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Manuscript Details

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Article type	Full Length Article

Abstract

This paper reports work aimed at extending previous studies of the sonochemical method for forming microspheres. It shows that a previously reported method for encapsulating and delivering hydrophilic species using a 'double emulsion' method can be used with chitosan or thiolated poly(methacrylic acid), PMAASH, based systems. One particular application involves targeted catalysis where gold nanoparticles are incorporated into chitosan microspheres and can be released to catalyse the borohydride reduction of 4-nitrophenol. Also reported is the use of ultrasound to 'trigger' the reduction reaction of 4-nitrophenol by rupturing nanoparticle-containing microspheres to release the catalyst. We also demonstrate that more sustainable and potentially lower environmental impact processes can be prepared by substituting commercial vegetable oil for the hydrocarbons used previously. We also report for the first time the use of responsive block copolymers to form microspheres. The copolymers consist of PMAASH blocks around a central, responsive block of poly(ethylene glycol), poly(4-vinylphenyl boronic acid) and poly(N-isopropyl acrylamide) to give systems that potentially respond to pH, sugar concentrations or temperature.

Keywords	sonochemistry, polymer microsphere, responsive material, sonochemical delivery, sonochemical catalysis
Manuscript category	Synthesis of organic and polymer materials

Corresponding Author	Gareth Price
Corresponding Author's Institution	University of Bath
Order of Authors	Gareth Price, Jasmin Bone, Karina Cochintoiu, James Courtenay, Robin James, Lauren Matthews, Ruth Simmons
Suggested reviewers	Sivakumar Manickam, Meifang Zhou, Artur Cavaco-Paulo, Judy Lee, Muthupandian Ashokkumar

Submission Files Included in this PDF

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Figure 1.JPG [Figure]

Figure 2.JPG [Figure]

Figure 3.JPG [Figure]

Figure 4.JPG [Figure]

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Figure 6.JPG [Figure]

Figure 7.JPG [Figure]

Figure 8.JPG [Figure]

Figure 9.JPG [Figure]

Figure 10.JPG [Figure]

Figure 11.JPG [Figure]

Scheme 1.JPG [Figure]

Scheme 2.JPG [Figure]

Scheme 3.JPG [Figure]

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Professor M. Ashokkumar
Editor-in-Chief
Ultrasonics - Sonochemistry

Ref: ULTSON_2018_1327

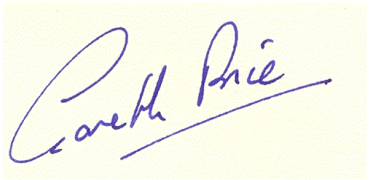
Thank you for sending the Reviewers' comments on our submitted manuscript entitled "Sonochemical production of responsive polymer microspheres".

We are grateful for their valuable suggestions. We have carefully considered them and our responses are listed below. The manuscript has been revised in line with the suggestions (with changes marked in red).

I hope that you as Editor will be happy with our responses and will now consider the paper to be publishable. We look forward to hearing from you.

Thanking you in anticipation

Yours Sincerely,



Gareth Price *on behalf of the authors.*

-**Reviewer 1**

The manuscript titled "Sonochemical production of responsive polymer microspheres" is reporting images of microspheres made with different shell material and encapsulating emulsions, vegetable oil and gold nanoparticles, and the response of some of the microspheres under pH and sonication to release the encapsulated content. Although the work is interesting, I have reservations on the suitability for the Journal of Ultrasonic Sonochemistry due to the lack of investigation on the sonication parameters and the lack of quantification and analysis provided. Therefore, in the current format, the manuscript is not suitable for publication and can be considered if the following issues are addressed.

We recognise this criticism and indeed were conscious of it when writing the paper. It is a criticism that I often make when refereeing for *Ultrasonics Sonochemistry*. It is often rebutted by Authors making comments suggesting that the scope of the journal covers *applications* of sonochemistry as well as its fundamentals. This is the case for this paper, the aim of which was to illustrate and extend the range of systems to which the sonochemical method can be applied. A little extra latitude might also be granted since the paper is effectively a report of a conference proceeding. Nonetheless, it is a valid criticism and we have tried to include additional detail as requested and also described in more depth the optimisation of conditions for each process which was rather 'taken as read' in the original manuscript. We also feel (our fault as it was not stressed sufficiently) that the reviewers have not acknowledged the use of ultrasound in triggering the nitrophenol reduction by rupturing the microspheres as a novel application.

-Could the reported microspheres be generated under different sonication conditions and time? Could the microspheres be generated by simple homogeniser or other high shear devices, and how would the stability of these microspheres compare with the microspheres synthesised using ultrasound. Details of the temperature of synthesis and sonication device should be included.

We have included some comments – but only briefly since there is sufficient Literature in this area to recognise that high shear will form the droplets but that the chemical action of ultrasound in forming radicals is also needed. For example, see pg 8 regarding chitosan, e.g. p 18 for the polymer systems.

-Statement " Chitosan microspheres containing tetradecane… were uniform and up to 10um in diameter." What is it meant by uniform? It would be useful for the authors to quantify the microsphere size distribution.

We have added some comments on the size distribution taken from reanalysing some of the images.

