

Synthesis of Uniform Polymer Microspheres with “Living” Character Using ppm Levels of Copper Catalyst: ARGET Atom Transfer Radical Precipitation Polymerisation

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ABSTRACT

The first example of activators regenerated by electron transfer atom transfer radical polymerisation (ARGET ATRP) under precipitation polymerisation (PP) conditions is reported. This new method for polymer synthesis (called ARGET ATRPP) requires only very low levels of metal catalyst for success, a distinctive feature that enables the production of uniform polymer microspheres under reversible-deactivation radical polymerisation control. The influence of the polymerisation conditions (monomer concentration, reaction time, initiator and catalyst concentrations) on the polymerisations and microsphere products were investigated and optimised. The ARGET ATRPP methodology was used to prepare polymer microspheres with narrow particle size distributions and mean particle diameters in the micron-size range, under dilute monomer conditions (2%) whilst using very low copper catalyst concentrations (down to 1.7 ppm). The “living” characteristics of the polymer microspheres enabled poly(methyl methacrylate) brushes to be *grafted-from* the microspheres directly without the need for any additional surface functionalisation step to immobilise initiator moieties.

Keywords: activators regenerated by electron transfer; atom transfer radical polymerisation; precipitation polymerisation; ARGET ATRPP; polymer microspheres.

1. INTRODUCTION

Polymer microspheres have attracted considerable attention in recent years due to their wide range of uses [1, 2]. From a number of synthesis techniques available for polymer microsphere production, precipitation polymerisation has been adopted widely due to its ease of operation and ability to deliver high quality products with narrow particle size distributions but without any need for the inclusion of stabilizers or surfactants during synthesis to prevent coagulation [3]. The precipitation polymerisation of vinyl monomers by free radical polymerisation starts with a homogenous, dilute solution of monomer(s) and initiator in a near- θ solvent. As the polymerisation ensues, soluble oligomers aggregate and precipitate out of solution to nucleate seed particles which then grow by the continuous capture of oligomers from solution. Typically, the crude reaction products are returned in the form of milky suspensions of polymer particles, from which the polymer microspheres can be isolated with ease by filtration or centrifugation.

Many refinements and extensions to the precipitation polymerisation methodology reported by Stöver *et al.* in 1993 [3] have been described in the literature, including distillation precipitation polymerisation [4] and photo-initiated precipitation polymerisation [5]. Within our own group, we have used precipitation polymerisation extensively for a range of purposes, including in molecular imprinting studies and for the production of hypercrosslinked polymer microspheres *inter alia* [6]. Typically, the polymer microspheres that are formed are crosslinked but have an appreciable level of pendent, unreacted vinyl groups (derived from crosslinker) decorating their surfaces. Whilst these vinyl groups can be used as functional handles for polymer-analogous reactions [7], functionalisation of the polymers to install polymerisation initiator moieties is an essential prerequisite to *grafting-from* studies. Motivations for conducting *grafting-from* studies from polymer surfaces include the opportunity to incorporate additional, special features into polymers such as thermo-responsiveness [8, 9], water compatibility [10] and layers of controllable thickness [11], however the installation of initiator moieties can be tedious and is complicated by the fact that the chemical reactions are heterogeneous in nature. Therein lies an opportunity to develop a streamlined synthetic methodology for the production of grafted polymer microspheres that do not require a polymer-analogous reaction step to immobilise polymerisation initiators.

Since its discovery in 1995 [12, 13], atom transfer radical polymerisation (ATRP) has evolved into one of the most versatile synthetic methods for the preparation of vinyl polymers with predictable

molecular weights, narrow molecular weight distributions and controlled architecture. The practical success of ATRP relies upon the establishment of a dynamic equilibrium between active (propagating) free radicals and dormant alkyl halides by means of continuous halogen atom exchange between transition metal complexes and propagating free radical centres. So far, ATRP has been used to prepare linear, star [14, 15], block [16] and graft [17] copolymers. Zhang's group were the first to report the use of ATRP in the context of precipitation polymerisation [18]; this new technique, called Atom Transfer Radical Precipitation Polymerisation (ATRPP), has been used to prepare molecularly imprinted polymer (MIP) microspheres, and MIPs prepared by this method showed significantly higher binding capacities than analogous microspheres prepared using free radical polymerisation methods. More recently, photo-initiated ATRP was used by Haupt *et al.* to prepare imprinted nanomaterials and nanocomposites [19].

Despite its applications within many fields, conventional ATRP does have some drawbacks, one of which is contamination of the polymer products by residual metal complex catalysts (which are often copper-based). Residual metal catalyst limits ATRP applications in various fields, such as microelectronics, biomaterials, *etc.* [20, 21]. Unsurprisingly, methods to remove residual catalyst have been developed, including the use of ion-exchange resins to capture soluble catalyst [22] and the immobilisation of catalysts onto solid surfaces [23]. More recently, greener ATRP techniques that require lower levels of catalyst [24], or even no metal complex catalyst at all [25], have been developed to address the metal catalyst contamination issue.

