids,⁴⁹ alcohols and alcohols/water mixtures.⁴⁶⁻⁴⁸

One limitation associated to traditional ATRP methods was the use of organic solvents to afford homogenous reaction conditions.^{7,50} Over the years, different systems have been developed to replace these volatile and potentially hazardous organic solvents by “green” solvents^{10,51} like supercritical carbon dioxide,^{52,53} ionic liquids^{49,54-56} or water.^{21,26,30,57} Aqueous ATRP usually results in polymers with relatively high dispersity () indicating either poor control or the loss of chain-end functionality.⁵⁷ This fact is due to several reasons: the large ATRP equilibrium constant in aqueous media,^{21,35} which generates high radical concentrations and consequently an increased rate of irreversible termination reactions;⁵⁸ the partial dissociation of the halide anion from deactivator complex, leading to inefficient deactivation of the propagating radicals;⁵⁸ some Cu(I)/ligand (L) complexes can disproportionate or undergo partial dissociation;⁵⁸ and lastly, hydrolysis of the carbon-halogen bond that diminishes the chain-end functionality.^{21,57,58} To suppress these problems, aqueous ATRP was traditionally performed with high copper concentrations that could mitigate the deactivator dissociation, and low ratios of Cu(I)/L:Cu(II)/L to reduce the radical concentration.^{21,57,59} Aqueous ICAR²⁶ and ARGET²¹ ATRP methods were developed aiming to control the polymerization at low Cu concentrations. In the presence of large excess of halide salts (e.g. NaCl or NaBr), well-controlled polymers of oligo(ethylene oxide) methyl ether (meth)acrylate (OEOMA) were obtained using catalyst concentration lower than 100 ppm.^{21,26} After the optimization of reaction conditions, ICAR and ARGET ATRP presented linear first-order kinetics, linear evolution of molecular weight with

conversion, and final $\bar{M}_n$ below 1.3. Thermoresponsive block copolymers and protein-polymer hybrids were synthesized using these techniques.^{21,26}

This study methodically investigates the different parameters of SARA ATRP of oligo(ethylene oxide) methyl ether acrylate (OEOA) in aqueous media catalyzed by Cu(II)Br₂/TPMA using a slow feeding of inorganic sulfites (e.g. Na₂S₂O₄) at room temperature (30 °C) (Scheme 1). This manuscript reports the optimization of reaction conditions in aqueous media for the synthesis of well-defined water-soluble polymers using low Cu concentrations. The reported system is also extended to the synthesis of block copolymers using a “one-pot” method and the preparation of a protein-polymer hybrid.

Results and discussion

Inorganic sulfites, such as Na₂S₂O₅, Na₂S₂O₄ and NaHSO₃, have recently been reported by our research team as supplemental activators and reducing agents in ATRP of (meth)acrylates.⁴⁵⁻⁴⁸ The possibility to control these polymerizations in aqueous media using only low ppm level of catalyst/L make these systems very promising for the preparation of (co)polymers or more complex macromolecular structures to be used in biomedical applications.

In aqueous ATRP, one of the biggest concerns is the partial dissociation of the halide anion from deactivator complex, leading to inefficient deactivation of the propagating radicals, which affects the $\bar{M}_n$ of the polymer.⁶⁰ This issue can be mitigated by adding halide salts to increase the concentration of the XCu(II)/L species that means promote an efficient deactivation.^{21,60} For that reason, the set of experiments were planned considering the addition of an excess of halide salt (NaCl).

In our previous studies Na₂S₂O₄ has proven to be an extremely efficient reducing agent that quickly converts Cu(II)X₂ to Cu(I)X species.⁴⁵ Due to its poor solubility in organic solvents,⁶¹ the concentrations of S₂O₄²⁻ anion and consequently the concentrations SO₂^{•-} resulting from dithionite anion dissociation are very small. In these solvents, an excess of Na₂S₂O₄, was employed,^{45,46} and the concentration of SO₂^{•-} species was maintained low and constant during the course of the polymerization. In aqueous media (more than 75% of water content) the Na₂S₂O₄ can be fully soluble, and consequently can generate a very high concentration of SO₂^{•-} species. In the first approach, an initial experiment was carried out by adding the total amount of Na₂S₂O₄ at the beginning of the polymerization using the following conditions: OEOA₄₈₀/HEBiB/Na₂S₂O₄/CuBr₂/TPMA = 250/1/1/0.05/0.4 (ESI Fig.S1[†] and Table 1, entry 1).

The kinetic results (ESI Fig. S1[†]) showed that the SARA ATRP of OEOA₄₈₀ proceeded in an uncontrolled manner. Most probably, the rapid dissolution of Na₂S₂O₄ at the beginning of the reaction led to a very high rate of Cu(I) (re)generation, depletion of [Cu(II)] and the occurrence undesired termination reactions. This effect is illustrated by the kinetic plot that does not follow linear first-order kinetics (ESI Fig. S1(a)[†]). Also, the $\bar{M}_n^{\text{GPC}}$ values were

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much higher than M_n^{th} (ESI Fig. S1(b)[†]) and a very small shift to high molecular weight fractions was observed in polymer GPC traces (ESI Fig. S1(c)[†]).

Previously in aqueous based systems,²¹ it was shown that the feeding rate of reducing agent (FR) had a major influence on the rate and the control over polymerization. Indeed, the continuous feeding of small amounts $\text{Na}_2\text{S}_2\text{O}_4$ over an extended period of time is essential to maintain its concentration at a relatively low level during the polymerization and achieve an efficient control over the polymerization.