-It is stated that the larger spheres observed in Fig 1 are likely to be air filled because or unencapsulated organic phase because they are mobile. Why would tetradecane filled microspheres not be mobile?

They would – but the density difference from the surrounding aqueous phase is much less so that they would be much less than air bubbles. Unencapsulated tetradecane droplets coalesce to form larger drops whereas microbubbles are stable. A comment has been added to clarify our meaning.

-" These microspheres ranged from 5-20 µm, larger than with an oil phase alone. These microspheres also were less stable, increasing in size after 3-4 days." Microsphere size distribution would support the statement that the spheres are range 5-20um and larger than oil phase alone. Also could the authors quantify the stability either by reporting the microsphere distribution or concentration as a function of time?

We have added some comments on the size distribution taken from reanalysing some of the images. However, we do not have the concentration-time data.

-Page 11 "stable dual phase within the chitosan shell", unclear from the images if the encapsulated material is dual phased. Could the authors verify the content by breaking the microspheres and releasing the content or other anlaysis or better images?

We feel that the images at higher resolution do show the two=phase nature of the contents. However, the 'release' experiment that is described is exactly what the final section of the paper described – the release of gold nanoparticles from an aqueous/tetradecane emulsion contained in microspheres.

-"If a peptide or other small molecule that targets leaves may be bound" unclear what authors mean by leaves.

'Leaves' – plural of leaf, green plant material on grasses or trees. The point has been clarified.

-"This was confirmed as the microspheres circled in red in Figure 10(d) appear to have a visibly different texture as a result of chitosan forming larger microspheres than $PMAA_{SH}$ therefore greater volumes of emulsion can be encapsulated". This statement needs to be further supported as the authors have only provided a visual observation. Also the size of the spheres circled are visibly larger than other microspheres, and this difference could make the outer edges more distinct than the smaller microspheres. SEM images would give better conclusive determination of the surface texture.

We agree that SEM might well give better evidence. However, the specialised equipment is not available to us; putting the microspheres into our SEM under high vacuum would evaporate the organic phase and rupture the spheres, making any conclusions doubtful.

Comments on images, structure and English.

-Scale on nearly all the images needs to be improved. Done

-The red circles in Fig. 1a needs to be explained in the caption. Done

-It is suggested that some of the discussions in the results and discussion section on the methodology and the schemes be moved to the methods section. i.e. page 16 "Each copolymer was characterised by NMR spectroscopy while DOSY NMR was used to confirm the formation of block copolymers. The presence of a block copolymer was further confirmed using GPC chromatography in which the molecular weight of the polymer was found to be M_n = 13100 $gmol^{-1}$."

We disagree here. The GPC results are simply reporting the result and not the experimental method. Whether the schemes are in the Discussion section or the Experimental section is a matter of style and we think for this particular paper it tells a more coherent story by have them in the discussion.

-Fig 7 there are two images (a) and (b), these are not explained in the figure captions.

-English needs to be revised some examples are:

*page 4 "diluted ith water" Typo corrected

*page 8 "Addition of a few of drops of dilute" No correction needed

*page 10 "prepared and this used as" Sentence has been clarified

*page 13 "observed when The MNPs were" Typo corrected

*page 14 "also varyng amounts of" Typo corrected

*page 16 "microspheres but that those lacking" Sentence has been clarified

-**Reviewer 2**

This paper deals with sonochemical method to synthesize various microsphere systems.

This work is interesting and well furnished. My main objection is that the role of ultrasound is too poorly addressed for a journal specialized in the use of ultrasound. Indeed, the part of ultrasound should be far more important. To begin, a scheme of the experimental setup must be presented with proper characterization information. More importantly, the discussion must be more focused around ultrasound effects during the different synthesis. What are the differences (physical and chemical) for systems synthesized with and without ultrasound? What is the mechanism proposed to explain such behavior when ultrasound is used? Maybe also a brief discussion about the influence of frequency and most importantly about the influence of power of ultrasound…

This comment has largely been addressed in the response to the first comment of Reviewer 1. In addition, we respectfully disagree that a 'scheme of the experimental set-up must be presented'. There is sufficient literature precedent referred to in the text to make this superfluous and a waste of journal space. We feel that the comments regarding the mechanism are addressed in terms of the chitosan systems. The aim of this paper was not to investigate any frequency effects and some additional comments have been made regarding the effect of intensity.

I think this paper could be published in Ultrasonics Sonochemistry after these issues are properly addressed.

I also have a few other remarks:

- Page 1: Abstract, please remove "s" from "gives" in the last sentence. Corrected
- Page 4: paragraph 2.2, "diluted With water"; "w" is missing. Corrected
- Page 5: paragraph 2.2, please remove "d" from "to prepared" Corrected
- Page 8: "Further confirmation was gained by carrying out the reaction in solutions saturated with nitrogen gas while excluding oxygen." Could you explain this point please? Further comments have been added to clarify this point.
- Page 9: "(…) and it is solvation in acid rather than sonication which alters the chitosan bonds". How would this happen? Under what mechanism? Presumably protonation at low pH. A comment has been added to clarify.
- Page 23: please remove "s" form "an interactions" Corrected