Herein, we describe a new ATRP-based methodology (ARGET ATRPP) for the production of polymer microspheres, a method which uses very low (ppm) levels of metal complex catalyst. Moreover, since the polymers have high levels of chain-end functionality [26] and the chain-end functionality is polymer-bound, the microspheres are set-up for immediate *grafting-from* studies to give grafted products with high grafting densities (no need for polymer-analogous reactions to install initiator moieties). To the best of our knowledge, this is the first report on the synthesis of polymer microspheres *via* a combination of ARGET ATRP and precipitation polymerisation.

2. EXPERIMENTAL

2.1. Materials

Technical grade divinylbenzene (DVB-55; comprising 55% DVB isomers and 45% ethylvinylbenzene isomers) and methyl methacrylate (MMA, 99%), were purchased from Sigma and passed through an activated basic alumina column prior to use. *L*-Ascorbic acid (Aldrich, 99%), acetonitrile (ACN, Aldrich, 99.8%), ethyl 2-bromoisobutyrate (EBiB, Aldrich, 98%), copper(I) bromide (CuBr, Aldrich, 99%), copper(II) bromide (CuBr₂, Aldrich, 99%) and *tris*[2-(dimethylamino)ethyl]amine (Me₆Tren, Aldrich, 97%), were used as received.

2.2. Instrumentation

All precipitation polymerisations were carried out in an incubator oven (UVP hybridizer oven, Analytik Jena, Jena, Germany) at a temperature of 65 °C and a rotation speed of ~ 8 rpm. Scanning Electron Microscopy (SEM) images were acquired using a Stereoscan 90 (Cambridge Instruments) instrument. Polymer microspheres were sputter-coated with gold using a Polaron SC500A Sputter Coater Fison Instrument prior to analysis. Image analysis of the SEM micrographs was performed using Image J47 software, on a population of 100 microspheres. The following equations were used to calculate the particle sizing characteristics:

$$U = \frac{D_w}{D_n}; D_n = \sum_{i=1}^k (n_i D_i) / \sum_{i=1}^k (n_i); D_w = \sum_{i=1}^k (n_i D_i^4) / \sum_{i=1}^k (n_i D_i^3)$$

where *U* is the polydispersity index, *D_n* is the number-average diameter, *D_w* is the weight-average diameter, *N* is the total number of the measured particles, and *D_i* is the particle diameter of the microspheres. FT-IR spectra were recorded on an Agilent ATR FT-IR spectrometer operating in the range 4700–650 cm⁻¹, with a resolution of 2 cm⁻¹ and with the number of scans set to 4.

2.3. General Procedure for the Preparation of Polymer Microspheres by ARGET Atom Transfer Radical Precipitation Polymerisation

Polymer microspheres were prepared *via* ARGET Atom Transfer Radical Precipitation Polymerisation according to the following procedure: DVB-55, ascorbic acid, acetonitrile and CuBr₂ were added to a borosilicate glass Kimax tube. The reaction mixture was purged with oxygen-free nitrogen gas for 15 min. and Me₆TREN then added. After a further 15 min. of nitrogen

gas purging, EBiB was added. The Kimax tube was then sealed under nitrogen and transferred to the incubator oven where it was rolled around its long axis at 8 rpm for 24 h at 65 °C. At the end of the reaction period, the crude reaction mixture was returned in the form of a milky suspension of polymer particles, from which the polymer microspheres were isolated by vacuum filtration on a nylon membrane filter. The polymer microspheres were washed sequentially on the filter with acetonitrile, water and acetone prior to drying overnight *in vacuo* (50 mbar) at 60 °C.

2.4. Grafting of Polymer Brushes from Polymer Microspheres

Polymer microspheres (14 mg), MMA (159.8 μL , 1.5 mmol) and acetonitrile (2 mL) were added to a 25 mL round-bottomed flask. The flask contents were purged with nitrogen gas for 10 min., CuBr (3.6 mg, 0.025 mmol) in Me₆TREN (13.3 μL , 0.05 mmol) added and the flask contents purged with nitrogen gas for a further 5 min. The flask was then sealed under nitrogen and transferred to an oil bath; the contents were stirred at 300 rpm for 24 h at 65 °C. The polymer microspheres were then collected by centrifugation and washed sequentially with acetonitrile, water and acetone before drying overnight *in vacuo* (50 mbar) at 60 °C.

3. RESULTS AND DISCUSSION

For polymerisation control, ATRP relies upon the equilibrium that is established between active (propagating) free radicals and dormant alkyl halides by continuous halogen atom exchange between transition metal complexes and the propagating radical sites. Normally, this equilibrium is adjusted to favour the dormant state since this lowers the active radical concentration and suppresses the radical termination reactions that are an integral feature of free radical polymerisations (Figure 1). However, a portion of chain-end functionality is inevitably lost during polymerisation, not only because radical termination reactions are not suppressed entirely but also because of side-reactions between the propagating radicals and the copper catalyst. The level of chain-end functionality in a linear polystyrene was reported to be 48% for the ATRP of styrene after 92% of the monomer had been consumed [26].