Effect of the feeding rate of sulfite (FR_S) on the polymerization

Based on the previous results, polymerizations with varied FR_S were performed. The feeding rate of $\text{Na}_2\text{S}_2\text{O}_4$ (FR_S) was varied from 8 to 128 nmol/min to determine the effect of the amount of $\text{Na}_2\text{S}_2\text{O}_4$ in the rate of the polymerizations (Fig. 1, ESI Fig. S2[†] and Table 1, entries 2-6). Indeed, higher FR_S led to higher polymerization rates. For a feeding rate of 8 nmol/min, an induction time of around 10 h was observed. A higher feeding rate of 128 nmol/min caused a significant increase of k_p^{app} during the polymerization. This increase of the k_p^{app} could result from either a high termination rate or inefficient deactivation steps. When feeding rates varied from 16 to 64 nmol/min, first-order kinetic plots and good agreement between experimental and theoretical molecular weights were obtained. In addition, the PDI of the final polymer were always below 1.32. The GPC traces showed that the distributions were unimodal and moved clearly toward higher molecular weight with the increasing conversions.

Fig. 2 shows a linear dependence between k_p^{app} and $\text{FR}_\text{S}^{1/2}$ within the controlled range of FR_S values (8 – 64 nmol/min).

In an attempt to enhance the polymerization rate while maintaining the control over the polymerization, two different FR_S were used in the course of a same experiment (ESI Fig. S3[†] and Table 1, entry 7). The results obtained highlighted the role of the concentration of $\text{Na}_2\text{S}_2\text{O}_4$ in the rate of the polymerization, but no significant improvement in k_p^{app} values was achieved when compared the experiment carried out using a constant $\text{FR}_\text{S} = 64$ nmol/min during the entire polymerization. Therefore, this FR_S was used for all subsequent experiments targeting a faster and well-controlled aqueous SARA ATRP.

Model experiments to elucidate the SARA ATRP mechanism The next set of experiments was envisaged to clarify the mechanism

The next set of experiments was envisaged to clarify the mechanism of aqueous ATRP using $\text{Na}_2\text{S}_2\text{O}_4$. The initial experiment was carried out using only monomer (OEOA), ATRP initiator (HEBiB), and deactivator ($\text{Cu}(\text{II})\text{Br}_2/\text{TPMA}$) in water (i.e., in absence of $\text{Na}_2\text{S}_2\text{O}_4$), and no polymerization occurred (Table 2, entry 2). This result indicates that the presence of $\text{Na}_2\text{S}_2\text{O}_4$ is mandatory to promote the *in situ* formation of the active $\text{Cu}(\text{I})/\text{L}$ species. Using $\text{Na}_2\text{S}_2\text{O}_4$ ($\text{FR}_\text{S} = 64$ nmol/min), even without the presence of $\text{Cu}(\text{II})\text{Br}_2/\text{TPMA}$ complex, POEOA were obtained both in the presence (Fig. 3 and Table 2, entry 3) and in the absence (Fig. 4 and Table 2, entry 4) of the ATRP initiator (HEBiB). As verified by the very high values, the polymerization of OEOA was uncontrolled in both cases. The rate of polymerization was much higher in the presence of the ATRP initiator, suggesting that

sulfites can act as supplemental activators of alkyl halides in aqueous media. Similar results have been obtained when sulfites were used to polymerize (meth)acrylates in organic solvents.^{45,46} In the absence of the ATRP initiator (Table 2, entry 4), the polymerization was much slower (~ 230 times), which indicated that the only a very few polymer chains were initiated directly from the $\text{SO}_2^{\bullet-}$ radical anions.

To evaluate the role of sulfites as supplemental activators of alkyl halides in aqueous media, the supplemental activator apparent rate constant (k_{a0}^{app}) was determined for HEBiB using $\text{Na}_2\text{S}_2\text{O}_4$ (Fig. 5 and Table 2, entry 5). Fig. 5 confirms that the $\text{SO}_2^{\bullet-}$ formed by dissociation of $\text{S}_2\text{O}_4^{2-}$ can activate the C-Br bond from HEBiB, as judged by ^1H NMR spectrometry (Fig. 5(a)).

UV-vis spectroscopy was used to study the reduction process of $\text{Cu(II)Br}_2/\text{TPMA}$ by $\text{Na}_2\text{S}_2\text{O}_4$ in aqueous media (Fig. 6, Fig. 7 and Table 2, entry 6). In the first experiment (Fig. 6), a solution of $[\text{CuBr}_2]_0/[\text{TPMA}]_0/[\text{NaBr}]_0 = 2.5/20/50$ mM in water was used by adding all $\text{Na}_2\text{S}_2\text{O}_4$ ($[\text{CuBr}_2]_0/[\text{Na}_2\text{S}_2\text{O}_4]_0 = 1/1$) at the beginning of the experiment. The results showed that $\text{Na}_2\text{S}_2\text{O}_4$ acted as a powerful reducing agent in water, converting all of Cu(II) species into Cu(I) activators within about 1 second. This result corroborates the data obtained for the experiment carried out using the total amount of $\text{Na}_2\text{S}_2\text{O}_4$ in the beginning of the reaction (ESI Fig. S1[†]). The high concentration of Cu(I)/X species led to an high concentration of radicals, which inevitably increased the termination reactions.