Sonochemical production and activation of responsive polymer microspheres

Gareth J. Price*, Jasmine Bone, Karina Cochintoiu, Jamie Courtenay, Robin James, Lauren Matthews and Ruth Simmons

Department of Chemistry, University of Bath, BATH, BA2 7AY, United Kingdom

Highlights

- Chitosan and poly(methacrylic acid) microspheres have been prepared to encapsulate water-in-oil emulsions
- Sonochemical conditions have been optimised for microsphere synthesis
- Sustainable delivery systems using chitosan and vegetable oil have been developed
- Microspheres have been prepared using responsive block copolymers
- Gold nanoparticle catalysts have been encapsulated in microspheres

Keywords: sonochemistry, polymer microsphere, responsive material, sonochemical delivery, sonochemical catalysis

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Abstract

This paper reports work aimed at extending previous studies of the sonochemical method for forming microspheres. It shows that a previously reported method for encapsulating and delivering hydrophilic species using a 'double emulsion' method can be used with chitosan or thiolated poly(methacrylic acid), $PMAA_{SH}$, based systems. One particular application involves targeted catalysis where gold nanoparticles are incorporated into chitosan microspheres and can be released to catalyse the borohydride reduction of 4-nitrophenol. Also reported is the use of ultrasound to 'trigger' the reduction reaction of 4-nitrophenol by rupturing nanoparticle-containing microspheres to release the catalyst.

We also demonstrate that more sustainable and potentially lower environmental impact processes can be prepared by substituting commercial vegetable oil for the hydrocarbons used previously. We also report for the first time the use of responsive block copolymers to form microspheres. The copolymers consist of $PMAA_{SH}$ blocks around a central, responsive block of poly(ethylene glycol), poly(4-vinylphenyl boronic acid) and poly(N-isopropyl acrylamide) to give systems that potentially respond to pH, sugar concentrations or temperature.

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1. INTRODUCTION

There is widespread interest in the production of polymer microspheres for a range of applications [1]. The microspheres are capsules with sizes in the range 1 μm – 100 μm and usually consist of a thin shell formed from a synthetic material or a biopolymer. The contents of the microspheres can be gases such as air, inert gases or fluorocarbons, these finding use as contrast agents in medical scanning. Liquids or solutions can also be encapsulated and applications here have included pharmaceuticals, drugs, flavours, fragrances and agrochemicals

Sonochemistry offers a particularly straightforward and rapid method for the preparation of microspheres. Since the first report by Suslick and Grinstaff [2] who encapsulated a selection of hydrocarbons in albumin microspheres, a number of proteins and related materials have been shown to readily undergo microsphere formation. Further examples include haemoglobin [3], avidin [4], DNA [5] and lysozyme [6]. More recently, biopolymers such as chitosan have been used [7, 8]. Synthetic polymers based on poly(methacrylic acid) have also been shown to be suitable as shell materials [9].

The sonochemical method relies on partitioning of the polymer to the interface between the polymer solution and a gas or immiscible liquid distributed through the solution by the streaming and mixing effects of ultrasound. Suslick *et al.* [3] also showed that chemical effects were important as superoxide radicals formed in the aqueous phase promoted crosslinking of the protein by producing disulphide bonds between thiol groups in cysteine residues in the protein which stabilised the microsphere shells. Thiol links are not necessary and other systems have been discovered that are stabilised by hydrogen bonding and other interactions [5, 10] although some degree of crosslinking does seem to be needed for long term (multi-week) stability.

Recently reported work from our laboratory [11] extended the types of system that could be produced by encapsulating an water-in-oil emulsion in a lysozyme shell. This opens up the possibility of storage and delivery of hydrophilic and water-soluble species. The work reported here extends that concept to explore different shell materials that could be used and to illustrate one potential application for such systems in the targeted delivery of catalysts. Recent advances in controlled polymer synthesis offer the possibility of preparing materials with unprecedented control over their structure and properties and we have been exploring the use of polymers that are responsive towards some environmental condition as potential shell materials. This should open up the possibility of targeting microspheres to release their

contents in response to some external stimulus. The ultimate aim is to limit waste by e.g. releasing catalysts, agrochemicals and ultimately pharmaceuticals only when and where they are needed.

2 EXPERIMENTAL

2.1 Materials and characterization methods

All reagents used as received from Sigma Aldrich unless stated otherwise. A Sonics and Materials Vibra Cell VC600 generator fitted with a 20 kHz horn and a 3 mm diameter micro tip attachment was used for the microsphere synthesis and release studies. Optical micrographs were captured using a GX optical L3001 microscope fitted with a Nikon SLR camera. All samples for optical micrography were deposited on the microscope slide using a capillary tube and a cover slip was placed on top. Laser scanning confocal microscopy experiments were carried out using a Zeiss LSM 510 META microscope. All images were analysed for scale using the program ImageJ [12] which was also used to estimate size distributions. FTIR spectra were recorded on a Perkin Elmer Spectrum 100 spectrometer after drying the samples overnight at 110 °C. UV-vis spectra were obtained from an Agilent 8453 UV-Vis spectrophotometer using 1 cm quartz crystal cuvettes. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100Plus system.