Activators regenerated by electron transfer (ARGET) ATRP [27] enables the ATRP of vinyl monomers using low levels of catalyst (concentration 10,000 to 10 ppm or lower) whilst still retaining control of the polymerisation. This is achieved by continuously reducing the metal complex in its higher oxidation state with an FDA-approved reducing agent such as ascorbic acid

or with tin(II) 2-ethylhexanoate ($\text{Sn}(\text{EH})_2$). Lowering the metal complex catalyst concentration to well below the levels used in conventional ATRP makes the process not only more environmentally friendly but also reduces the number of undesirable side-reactions between propagating radicals and catalyst and enhances the level of polymer chain-end functionality. For example, linear polystyrene prepared by ARGET ATRP was reported to have 87% chain-end functionality at 92% monomer conversion [26]. In the light of these reports, it is to be anticipated that DVB-based polymers prepared by ARGET ATRPP will also have high levels of chain-end functionality, which will then permit the preparation of densely grafted copolymers *via a grafting-from* approach. In addition to the need for significantly less catalyst, ARGET ATRP requires less stringent polymerisation conditions than conventional ATRP since the presence of an excess of reducing agent counters the oxidising potential of any adventitious oxygen and counters the effects of unwanted termination processes. One positive practical consequence of this is that simple methods for the deoxygenation of monomer solutions can be used for ARGET ATRP.

In the present work, ARGET ATRP was applied and adapted for precipitation polymerisation to allow the ARGET ATRP methodology to be extended to include the production of crosslinked polymer microspheres. In this regard, DVB-55, EBiB, $\text{Cu}(\text{II})\text{Br}/\text{Me}_6\text{TREN}$ and ascorbic acid were used as crosslinker, initiator, catalyst/ligand and reducing agent, respectively (Figure 1). When $\text{Cu}(\text{II})$ is reduced by ascorbic acid, the resulting $\text{Cu}(\text{I})$ is able to abstract the halogen from the EBiB initiator to give an initiating radical. Unlike conventional precipitation polymerisation, where soluble oligomer radicals are captured *via* vinyl groups pendent to the growing microspheres during the growth phase of the polymerisation [28], for ARGET ATRPP it is monomers that are captured from solution during microsphere growth in a manner similar to surface-initiated polymerisation [29]. This is a very significant differentiating mechanistic feature between precipitation polymerisation conducted under free radical polymerisation conditions on the one hand and ARGET ATRPP conditions on the other hand.

3.1. Effect of monomer concentration

Monomer concentration is known to have a significant influence on precipitation polymerisations [3, 30], therefore the effect of monomer concentration on particle size and particle size distribution was investigated first. SEM images of the polyDVB-55 polymer microspheres prepared at different monomer concentrations in acetonitrile are shown in Figure 2. The initiator concentration was set

to 1 mol% relative to monomer, and the monomer concentration was varied from 1% (v/v) to 6% (v/v) relative to porogen (these are typical monomer concentrations for precipitation polymerisations). When the monomer concentration was 1%, no insoluble polymer microspheres were isolated from the reaction medium, however when the monomer concentration was increased from 1% (v/v) to 2% (v/v) then uniform polymer microspheres were isolated, albeit in a low yield (Table 1). Low yields of insoluble polymer product are to be expected with DVB-55 as the crosslinker, since 45% of technical grade DVB-55 is comprised of ethylvinylbenzene and incorporation of monovinyl ethylvinylbenzene into the polymer will suppress precipitation. As the monomer concentration was increased to above 2% (v/v) it was found that the yields of microspheres improved, although at a monomer concentration of 6% (v/v) the microspheres were found to aggregate. These trends are similar to trends reported in the literature for the preparation of polydivinylbenzenes by precipitation polymerisation using free radical polymerisation [29]. Subsequent polymerisations were performed using a monomer concentration of 2% (v/v) since the microspheres formed under these conditions were of high quality (low CV).

3.2. Effect of polymerisation time

During the particle growth phase of a precipitation polymerisation, as more monomer is consumed the mean particle size of the microspheres is expected to increase provided that the formation of new particles by secondary nucleation processes does not dominate. Given the low yields of microspheres obtained after the polymerisation of DVB-55 at varying concentrations for 24 h, the polymerisation time was increased stepwise up to 96 h to see if this would increase the yield of the insoluble microsphere products. However, no significant improvements in yield were recorded and there was no measurable changes to mean particle diameters (Figure 3); we ascribe these outcomes to the slow kinetics expected for increasingly dilute monomer solutions and the modest increases in particle diameters that were anticipated. Thus the polymerisation time was fixed at 24 h.

3.3. Effect of initiator concentration

The initiator concentration is known to have an effect on precipitation polymerisations [31, 32]. In conventional precipitation polymerisation conducted under free radical polymerisation conditions and precipitation polymerisation conducted under ATRP conditions, the particle sizes and yields of product increase as the initiator concentration is raised. Thus, the influence of initiator

concentration on the particle sizes and yields of the polymer microspheres in the present work were investigated, with the monomer concentration and the polymerisation time being held constant. All polymerisations were conducted at 65 °C for 24 h, at a monomer concentration of 2% (v/v) by increasing the initiator to crosslinker mole ratio in the range: 1:100 (I-10), 1:50 (I-20), 1:33 (I-30), and 1:25 (I-40). As can be seen in Table 2, increasing the initiator concentration increased the yield of microspheres up to 15% when the initiator to crosslinker mole ratio was 1:40, which is in agreement with the results obtained from ATRPP [29]. Whilst a yield of 15% is modest, such a yield compares favourably with the yields of (molecularly imprinted) polymer nanogels prepared under high dilution conditions, plus the microsphere quality is high. However, a coagulum was obtained when the initiator concentration was increased to 4 mol% (Figure 4).