In order to determine the reduction rate of $[\text{Cu(II)Br}_2]_0/[\text{TPMA}]_0$ in water at 30 °C, under similar conditions used for the polymerization, a solution of $[\text{CuBr}_2]_0/[\text{TPMA}]_0/[\text{NaCl}]_0 = 10/80/100$ mM in water was used. The feeding of $\text{Na}_2\text{S}_2\text{O}_4$ was set as $\text{FR}_S = 64$ nmol/min (Fig. 7 and Table 2, entry 6). As expected, the reduction rate was much slower when the compared with the total addition of $\text{Na}_2\text{S}_2\text{O}_4$ (Fig. 6). For the feeding rate of $\text{Na}_2\text{S}_2\text{O}_4$ ($\text{FR}_S = 64$ nmol/min) approximately half of Cu(II) species were reduced to Cu(I) in about 140 min.

The results allowed concluding that, in aqueous systems, inorganic sulfites act both as supplemental activators and reducing agents, following the SARA ATRP mechanism. This conclusion has also been verified in the SARA ATRP of (meth)acrylates in the presence of organic solvents (e.g. DMSO, alcohols).^{45,46}

Effect of the ligand (L) and its concentration on the polymerization

As it has been observed in aqueous ATRP, the initial presence of an excess of L compared to Cu increased the concentration of activator and consequently the polymerization rate.²¹ In addition, this aqueous SARA ATRP requires low concentrations of catalyst, and any partial dissociation of L from the metal complex could have a strong impact on the control over polymerization.^{21,30} This assumption was evaluated with two experiments performed using the L/Cu ratios of 2/1 and 8/1 (Fig. 1(d, e and f), ESI Fig. S4[†] and Table 3, entries 1-2). As expected, the kinetic data revealed that for 8-fold excess of L the polymerization was twice faster. Additionally, narrow molecular weight distributions even for high monomer conversions were observed ($\text{MWD} = 1.28$). The data obtained when L/Cu ratio of 2/1 was employed suggest that despite of the relatively high stability of Cu/TPMA complex, due to

low catalyst concentrations used in this aqueous SARA ATRP system, may not be enough to stabilize catalyst complexes. Only a larger excess of L could shift equilibrium toward the Cu(I)/L species.

Another important parameter that has a decisive impact in aqueous SARA ATRP concerns the L used, because it defines the activity and stability of the catalyst.⁶² The substitution of TPMA for Me₆TREN, a L binding stronger to Cu(II) but weaker to Cu(I),^{47,62} (Fig. 1(d, e and f), ESI Fig. S5[†] and Table 3, entries 1 and 3), resulted in polymerization almost 4 times faster. However, the use of Me₆TREN resulted also in broader molecular weight distributions, which revealed an inferior control over the polymerization.

Based on the previous results, TPMA was used as L with a L/Cu ratio of 8/1 for the following experiments targeting a well-controlled aqueous SARA ATRP.

Effect of an added salt and its concentration on the polymerization

It is known that, bromide chain ends are typically 10–100 times more ATRP active than chlorine chain ends, but carbon-chlorine bonds are more stable to hydrolysis.^{21,57,62} Based on that, the following studied parameter was the influence of nature of halogen species on control over polymerization. The results of the experiments, conducted in the presence of three different added salts (NaCl, TEACl and NaBr) are shown in (Fig. 1(d, e and f), ESI Fig. S6[†] and Table 4.

The polymerization in the presence of TEACl (ESI Fig. S6(a, b and c)[†] and Table 4, entry 2) is similar to the polymerization with NaCl (Fig. 1(d, e and f) and Table 4, entry 1) regarding the polymerization rate (similar k_p^{app}) and the control over polymerization (similar $\alpha < 1.28$ even for high monomer conversions). Additionally, both polymerizations presented linear first-order kinetics, linear evolution of molecular weight with conversion, and good correlation between the experimental and theoretical molecular weight. This indicated that probably the anion, unlike the cation, affects the polymerization. To validate this result, NaBr was also tested at the same concentration (100 mM) (ESI Fig. S6(d, e and f)[†] and Table 4, entry 3). The polymerization was faster compared to the ones observed for two chloride salts, but with a poor control of the polymerization ($\alpha \sim 1.41$) (mainly for high monomer conversions) due to the lower hydrolytic stability of the carbon-bromide bond. The addition of halide salts shifts the ATRP equilibrium toward the formation of a stable deactivator, contributing to the improvement of the control over polymerization. The effect of the concentration of the salt on the polymerization was also studied. The influence of varying the NaCl concentration from 10 mM to 100 mM is shown in entries 1 and 4 of Table 4. The results showed that for higher salt concentration slower polymerization was obtained. This observation can be due to: a higher concentration XCu(II)/L; the formation of inactive XCu(I)/L species; or the replacement of TPMA ligand by halide anions.^{21,26,57} As it is mentioned before, the 100 mM concentration of NaCl provided a perfect control over polymerization (Fig. 1(d, e and f)). For lower salt concentrations, faster polymerization was obtained but α values increased to ~ 1.36 (ESI Fig. S6(g, h and i)[†]), which suggests that a NaCl concentration of 10 mM was not enough to prevent the partial dissociation of the deactivator complex.