2.2 Formation of Microspheres

0.5 g of chitosan was dissolved in 100 mL 0.27 M acetic acid. This had a gel like consistency so a 3:1 water diluted solution was used to prepare the microspheres. 100 μL of tetradecane (or tetradecane containing saturated nile red or Sudan III dyes) was layered on 1 mL of the chitosan solution and this sonicated at 14 Wcm^{-2} for 30 s or 60 s. The tip of the ultrasound horn was placed as accurately as possible at the interface between the organic and aqueous phases. Ultrasound intensities were measured calorimetrically [12]. Sonication was carried out at room temperature (17 – 22 °C) in open tubes in air except when saturated with nitrogen. The resulting solutions were left for a few minutes to settle, diluted with water and photographed using the optical microscope. For longer-term storage, samples were kept in the dark at room temperature.

The same method was used when replacing the tetradecane with commercial vegetable oil purchased from a local supermarket. Experiments involving encapsulation of an emulsion replaced the tetradecane with freshly prepared 60 vol% tetradecane/vegetable oil

and 40 vol% 1 M NaCl containing 1 wt% Span80 surfactant. Sonication was carried out for 60 s at 14 Wcm^{-2}.

Labelling the microspheres with a fluorescent probe was conducted [7] by reacting 200 µL of a suspension of chitosan microspheres with 200 µL of 0.2 mg mL^{-1} fluorescein isothiocyanate (FITC) in phosphate buffered saline (PBS) at pH 7.4. The mixture was shaken at room temperature for 2 hr. The reacted microspheres were washed with water to remove unreacted probes.

The same basic sonication procedure was used to prepare microspheres from thiolated poly(methacrylic acid), $PMAA_{SH}$. 1 ml of 40 mM tris acetate-EDTA buffer solution was added to a centrifuge tube with 0.0348 g $PMAA_{SH}$. 100 µL of the organic phase (tetradecane, vegetable oil or water-in-oil emulsion) was added to the tube and the ultrasound horn tip was placed in the middle of the tube at the oil-water interface. The sample was then sonicated for 60 s at 14 W cm^{-2}, whilst sitting in an ice bath.

2.3 Polymer synthesis

2.3.1 Poly (MAA_{SH}–*b*-PEG-*b*-MAA_{SH}) block copolymer

28 g of bis hydroxyl terminated PEG (M = 2000) was dried at 50°C under vacuum and dissolved in 60 mL dry dichloromethane to which 5.5 g 4-(dimethylamino)pyridine (DMAP) had been added. After cooling in an ice bath, 4.2 mL Triethylamine (TEA) was added followed by 7.3 mL of 2-bromoisobutyryl bromide in 60mL dry DCM added over the course of 2 hr. The resulting solution was left to stir for 16 hr. The solution was filtered to remove a salt and then reduced using rotary evaporation to a quarter of its original volume. The product was recoved by precipitation in ~500mL cold diethyl ether and recrystallized from ethanol before drying under vacuum at 40°C for 12 hr.

The functionalised PEG was reacted by ATRP. 1.2668g of the macroinitator in 10 mL HPLC grade toluene was purged with nitrogen for 30 min and 0.03 g copper (I) chloride added. To this was then charged 10 g of degassed *tert*-butyl methacrylate (^{t}BMA) (purified using an inhibitor removal column) and 0.0626mL of N,N,N',N',N'-pentamethyldiethylenetriamine PMDETA. The solution was stirred at room temperature for 10 minutes before being heated to 85°C for 3 hours. The block copolymer was recoved by precipitation in cold *n*-hexane.

The ^{t}BMA blocks were hydrolysed to methacrylic acid by stirring at room temperature for 16 hr with 5 eq of trifluoroacetic acid (TFA) in dichloromethane. The product was recovered by removing the dichloromethane under vacuum, dissolving in the minimum

amount of methanol and reprecipitating from cold diethyl ether. NMR spectroscopy showed that removal of 90+% was achieved.

The final step was to introduce the thiol groups. Following Cavalieri *et al.* [9] 1.0 g of the PMAA-PEG-PMAA copolymer was reacted with 0.719 g cysteamine hydrochloride in 10 mL of pH7 phosphate buffer in the presence of 0.6505g *n*-(3-dimethylaminopropyl)*n′*-ethylcarbodiimide hydrochloride (EDC) and 0.7069 n-hydroxysuccinimide (NHS) in 15mL methanol. The solution was stirred at room temperature for 16 hours and the product isolated by drop-wise addition to cold diethyl ether and filtration before drying under vacuum for 8 hours at 40°C. NMR spectroscopy showed the level of thiolation to be 25%.

2.3.2 Poly (MAA_{SH}–*b*-4VPBA) block copolymer

The procedure used for the preparation of the boronic acid containing copolymer followed from the work of Maji et al. [14]

Methacrylic acid, MAA, was passed through an inhibitor removal column. 10.0 g MAA in 30 ml methanol (Fluka Analytical) was stirred under nitrogen for 30 min with 0.245 g S-(thiobenzoyl)thioglycolic acid (the RAFT agent) 0.0805 g 4,4'-azobis(4-cyanovaleric acid as the initiator. The reaction vessel was then connected to a Schlenk line, where three freeze-pump-thaw cycles were carried out before heating to 61ºC for 24 hr. The polymer was recovered by precipitation in ice-cold diethyl ether. 0.317 g of this polymer, 0.515 g 4-vinylphenyl boronic acid and 1.80 mg AIBN was then dissolved in 5 mL dimethyl formamide, DMF. After three freeze-thaw cycles, the reaction was heated for 7 hr at 110°C. The polymer was recovered by precipitation in ice cold ethyl acetate and dried overnight under vacuum. Thiolisation was conducted using cysteamine hydrochloride as described in Section 2.3.1.