3.4. Effect of catalyst concentration and reducing agent

Contamination of polymer products with residual catalyst is one of the main limitations of ATRP, and several approaches have been developed to address this problem [22, 33, 34]. Whilst metal-free ATRP enables the polymerisation of vinyl monomers without the need for any metal catalysts, in our hands this methodology did not yield any polymer microspheres when the method was applied to precipitation polymerisation, most probably due to the very diluted reaction medium. Therefore, a series of experiments was conducted to ascertain the effect of catalyst concentration on particle size and yield and to establish the minimum catalyst concentration required to run a successful ARGET ATRPP. We began by setting the [DVB-55]/[EBiB]/[Ascorbic Acid]/[Me₆TREN]/[CuBr₂] mole ratio to 100/1/1/1/1. Spherical particles with a mean diameter of 1.4 μm were obtained under these conditions (catalyst concentration: 17 ppm). Interestingly, when the catalyst to monomer ratio was decreased to 1:200, 1:330, 1:500, and 1:1000, no change in the mean particle size was observed, although the yields of products decreased slightly. This suggests that the metal catalyst is less active under such dilute conditions. In this context, it was observed that precipitation polymerisations could be conducted successfully with catalyst concentrations as low as 1.7 ppm when the [DVB-55]/[EBiB]/[Ascorbic Acid]/[Me₆TREN]/[CuBr₂] mole ratio was 1000/10/10/10/1. However, when the catalyst concentration was decreased to levels below 1.7 ppm (0.67 ppm and 0.17 ppm), no isolable, insoluble product was observed after 48 hours.

Various reducing agents, including tin(II) 2-ethylhexanoate (Sn(EH)₂), glucose and ascorbic acid, can be used to good effect in ARGET ATRP. However, the careful selection of reducing agent is

critical since controlled polymerisations can only be realised when the reducing agent is slightly soluble in the reaction medium because the ATRP equilibrium must favour the dormant state [35]. In the present work, ascorbic acid was selected as the reducing agent due to its limited solubility in acetonitrile. With ascorbic acid as reducing agent, uniform polymer microspheres were obtained at a 10:1 ascorbic acid: Cu mole ratio. No differences in particle size (2.3 μm) or reaction yield were observed upon moving to a 20:1 ratio. As the ratio was increased further to 40:1 and 60:1, there was a negligible decrease in particle size (2.1 μm) and no change in yield. When ascorbic acid was absent, and ARGET ATRPP impossible, no polymer microspheres were formed.

3.5. Grafting of polymer brushes from polymer microspheres

Surface-initiated controlled radical polymerisations have great potential in surface and interface engineering since they enable the growth of polymer brushes from surfaces with high grafting densities [36]. To achieve this goal, an initiator must be immobilised onto the surface which normally necessitates the chemical modification of the surface [37]. On the other hand, polymer particles prepared by reversible-deactivation radical polymerisations already have initiating moieties on their surfaces that enables the growth of polymer brushes from their surfaces without the need for an additional modification step [38-40]. To illustrate the “living” nature of the polymer microspheres produced *via* ARGET ATRPP, surface initiated ATRP (SI-ATRP) was performed with methyl methacrylate as monomer. For this grafting work, the $[\text{Me}_6\text{TREN}]:[\text{Cu}(\text{I})\text{Br}]$ mole ratio was fixed at 10:1 to ensure complex formation under the dilute monomer conditions applied (for grafting experiments with relatively higher monomer concentrations, a mole ratio of 2:1 is more typical). After 24 hours of grafting, the previously white particles took on a blue hue, due to the presence of copper, and a mass increase of 11% was recorded. The products were characterised by FT-IR spectroscopy. For comparative purposes, polyDVB-55 particles were synthesized by free radical precipitation polymerisation (FRPP) according to a procedure described elsewhere [3]. All polymers showed signals in the FT-IR spectra characteristic of polyDVB: 3010 cm^{-1} (aromatic C-H stretch), 2915 cm^{-1} (aliphatic C-H stretch), 1585 cm^{-1} (aromatic C=C bend), 1440 cm^{-1} (aliphatic C-H bend). As expected, the FT-IR spectrum of the polyDVB-55 microspheres prepared by FRPP showed no carbonyl signal at 1736 cm^{-1} (Figure 6a). On the other hand, the polymer microspheres that were prepared by ARGET ATRPP gave rise to a weak carbonyl stretching signal in the FT-

IR spectrum at 1736 cm^{-1} due to the presence of ATRP initiating species (EBiB) within the microspheres (Figure 6b). This signal becomes much more intense following the SI-ATRP due to an overlapping signal assigned to the carbonyl groups in the polyMMA brushes (Figure 6c). In addition, a distinct band appears from 1100 cm^{-1} to 1275 cm^{-1} , which is assigned to the C–O–C stretching vibration for grafted polyMMA brushes (Figure 6c). These results demonstrate the successful grafting of PMMA brushes from the polymer microspheres.