With no addition of any halide salts (ESI Fig. S6(j, k and l)[†]), the polymerization was significantly faster (Table 4), but the control over polymerization was not so effective (~ 1.47). The GPC traces showed that polymers synthesized in the absence of an added salt exhibited broader molecular weight distributions, which could be due to termination reactions caused by the higher radical concentration. These results confirm that, in this aqueous SARA ATRP, the addition of extra halide species is necessary to promote an efficient deactivation of growing radicals.

Effect of sulfite (or SARA agent) used on the polymerization

The next series of experiments were carried out to compare the efficiency of the three most commonly used inorganic sulfites ($\text{Na}_2\text{S}_2\text{O}_5$, $\text{Na}_2\text{S}_2\text{O}_4$ and NaHSO_3) in aqueous SARA ATRP (Fig. 1(d, e and f), Fig. 8, Fig. 9 and Table 5). The polymerization rates were first order with respect to monomer concentration, the molecular weights determined by GPC matched the theoretical values, proving the efficient initiation and excellent control during polymerizations (α always < 1.28), regardless of the inorganic sulfite used. Comparing the kinetic data, $\text{Na}_2\text{S}_2\text{O}_4$ provided a faster aqueous polymerization of OEOA (Table 5). Thus, $\text{Na}_2\text{S}_2\text{O}_4$ is a more efficient reducing agent, allowing a faster (re)generation of the active Cu(I)X/L catalyst. The polymerizations using $\text{Na}_2\text{S}_2\text{O}_5$ and NaHSO_3 were 25 and 38 times slower, respectively. The same conclusion was reached in the polymerization of (meth)acrylates in DMSO,⁴⁵ although the difference in polymerization rates were not as pronounced (3.5 and 2.3 times slower, respectively). Therefore, $\text{Na}_2\text{S}_2\text{O}_4$ was selected for the further set of experiments.

Variation of Cu concentration, targeted DP and monomer

For certain applications, namely biomedical and electronics, the presence of residual Cu could be a major hurdle. For that reason, it is imperative to find balance between the optimal (minimal) Cu concentration to control the polymerization and an acceptable polymerization rate. It is known that polymerization rate and α of the final product in ATRP are determined by $[\text{Cu(I)}]/[\text{Cu(II)}]$ ratio and Cu(II) concentration respectively.^{2,10,63} (Fig. 1(d, e and f), Fig. 10, Fig. 11 and Table 6 (entries 1-4) shows the effect of lowering the catalyst concentration (the Cu concentration was varied from 130 to 1.3 ppm) for different targeted DPs. The results suggest that lower initial Cu(II) concentrations lead to a poor control over polymerization, mainly defined by the final α values. Also, the polymerization rate decreased with increased targeted DP, due to the lower concentration of growing radicals. It is remarkable to note that even for a very high molecular weight ($M_n^{\text{GPC}} = 267\,500$) P(OEOA₄₈₀) (targeted DP = 1 000) (Fig. 10(a, b and c)), only 6.5 ppm of total copper concentration was enough to obtain acceptable values of dispersity ($\alpha < 1.38$). This result suggests that the effect of $\text{Na}_2\text{S}_2\text{O}_4$ on any side reactions should be small. In the attempt to even more reduce the initial copper concentration, it was tested using only 1.3 ppm (Fig. 10(d, e and f)). The results indicated that 1.3 ppm of Cu concentration was too low to control the polymerization, especially above 50% of monomer conversion.

The optimized conditions for aqueous SARA ATRP reported in this manuscript for OEOA₄₈₀ polymerization (Fig. 11 and Table 6, entry 4) was successfully extended to the polymerization of other monomer, OEOMA₅₀₀ (Fig. 12 and Table 6, entry 5). The results are

comparable to OEOA in terms of control over the polymerization and low dispersity values ($\text{M}_w/\text{M}_n < 1.22$). It is worth to mention that very high monomer conversions ($> 95\%$) were achieved in these two polymerizations (Table 6, entries 4-5) with an excellent control over molecular weight and .

Start/Stop polymerizations

In this work, inorganic sulfites (e.g. $\text{Na}_2\text{S}_2\text{O}_4$) were fed to the reaction in order to regenerate Cu(I) species and promote controlled polymerizations. Thus, one should expect that if the feeding of SARA agent is stopped, a significant buildup of the deactivator occurs shifting the ATRP equilibrium toward the dormant species. As it has been demonstrated in aqueous photoinduced ATRP⁴⁰ or aqueous ARGET ATRP,²¹ the next experiment (ESI Fig. S7 and Table S1[†]) proved that the polymerization can be started or stopped on demand by turning on or off the feeding of $\text{Na}_2\text{S}_2\text{O}_4$. $\text{Na}_2\text{S}_2\text{O}_4$ was continuously added ($\text{FR}_S = 64 \text{ nmol/min}$) to the reaction for 1 h and, for similar conditions (catalyst loading of 26 ppm), the polymerization proceeded at the same rate. It is perfectly visible in ESI Fig. S7[†], after the feeding of the $\text{Na}_2\text{S}_2\text{O}_4$ to be turned off the polymerization almost stopped. This procedure was repeated four more times until a monomer conversion of 75%. In the last cycle after the stop feeding (time = 9 h), the reaction was left during more 15 h and no relevant differences were observed. During the course of this experiment the of the obtained polymers were low and the molecular weights matched perfectly the theoretical ones.