2.3.3 Poly (MAA_{SH}–*b*-NIPAM-*b*-MAA_{SH}) block copolymer

Following Yang and Cheng [15], poly(methacrylic acid) was prepared by RAFT polymerization as described in the previous section. 1.02 g of PMAA and 1.28 g N-isopropyl acrylamide, NIPAM, were dissolved in 30 mL of dry methanol with 0.25 g 4,4'-azobis(4-cyanovaleric acid as the initiator. After three freeze-thaw cycles to remove dissolved oxygen, the reaction was heated at 61ºC for 64 hr. The polymer was recovered by precipitation in ice-cold diethyl ether and dried overnight under vacuum.

To add the third block, 0.768 g of this copolymer was dissolved in 30 mL methanol and 0.328 g MAA added with 2.70 mg 4,4'-azobis(4-cyanovaleric acid). After three freeze-

pump-thaw cycles the reaction was stirred at 61ºC for 24 hours. Recovery was again by precipitation in ice-cold diethyl ether. Thiolisation was conducted using cysteamine hydrochloride as described in Section 2.3.1.

2.4 Nanoparticle synthesis and catalysis

Magnetic iron oxide (Fe_3O_4) nanoparticles, MNPs, were synthesised [16] by reacting 4.30 g of iron (II) sulphate and 11.75 g of iron (III) chloride in the minimum amount water under a flow of nitrogen gas. The mixture was heated to 80 ˚C and 0.5 g of lauric acid dissolved in acetone (12.5 ml) and ammonium hydroxide (25 ml, 28% m/v) were added. Once at 80 ˚C, five further portions of 1.0 g of lauric acid dissolved in acetone (25 ml) were added over the course of 5 minutes. The solution was allowed to cool, at which point acetone (80 ml) and methanol (80 ml) were added to precipitate the nanoparticles which were then separated by use of a magnet. The nanoparticles were rinsed with further 50:50 mixtures of acetone and methanol until only magnetic particles remained. These were left to dry in a 60 ˚C oven overnight.

0.1 g of MNPs were added to 1 ml of chitosan or $PMAA_{SH}$ solutions prior to formation of microspheres as in Section 2.2.

Gold nanoparticles, AuNPs, were prepared [17] by adding 3 ml of 0.05 M aqueous sodium citrate to 300 ml of boiling 1.25 x 10^{-4} M $HAuCl_4$ solution. On cooling, the pale purple solution turned ruby red, indicating the reduction of water soluble Au(I) into insoluble Au(0). Higher concentrations of AuNPs were obtained through a previously reported method [18] where 1 g of Pluronic P85 and 0.02 g of $HAuCl_4$ were added to 100 ml of deionized water while stirring. Once dissolved 0.02 g of sodium citrate was added. The solution was stirred for 5 minutes, covered with foil, and left to react at room temperature for 24 hr.

The AuNP suspension was incorporated into microspheres by forming an emulsion with tetradecane. Tetradecane (1 ml) and gold colloid solution (1 ml) were added to a plastic tube. 0.05 g of span 80 was added as an emulsifier. The mixture was sonicated with an ultrasonic horn for 30 s at 14 W cm^{-2}.

Catalysis testing was carried out as described by Bibi *et al.* [17]. 4-nitrophenol (2 ml, 0.8 mM) was mixed with freshly prepared sodium borohydride (5 ml, 10 μM). Varying volumes of colloidal gold nanoparticles (1 - 4 ml) were added and the UV-vis spectrum recorded every minute until no more change was seen.

3 RESULTS AND DISCUSSION

3.1 Sustainable chitosan microspheres for hydrophilic solutes

Chitosan microspheres were prepared sonochemically from a solution of the biopolymer in acetic acid solutions at varied concentrations. The solubility of chitosan at basic pH is low [7] and even under acidic conditions, high concentrations of chitosan formed a gel. Therefore, relatively dilute solutions (< 0.5 wt% in 0.27 M acid) were used for microsphere synthesis. Chitosan microspheres containing tetradecane, shown in Figure 1(a) were uniform and up to 10 μm in diameter. Image analysis suggested a mean size of 8 ± 3 μm for samples after allowing the sample to settle for 30 min. The larger spheres present are likely air bubbles or unencapsulated organic phase as they were mobile and quickly coallesced or moved out of the field of view on the microscope slide. The use of larger volumes of tetradecane made the oil / water interface easier to see and to locate the horn tip but did not increase the amount of microsphere formation. Addition of a few of drops of dilute sodium hydroxide solution to the microspheres post sonication also gave stable, well-formed microspheres with a defined spherical shell which were stable for several weeks. The addition of base deprotonates chitosan and therefore precipitates it, increasing the long-term stability.