CONCLUSIONS

We have reported the first example of ARGET ATRP conducted under precipitation polymerisation conditions. This new method, termed ARGET ATRPP, yields high quality polymer microspheres in a size range (1-3 μm) attractive for many applications. Whilst the yields of microspheres are lower than the yields obtained for conventional precipitation polymerisations conducted under free radical polymerisation conditions, the products are of high quality and can be used directly in *grafting-from* experiments without any need for the installation of initiator moieties *via* polymer-analogous reactions. Given the popularity of precipitation polymerisation within the molecular imprinting community, we are confident that ARGET ATRPP will be attractive for the template-directed synthesis of imprinted polymers and that the requirement to include additional (ARGET) components within polymerisation can be accommodated by rational polymer design and product optimisation work. We expect the materials produced to be particularly attractive for analytical scale operations and/or where post-polymerisation chemical modification of imprinted polymers is required prior to exploitation. Furthermore, the requirement to use low levels of metal complex catalyst only, combined with the oxygen-tolerant nature of ARGET ATRP, makes ARGET ATRPP an attractive method for the facile production of polymer microsphere under reversible-deactivation radical polymerisation conditions.

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DATA AVAILABILITY

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical limitations and because the data also forms part of an ongoing study.

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Figure and Table captions:

Figure 1. Mechanism of particle formation in ARGET ATRPP

Figure 2. SEM images of polyDVB-55 microspheres prepared *via* ARGET ATRPP at a monomer concentration (v/v) of 2% (a), 3% (b), 4% (c) and 6% (d), respectively.

Figure 3. SEM images of polyDVB-55 microspheres prepared *via* ARGET ATRPP where the polymerisation time was 24 h (a), 48 h (b), 72 h (c) and 96 h (d), respectively.

Figure 4. SEM images of polyDVB-55 microspheres prepared *via* ARGET ATRPP at an initiator to catalyst mole ratio of 10 (a), 20 (b), 30 (c), and 40 (d), respectively.

Figure 5. SEM images of polyDVB-55 microspheres prepared *via* ARGET ATRPP at a Cu: Ascorbic Acid mole ratio of 1:10 (a), 1:20 (b), 1:40 (c) and 1:60 (d), respectively.

Figure 6. FT-IR spectra of polyDVB-55 microspheres prepared by conventional free radical precipitation polymerisation [FRPP] (a) and ARGET ATRPP (b), and poly(DVB-55-g-MMA) microspheres prepared by ARGET ATRPP of DVB-55 followed by SI-ATRP of MMA (c).

Table 1. Effect of monomer concentration on particle size, dispersity, and yield. [DVB-55]/[EBiB]/[Ascorbic Acid]/[Me₆TREN]/[CuBr₂]: 1000/10/10/10/1. All polymerisations were conducted at 65 °C for 24 h. ^a Number average diameter; ^b Polydispersity index; ^c Coefficient of variation of the polymer particles; ^d Determined gravimetrically.

Table 2. Polymerisations conducted using variable initiator concentrations. All polymerisations were conducted at 65 °C for 24 h, at a monomer concentration of 2% (v/v) and by varying the initiator crosslinker to ratio: 1:100 (I-10), 1:50 (I-20), 1:33 (I-30), and 1:25 (I-40).

Figure 1.

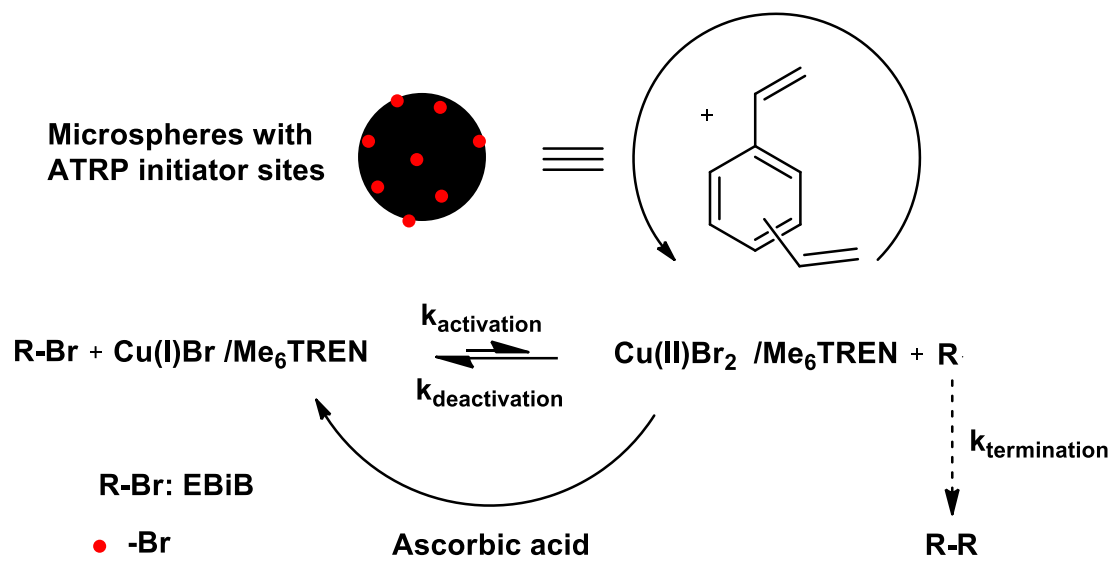


Figure 2.