Chain Extension Experiments

The possibility to synthesize polymers with active chain-ends, which can be functionalized or reinitiated to afford complex macromolecular structures, is one of the crucial advantages of RDRP techniques over conventional radical polymerizations. “One-pot” re-initiation and copolymerization experiments were performed to evaluate the livingness of P(OEOA₄₈₀) (Table 6, entry 4) and P(OEOMA₅₀₀) (Table 6, entry 5) chains synthesized using aqueous SARA ATRP. As shown in Fig. 13(a) and Table 7 (entry 1), a complete shift of the GPC traces during the “one-pot” chain extension experiment was achieved. The molecular weight of the starting P(OEOA₄₈₀)-Cl (95% of monomer conversion, $M_n^{\text{th}} = 23\,000$, $M_n^{\text{GPC}} = 21\,100$, $\text{M}_w/\text{M}_n = 1.14$) shifted toward very high molecular weight when fresh monomer was supplied (45% of monomer conversion, $M_n^{\text{th}} = 214\,600$, $M_n^{\text{GPC}} = 201\,200$, $\text{M}_w/\text{M}_n = 1.31$). Also, “one-pot” P(OEOMA₅₀₀)-*b*-P(OEOA₄₈₀) diblock copolymer (43% of monomer conversion, $M_n^{\text{th}} = 206\,500$, $M_n^{\text{GPC}} = 222\,700$, $\text{M}_w/\text{M}_n = 1.29$) was synthesized from a P(OEOMA₅₀₀)-Cl macroinitiator (98% of monomer conversion, $M_n^{\text{th}} = 24\,600$, $M_n^{\text{GPC}} = 26\,200$, $\text{M}_w/\text{M}_n = 1.22$) (Fig. 13(b) and Table 7, entry 2). These results demonstrate the “living” character of the obtained polymers and the possibility of using this aqueous catalytic system in the synthesis of more complex macromolecular structures.

Synthesis of a protein–polymer hybrid

In order to explore the biologically friendly conditions of the aqueous SARA ATRP, as proof-of-concept a protein-polymer hybrid was synthesized by “grafting from” approach. Bovine serum albumin (BSA) was used as a model protein, which contained 30 ATRP initiating sites. The reaction was performed in phosphate buffered saline (PBS) (NaCl (137 mM – 1X) and NaH_2PO_4 (10 mM – 1X) salts) used for protein stabilization. The grafting of

P(OEOA₄₈₀) from BSA-O-[iBBR]₃₀ in PBS showed a linear first-order kinetic plot, linear evolution of molecular weight with conversion, an excellent correlation between experimental and theoretical molecular weight values, and a relatively low dispersity values (≤ 1.35) (Fig. 14 and Table 8). These results indicate that this system can be successfully used to afford protein-polymer hybrids.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) experiment was performed to measure the changes from the native BSA to BSA modified with ATRP initiator (BSA-O-[iBBR]₃₀), and protein-polymer hybrids (BSA-[P(OEOA₄₈₀)₃₀]: 20 μ L, 10 μ L and 5 μ L of sample mixture added to the gel slot) (Fig. 15). With the presence of protein markers, it is clear that the native BSA (66 kDa) was placed between 55 kDa and 72 kDa markers, and BSA-O-[iBBR]₃₀ (75 kDa) was placed slightly above 72 kDa line. As expected, the protein-polymer hybrid BSA-[P(OEOA₄₈₀)₃₀] showed broad bands above 180 kDa and complete consumption of protein initiators were observed.

In Fig. 16, the results obtained by dynamic light scattering (DLS) of BSA-O-[iBBR]₃₀ nanoparticles before polymerization (diameter ($D_{\text{BSA-O-[iBBR]30}}$) = 11.35 nm; dispersity ($PDI_{\text{BSA-O-[iBBR]30}}$) = 0.387) and BSA-[P(OEOA₄₈₀)₃₀] nanoparticles ($D_{\text{BSA-[P(OEOA480)]30}}$ = 57.54 nm; $PDI_{\text{BSA-[P(OEOA480)]30}}$ = 0.256) clearly showed an increase in size and absence of any undesirable aggregation.

Conclusions

The preparation of well-defined (co)polymers at ambient temperature (30 °C) in water using catalyst concentrations between 6 and 26 ppm was achieved by aqueous SARA ATRP of OEOA₄₈₀ and OEOMA₅₀₀ using inorganic sulfites. The critical parameters for preparation of well-controlled polymers were found to be: the slow continuous feeding of inorganic sulfites (Na₂S₂O₄) ($FR_S = 16 - 64$ nmol/min) to the reaction medium; the use of a large excess of halide salt (e.g. NaCl, $[NaCl]_0 = 100$ mM) and high ratio L/Cu (8/1) to ensure the stability of the deactivator complex. Among there different inorganic sulfites, Na₂S₂O₄ was the most efficient SARA agent for the studied system. A start/stop polymerization proved that the reaction can be stopped and (re)started at any point simply by controlling the feed of the SARA agent. The high retention of chain-end functionality was proved by successful “one-pot” chain extension and “one-pot” block copolymerization experiments. Finally, the very low Cu catalyst concentration used, together with the inexpensive, safe, and environmentally friendly inorganic sulfites employed, suggests this aqueous SARA ATRP to be very suitable for working with biological active molecules. As proof-of-concept, a BSA protein-polymer hybrid was successfully synthesized.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. (a, d and g) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b, e and h) plot of number-average molecular weights ($M_{n,\text{GPC}}$) and M_w/M_n vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c, f and i) GPC traces vs. time. Conditions: $[\text{OEOA}_{480}]_0/[\text{HEBiB}]_0/[\text{Cu(II)Br}_2]_0/[\text{TPMA}]_0 = 250/1/0.05/0.4$; $\text{FR}(\text{Na}_2\text{S}_2\text{O}_4) = 8$ (a, b and c), 64 (d, e and f) and 128 (g, h and i) nmol/min ; $[\text{NaCl}]_0 = 100 \text{ mM}$; $[\text{OEOA}_{480}]_0/[\text{Water}] = 1/3$.