It is difficult to quantify the concentrations of microspheres that form. In additiona to the natural variation in formation, the sample is highly heterogeneous and it is extremely difficult to take samples for analysis in a consistent manner. Hence, quantifying the effect of experimental variables is not straightforward and the observations are necessarily qualitative and lack quantitative detail. For each system optimisation of the preparation conditions in terms of the ultrasound intensity and sonication time was carried out. Briefly, there is competition between the two main processes responsible for microsphere formation – dispersion into droplets and radical formation to promote connection of the polymer chains – and the destruction of already-formed microspheres as they are subjected to the high shear forces around cavitation bubbles. Generally, the latter are more prevalent at higher ultrasound intensities and if sonication is performed for extended lengths of time. Hence, the preferred conditions involved sonication for short times (no more than 2 min and usually 30 – 60 s) at relatively low intensities.

The precise mechanism of microsphere formation and stabilization in chitosan solutions is not totally clear. Assuming that chitosan partitions to the water-air interface to form a shell is reasonable although there is no obvious route to provide the longer-term stability allowed by thiol crosslinking in many previous examples [1]. Conducting the

sonication in the presence of a radical trap, *t*-butanol, suppressed formation of stable microspheres and resulted in very low yields. This demonstrates that radical production in the aqueous phase is important for the crosslinking process to confer stability. Further confirmation of the importance of the involvement of sonochemically generated radical species was gained by carrying out the reaction in solutions saturated with nitrogen gas while excluding oxygen. Again, no stable microspheres were formed. The oil phase was distributed into an emulsion but the lack of stabilisation meant that phase separation occurred over the course of a few minutes. The implication of these observations is that modification of the chitosan, presumably crosslinking, under the action of sonochemically generated oxygen containing radicals is necessary for conferring stability onto the polymer shells to form microspheres.

Any changes to the structure of chitosan during sonication were investigated by drying a suspension of the microspheres and using FTIR spectroscopy (Figure 1(b)) to compare with spectra of native chitosan, chitosan that had been dissolved in acetic acid and chitosan that was separately sonicated in acid solution. The spectra showed that all the forms have the characteristic broad peaks for O-H and N-H. For chitosan, these occurred around 3290 cm^{-1} and 1654 cm^{-1} respectively. In comparison, these peaks occur at 3270 cm^{-1} and 1538 cm^{-1} for the chitosan dissolved in acetic acid, at 3268 cm^{-1} and 1537 cm^{-1} for the sonicated chitosan solution and at 3273 cm^{-1} and 1563 cm^{-1} for the chitosan microspheres. The similarity in the latter three and the shift to lower wavenumber from the chitosan precursor suggests that the species are essentially the same and that the peak shifts are due to solvation in acid and protonation rather than changes arising from sonication. The small shift in the peaks may also be attributed to the formation of an imine bond (-C=N) from free chitosan to the microspheres, between the free amine groups and the aldehydic end of the chitosan moiety [7]. This would suggest some reaction such as crosslinking upon formation of microspheres. Additionally, a small peak for C-H stretches seen for the first three samples at 2880 cm^{-1}, though is much sharper at 2921 cm^{-1} for the chitosan microsphere spectra, indicating the presence of the encapsulated tetradecane.

As noted above, a major aim of this work is to encapsulate and deliver water soluble or hydrophilic species. To facilitate this, a water in tetradecane emulsion was prepared and this emulsion used as the 'organic phase' during microsphere formation. Figure 1(c) shows that chitosan can be used to encapsulate such an emulsion although these microspheres are not so uniform in size. The presence of an aqueous phase inside the microspheres was confirmed using confocal microscopy as in Figure 1(d) where the fluorescence due to 5, 6-

carboxyfluorescein which is water soluble but tetradecane insoluble. These microspheres had a mean diameter of 15 μm and ranged from 5-20 μm, larger than with an oil phase alone. These microspheres also were less stable, increasing in size after 3-4 days. There is also some deformation of the spherical shape over time, indicating that the emulsion is not stable within the chitosan microspheres, possibly leading to the aqueous phase of the emulsion leaking out of the microspheres into the bulk solution. The emulsion is not so clearly defined in the internal structure of the chitosan microspheres as previously seen in lysozyme microspheres [11].

While tetradecane is a good laboratory model for the oil phase, it is not an environmentally friendly matrix for delivery of a drug, flavour or pesticide molecule in real world applications. Therefore, as shown in Figure 2(a), we attempted to use of a sustainable, commercial vegetable oil for encapsulation.