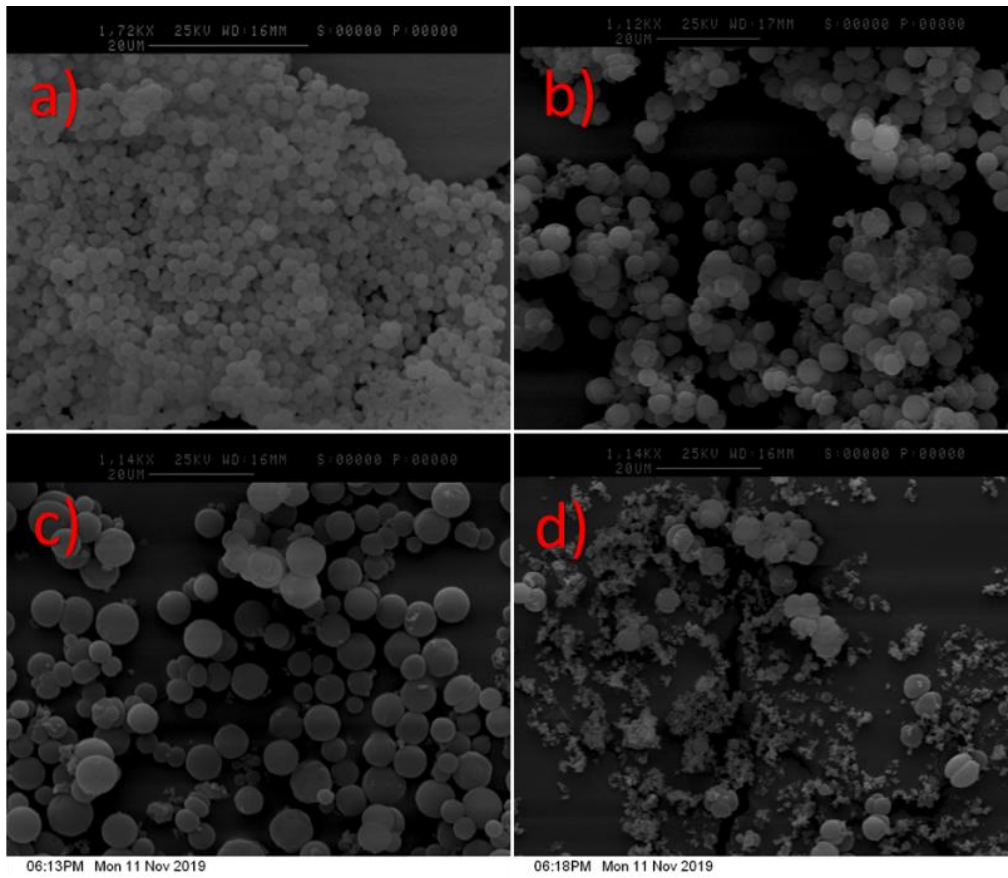


Figure 3.