Fig. 2.

k_p^{app} values of the SARA ATRP of OE₄₈₀ catalyzed by CuBr₂/TPMA in water at 30 °C vs. $FR(\text{Na}_2\text{S}_2\text{O}_4)^{1/2}$. Conditions: [OE₄₈₀]₀/[HEBiB]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 250/1/0.05/0.4; [OE₄₈₀]₀/[Water] = 1/3.

Fig. 3.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: $[OEOA_{480}]_0/[HEBiB]_0/[Cu(II)Br_2]_0/[TPMA]_0 = 250/1/0/0$; $FR(Na_2S_2O_4) = 64$ nmol/min; $[NaCl]_0 = 100 \text{ mM}$; $[OEOA_{480}]_0/[Water] = 1/3$.

Fig. 4.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: $[OEOA_{480}]_0/[HEBiB]_0/[Cu(II)Br_2]_0/[TPMA]_0 = 250/0/0/0$; $FR(Na_2S_2O_4) = 64$ nmol/min; $[NaCl]_0 = 100$ mM; $[OEOA_{480}]_0/[Water] = 1/3$.

Fig. 5. Determination of the supplemental activator apparent rate constant for HEBiB in water at 30 °C feeding $\text{Na}_2\text{S}_2\text{O}_4$. Conditions: $[\text{HEBiB}]_0 = 20 \text{ mM}$; $\text{FR}(\text{Na}_2\text{S}_2\text{O}_4) = 64 \text{ nmol/min}$.

Fig. 6.

UV-vis spectra of reduction of Cu(II)Br₂/TPMA by Na₂S₂O₄ in water at 30 °C; [CuBr₂]₀/ [TPMA]₀/[Na₂S₂O₄]₀/[NaBr]₀ = 2.5/20/2.5/50 mM.

Fig. 7.

UV vis spectra of reduction of Cu(II)Br₂/TPMA by Na₂S₂O₄ in water at 30 °C; [CuBr₂]₀/ [TPMA]₀/[Na₂S₂O₄]₀/[NaBr]₀ = 2.5/20/2.5/50 mM.

Fig. 8.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OE OA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: [OE OA₄₈₀]₀/[HEBiB]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 250/1/0.05/0.4; [OE OA₄₈₀]₀/[Water] = 1/3; FR(Na₂S₂O₅) = 64 nmol/min; [NaCl]₀ = 100 mM.

Fig. 9.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: $[OEOA_{480}]_0/[HEBiB]_0/[Cu(II)Br_2]_0/[TPMA]_0 = 250/1/0.05/0.4$; $[OEOA_{480}]_0/[Water] = 1/3$; $FR(NaHSO_3) = 64 \text{ nmol/min}$; $[NaCl]_0 = 100 \text{ mM}$.

Fig. 10.

(a and d) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b and e) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c and f) GPC traces vs. time. Conditions: (a, b and c) [OEOA₄₈₀]₀/[HEBiB]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 1000/1/0.05/0.4; (d, e and f) [OEOA₄₈₀]₀/[HEBiB]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 1000/1/0.01/0.08; [OEOA₄₈₀]₀/[Water] = 1/3; FR(Na₂S₂O₄) = 16 nmol/min; [NaCl]₀ = 100 mM.

Fig. 11.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: $[OEOA_{480}]_0/[HEBiB]_0/[Cu(II)Br_2]_0/[TPMA]_0 = 50/1/0.05/0.4$; $[OEOA_{480}]_0/[Water] = 1/3$; $FR(Na_2S_2O_4) = 64$ nmol/min; $[NaCl]_0 = 100$ mM.

Fig. 12.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOMA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: $[OEOMA_{500}]_0/[HEBiB]_0/[Cu(II)Br_2]_0/[TPMA]_0 = 50/1/0.05/0.4$; $[OEOMA_{500}]_0/[Water] = 1/3$; $FR(Na_2S_2O_4) = 64 \text{ nmol/min}$; $[NaCl]_0 = 100 \text{ mM}$.

Fig. 13.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: [OEOA₄₈₀]₀/[HEBiB]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 250/1/0.05/0.1; FR(Na₂S₂O₄) = 64 nmol/min; [NaCl]₀ = 100 mM; [OEOA₄₈₀]₀/[Water] = 1/3.

Fig. 14.

(a) Kinetic plots of conversion and $\ln[M]_0/[M]$ vs. time; (b) plot of $M_{n, GPC}$ and (M_w/M_n) vs. conversion for aqueous SARA ATRP of OEOA₄₈₀ at 30 °C; and (c) GPC traces vs. time. Conditions: [OEOA₄₈₀]₀/[BSA-O-[iBBBr]₃₀]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 250/1/0.05/0.4; [OEOA₄₈₀]₀/[Water] = 1/6; FR(Na₂S₂O₄) = 16 nmol/min.