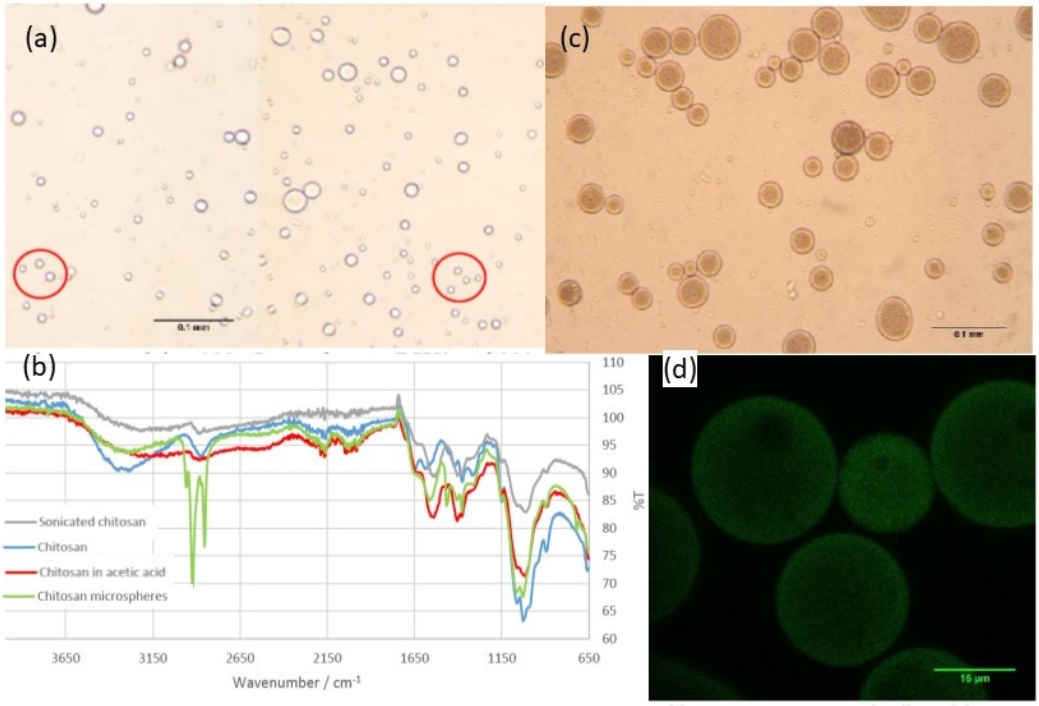


Figure 1. (a) Chitosan microspheres containing tetradecane. Red circles highlight microspheres; (b) FTIR spectra of chitosan and dried chitosan microspheres containing tetradecane; (c) chitosan microspheres containing a 40% aqueous $NaCl_{(aq)}$ in tetradecane emulsion; (d) confocal micrograph of chitosan microspheres sonicated with a 40% aqueous $NaCl_{(aq)}$ in tetradecane emulsion containing 5, 6-carboxyfluorescein.

Microspheres was again successfully formed and they were 5-15 μm in size. This is quite a broad range but is suitable for a range of applications [1]. Some droplets of vegetable oil were not encapsulated but over the course of a day or two these coalesced and left the solution whereas the suspension of microspheres remained stable over several weeks. The sample of microspheres settles over time, so that a large number are viewed in the sample above. The size of the microspheres remains at ~5-10 μm. The use of the fluorescent Nile Red dye in the vegetable oil organic phase illustrates the internal structure of the microspheres, demonstrating the encapsulation of the sustainable organic phase within the chitosan shell. Chitosan microspheres containing Nile Red as an easily viewed, model for a hydrophobic drug or pesticide were imaged using confocal microscopy (Figure 2(b)). This sample was viewed on the microscope 17 days after formation.

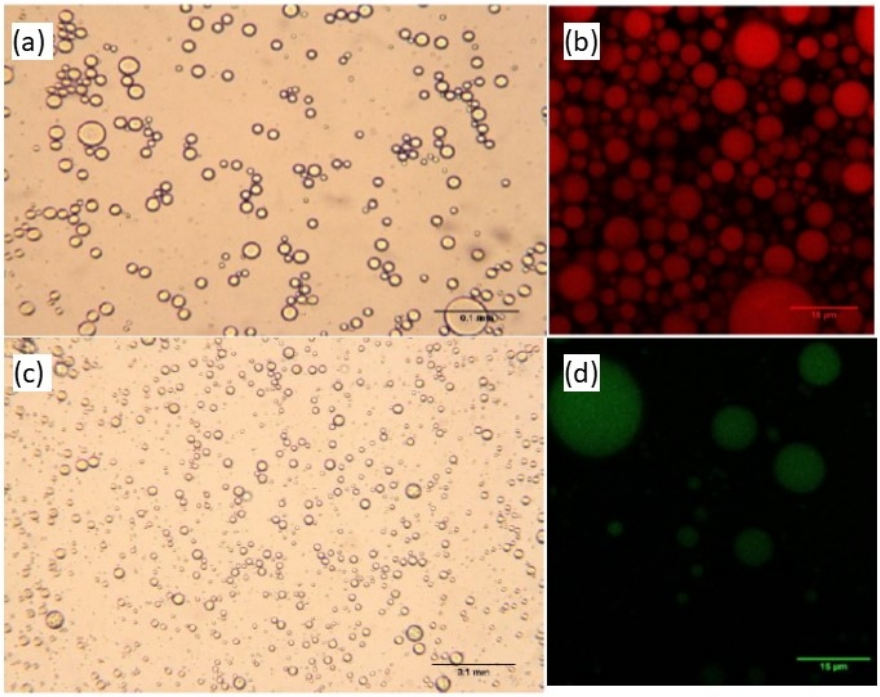


Figure 2. (a) Chitosan microspheres containing vegetable oil; (b) Confocal micrograph of vegetable oil with Nile Red encapsulated in chitosan; (c) Chitosan microspheres containing a of 40% (w/v) sodium chloride solution / vegetable oil emulsion; (d) Confocal micrograph of (c) with aqueous phase containing 5, 6-carboxyfluorescein

Initial trials at producing microspheres containing a vegetable oil / water emulsion resulted in microspheres that were very small in size, 1-5 μm, and fewer in number than when the oil was used alone. Additionally, the emulsion was not stable for more than a few hours.