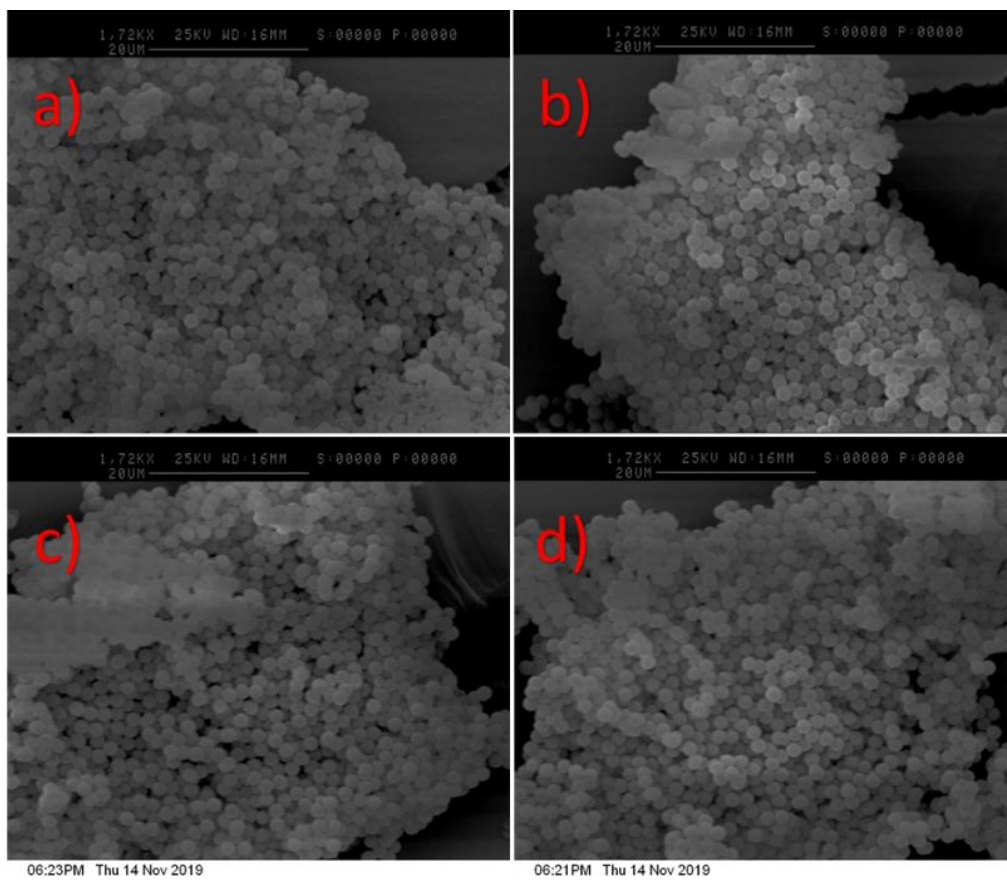


Figure 4.

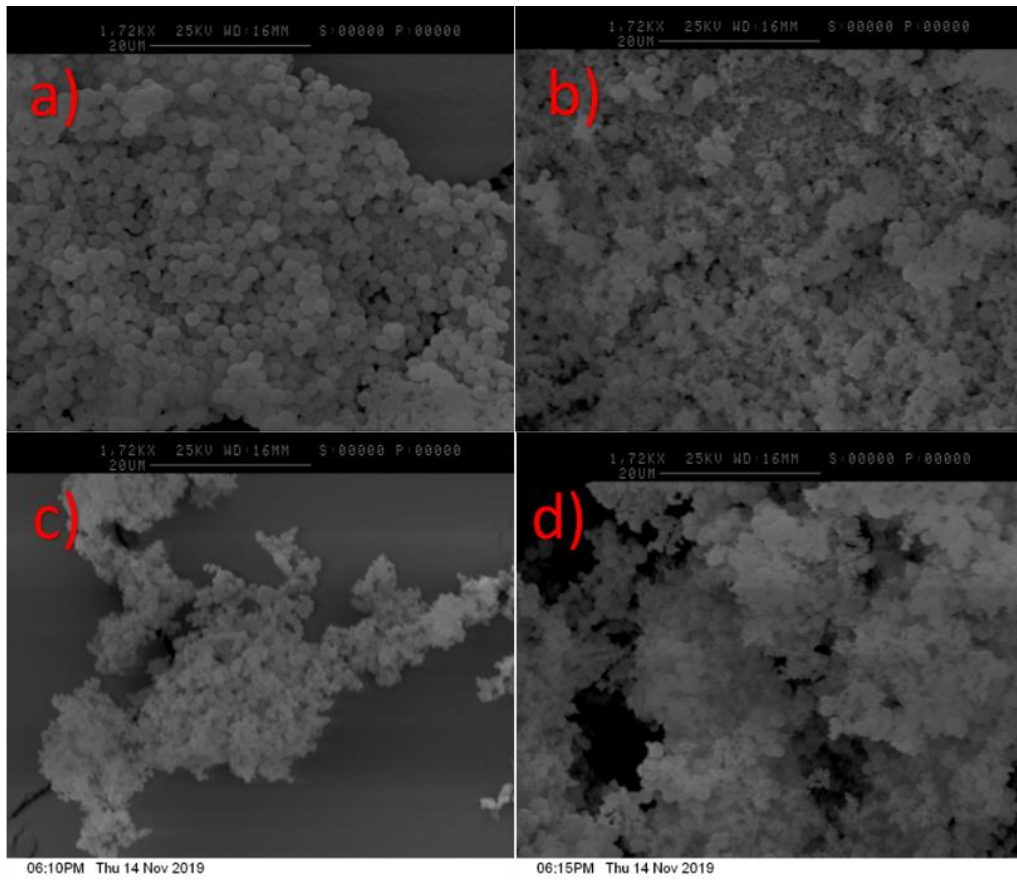


Figure 5.