Fig. 15. SDS-PAGE of native BSA (66 kDa), BSA-O-[iBBBr]₃₀ (75 kDa), various concentrations of BSA-[P(OEOA₄₈₀)₃₀] hybrids, and protein markers.

Fig. 16.

Dynamic light scattering distribution of BSA-O-[iBBR]₃₀ before polymerization (blue line) and BSA-[P(OEOA₄₈₀)]₃₀ nanoparticles; Conditions: [OEOA₄₈₀]₀/[BSA-O-[iBBR]₃₀]₀/[Cu(II)Br₂]₀/[TPMA]₀ = 250/1/0.05/0.4; [OEOA₄₈₀]₀/[Water] = 1/6; FR(Na₂S₂O₄) = 16 nmol/min.

Scheme 1.

Aqueous SARA ATRP of OEOMA₄₈₀ and OEOMA₅₀₀ by Feeding Inorganic Sulfites.

Table 1

Aqueous SARA ATRP of OEOA₄₈₀ with Varied Feeding Rate of Na₂S₂O₄ (FR_S).

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)Br ₂] ₀ /[TPMA] ₀	FR _S (nmol/min)	Cu ^b (ppm)	k _p ^{app} (h ⁻¹)	Time (h)	Conv. (%)	M _n th × 10 ⁻³	M _n ^{GPC} × 10 ⁻³
1	250/1/0.05/0.4	0	26	---	72	7	8.7	36.0
2	250/1/0.05/0.4	8	26	0.016	82	68	82.4	130.3
3	250/1/0.05/0.4	16	26	0.051	30	78	93.9	117.2
4	250/1/0.05/0.4	32	26	0.118	10	67	80.5	92.2
5	250/1/0.05/0.4	64	26	0.181	7	71	85.8	85.0
6	250/1/0.05/0.4	128	26	0.210	7	44	52.8	47.7
7 ^c	250/1/0.05/0.4	64	26	0.182	4.5	55	66.6	63.1
		8		0.034	32	83	99.4	106.5

^a All polymerizations were conducted with [M]₀ = 0.5 M, [I]₀ = 2 mM, and [NaCl]₀ = 100 mM.^b Calculated by the initial weight ratio of Cu to the monomer.^c FR_S = 64 nmol/min during 4.5h and FR_S = 8 nmol/min until 32h.

Table 2

Aqueous SARA ATRP using Inorganic Sulfites.

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)Br ₂] ₀ /[TPMA] ₀	FR _S (nmol/min)	Cu ^b (ppm)	k _p ^{app} (h ⁻¹)	Time (h)	Conv. (%)	M _n th × 10 ⁻³	M _n ^{GPC} × 10 ⁻³
1	250/1/0.05/0.4	64	26	0.181	7	71	85.8	85.0
2 ^c	250/1/0.05/0.4	0	0	---	72	0	---	---
3 ^d	250/1/0/0	64	0	0.363	1.5	41	50.0	344.0
4 ^e	250/0/0/0	64	0	0.0016	90	14	16.4	26.5
5 ^f	0/10/0/0	64	---	0.0196	96	85	---	---
6 ^g	250/1/0.05/0.4	64	26	0.181	7	71	85.8	85.0

^a All polymerizations were conducted with [M]₀ = 0.5 M, [I]₀ = 2 mM, and [NaCl]₀ = 100 mM.

^b Calculated by the initial weight ratio of Cu to the monomer.

^c Without Na₂S₂O₄: [Na₂S₂O₄]₀ = 0 mM and FR_S = 0 nmol/min.

^d Without Cu(II)Br₂/TPMA to prove the SARA ATRP with sulfites.

^e Determination of k₀^{app}: [M]₀ = 0.5 M in water feeding Na₂S₂O₄ (FR_S = 64 nmol/min).

^f Determination of Supplemental Activator Constant (k₀^{app}) for HEBiB ([I]₀ = 20 mM) in water feeding Na₂S₂O₄ (FR_S = 64 nmol/min).

^g Determination of Reduction Process Constant (k_{red}^{app}) by UV-vis spectroscopy of [Cu(II)Br₂]₀/[TPMA]₀ = 10/80 mM in water using [NaCl]₀ = 100 mM and feeding Na₂S₂O₄ (FR_S = 64 nmol/min).

Table 3Aqueous SARA ATRP of OEOA₄₈₀ with Varied Ligand (L) and L/Cu Ratio.

Entry ^a	[M] ₀ /[L] ₀ /[Cu(II)Br ₂] ₀ /[TPMA] ₀	L/Cu	Cu ^b (ppm)	k _p ^{app} (h ⁻¹)	Time (h)	Conv. (%)	M _n th × 10 ⁻³	M _n ^{GPC} × 10 ⁻³	PDI
1	250/1/0.05/0.4	8/1	26	0.181	7	71	85.8	85.0	1.28
2	250/1/0.05/0.1	2/1	26	0.092	12	66	79.2	70.1	1.32
3 ^c	250/1/0.05/0.1	8/1	26	0.688	2.5	82	98.4	101.8	1.44

^a All polymerizations were conducted with [M]₀ = 0.5 M, [L]₀ = 2 mM, [NaCl]₀ = 100 mM and FRS = 64 nmol/min^b Calculated by the initial weight ratio of Cu to the monomer.^c It was used Me₆TREN as L.