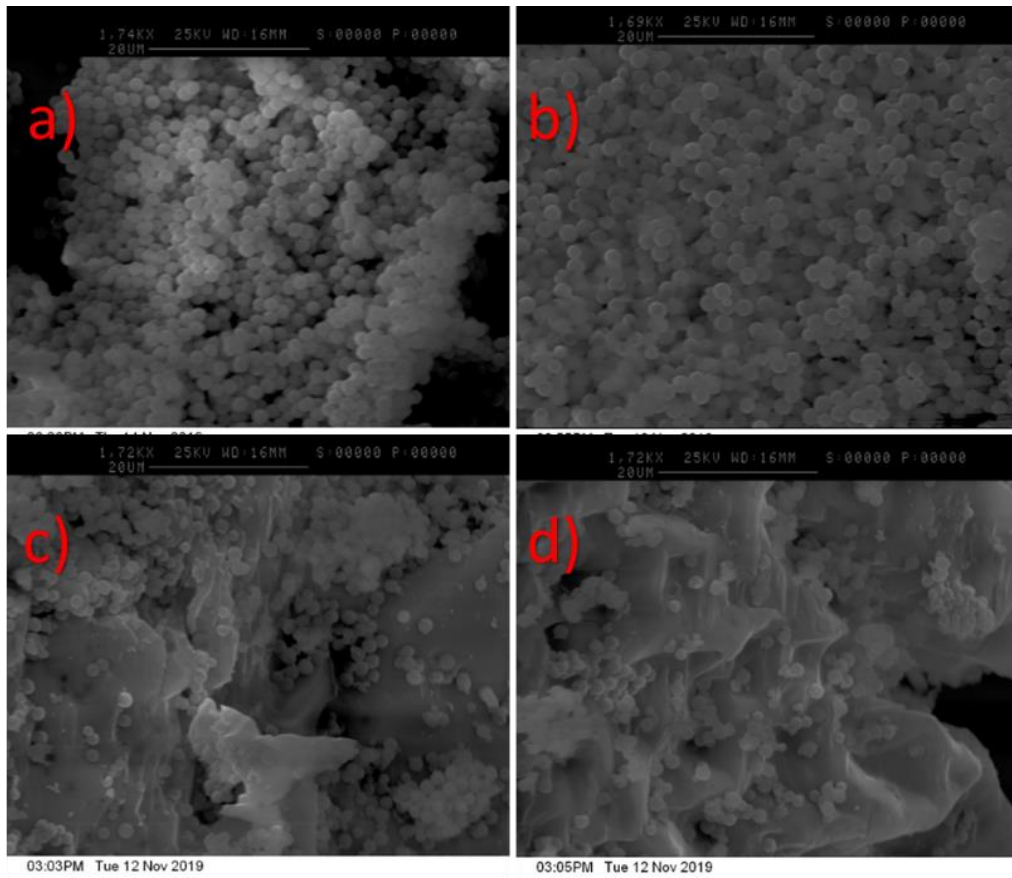


Figure 6.

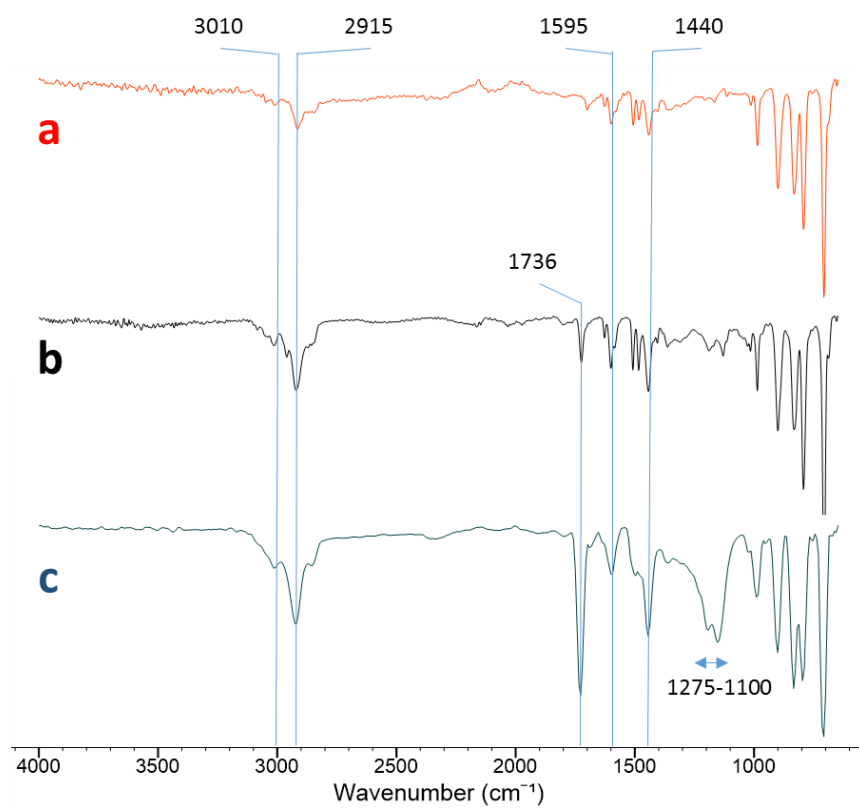


Table 1.

Monomer concentration (v/v)	D_n^a	U^b	CV %^c	Yield^d (%)
1	Not isolated	Not isolated	Not isolated	Not isolated
2	2.3	1.08	6.7	3
3	3.1	1.28	22.3	4
4	4.0	1.43	34.1	6
6	aggregated	aggregated	aggregated	13

Table 2.

	I-10	I-20	I-30	I-40
DVB-55	1000	1000	1000	1000
EBiB	10	20	30	40
Ascorbic Acid	10	10	10	10
Me₆TREN	10	10	10	10
CuBr₂	1	1	1	1
Yield (%)	2	4	9	15