Table 4Aqueous SARA ATRP of OEOA₄₈₀ with Varied Salt and Salt Concentration.

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)]/Br ₂ /[TPMA] ₀	Salt : mM	Cu ^b (ppm)	<i>k_p</i> ^{app} (h ⁻¹)	Time (h)	Conv. (%)	<i>M_n</i> th × 10 ⁻³	<i>M_n</i> ^{GPC} × 10 ⁻³	PDI
1	250/1/0.05/0.4	NaCl : 100	26	0.181	7	71	85.8	85.0	1.28
2	250/1/0.05/0.4	TEACl : 100	26	0.121	10	68	81.7	75.9	1.28
3	250/1/0.05/0.4	NaBr : 100	26	0.241	5.8	74	89.3	65.5	1.41
4	250/1/0.05/0.4	NaCl : 10	26	0.227	6.5	75	90.8	86.7	1.36
5	250/1/0.05/0.4	-- : --	26	0.915	4	93	112.1	81.0	1.47

^a All polymerizations were conducted with [M]₀ = 0.5 M, [I]₀ = 2 mM, and FRS = 64 nmol/min;^b Calculated by the initial weight ratio of Cu to the monomer.

Table 5Aqueous SARA ATRP of OEOA₄₈₀ with Various Sulfites.

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)]/Br ₂ /[TPMA] ₀	Sulfite	Cu ^b (ppm)	<i>k_p</i> ^{app} (h ⁻¹)	Time (h)	Conv. (%)	<i>M_n</i> th × 10 ⁻³	<i>M_n</i> ^{GPC} × 10 ⁻³	PDI
1	250/1/0.05/0.4	Na ₂ S ₂ O ₄	26	0.181	7	71	85.8	85.0	1.28
2	250/1/0.05/0.4	Na ₂ S ₂ O ₅	26	0.0073	150	67	80.5	67.3	1.22
3	250/1/0.05/0.4	NaHSO ₃	26	0.0048	164	55	65.7	58.5	1.22

^a All polymerizations were conducted with [M]₀ = 0.5 M, [I]₀ = 2 mM, [NaCl]₀ = 100 mM and FR_S = 64 nmol/min;^b Calculated by the initial weight ratio of Cu to the monomer.

Table 6Aqueous SARA ATRP of OEOMA₄₈₀ and OEOMA₅₀₀ with Varied Cu Concentration and DP.

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)]/Br ₂ /[TPMA] ₀	DP	Cu ^b (ppm)	k _p ^{app} (h ⁻¹)	Time (h)	Conv. (%)	M _n th × 10 ⁻³	M _n ^{GPC} × 10 ⁻³	PDI
1 ^c	250/1/0.05/0.4	250	26	0.181	7	71	85.8	85.0	1.28
2 ^c	1000/1/0.05/0.4	1000	6.5	0.124	7.5	60	287.6	267.5	1.38
3 ^c	1000/1/0.01/0.08	1000	1.3	0.206	5.5	68	325.4	323.6	1.85
4 ^c	50/1/0.05/0.4	50	130	0.238	12.5	95	23.0	21.1	1.14
5 ^d	50/1/0.05/0.4	50	130	0.208	18	98	24.6	26.2	1.22

^a All polymerizations were conducted with [M]₀ = 0.5 M, [NaCl]₀ = 100 mM, and FR_S = 64 mmol/min; [I]₀ = 2 mM for entry 1, [I]₀ = 0.5 mM for entry 2 and 3, [I]₀ = 10 mM for entry 4 and 5.^b Calculated by the initial weight ratio of Cu to the monomer.^c M = OEOMA₄₈₀.^d M = OEOMA₅₀₀.

Table 7

“One-Pot” Chain Extension Experiments from Poly(OEOA₄₈₀) and Poly(OEOMA₅₀₀).

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)Br ₂]/[TPMA] ₀	Monomer	Cu ^b (ppm)	Time (h)	Conv. (%)	$M_n^{th} \times 10^{-3}$	$M_n^{GPC} \times 10^{-3}$
1 ^c	1000/1/0/0	OEOA ₄₈₀	6.5	12	45	214.6	201.2
2 ^d	1000/1/0/0	OEOA ₄₈₀	6.5	20	43	206.5	222.7

^a All polymerizations were conducted with [M]₀ = 0.5 M, [MI]₀ = 2 mM, [NaCl]₀ = 100 mM and FR_S = 8 nmol/min;

^b Calculated by the initial weight ratio of Cu to the monomer.

^c The Poly(OEOA₄₈₀)-Cl (MI) was obtained in Table 5, entry 4;

^d The Poly(OEOMA₅₀₀)-Cl (MI) was obtained in Table 5, entry 5;

Table 8

SARA ATRP of OEOA₄₈₀ in PBS Initiated by BSA-O-[iBBT]₃₀.

Entry ^a	[M] ₀ /[I] ₀ /[Cu(II)Br ₂]/[TPMA] ₀	FR _S (nmol/min)	Cu ^b (ppm)	k _p ^{app} (h ⁻¹)	Time (h)	Conv. (%)	M _n th × 10 ⁻³	M _n ^{GPC} × 10 ⁻³	PDI
1	250/1/0.05/0.4	16	26	0.105	10	63	75.1	75.6	1.35

^a All polymerizations will be conducted with [M]₀ = 0.5 M, [I]₀ = 2 mM.

^b Calculated by the initial weight ratio of Cu to the monomer.