Various combinations of sodium chloride solutions and surfactants were tried and an optimum combination of 40% (w/v) sodium chloride solution with 1% (w/v) Span 80 surfactant solution as the aqueous phase resulted in microspheres that were consistently between 5-15 μm with well-formed, defined spherical shape (Figures 2(c), (d))which was stable over the course of a week. There were no large oil droplets as seen in previous examples. The internal structure of the chitosan microspheres again does not contain a clear illustration of an emulsion as for the lysozyme microspheres [11]. The size of the spheres also varied greatly. This indicates that further development would be required in order to reproducibly encapsulate a stable dual phase within the chitosan shell. However, the work does establish the precedent for the use of this particular combination of materials.

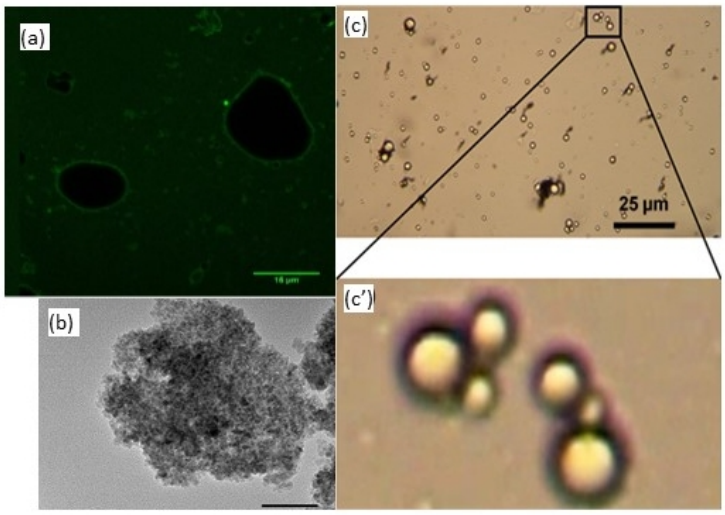


Figure 3. (a) FITC labelled chitosan microspheres; (b) TEM images for collections of individual iron oxide nanoparticles (scale 50 nm); (c) Microspheres formed from chitosan/MNP blend

In addition to varying their contents, modification of the shell of the microspheres is important from the point of view of targeting their use. To illustrate the potential, a marker, fluorescein isothiocyanate, FITC, was reacted with a suspension of chitosan microspheres. FITC has been shown to be an effective model for functionalising the shell through bonding to the free amine group [7] and as shown in Figure 3(a) the method is readily applicable to the microspheres developed here although some unreacted polymer aggregates in solution can also be seen.

The images illustrate that the core of the microspheres are filled with non-fluorescent tetradecane solution and the shell is composed of the chitosan polymer where free amine groups undergo reaction with FITC, as shown by the green fluorescent signal around the spheres. The background signal shows free FITC in solution. Conjugation of the amine functionality of chitosan to different targeting vectors illustrates the potential for chitosan microspheres to be bound to other small molecules, such as proteins. It has been shown that proteinaceous microspheres with the addition of RGD peptide may be used to target cancer cells and provide delivery of a drug to a specific site in the body. If chitosan microspheres may be used for targeted drug delivery, it may also have potential use for targeted pesticide delivery. If a peptide or other small molecule that targets particular parts of a plant or crop such as the leaf can be bound to the amine on chitosan, then a pesticide may be targeted to the plant; a more efficient and less damaging application of pesticides to the environment.

In an alternative modification procedure, microspheres were prepared with shells containing magnetic iron Oxide (Fe_3O_4) nanoparticles, MNPs. The MNPs were formed by coprecipitation of ferric and ferrous ions in solution and recovered by attraction to a permanent magnet. The powder x-ray-diffraction gave a suitable comparison with literature sources [19] and the TEM micrographs (Figure 3(b)) showed the sizes of the nanoparticles to be between 5 and 15 nm. The MNPs were incorporated into the microsphere shell by dispersion in the chitosan prior to microsphere formation. A similar method was used with poly(methacrylic acid) microspheres (see Section 3.2). In these preliminary experiments, the dispersion was not perfect and, particularly with chitosan solutions, the MNPs showed some aggregation. Upon sonication microspheres were formed (Figure 3(c)) and were larger than those without nanoparticles. It was notable that some of the microspheres were found in contact with, and moved on the slide in concert with, their neighbours, suggesting some degree of magnetism in the microspheres. These effects were not observed when the MNPs were not present, implying that the MNPs impart some degree of magnetism to the microspheres. The incorporation of iron oxide nanoparticles into $PMAA_{SH}$ microspheres was more successful and when a bar magnet was moved across the surface, movement of the microspheres could be observed. A video was taken and a still image is displayed in Figure 3(c).

The work builds on literature reports of the use of chitosan to form functional microspheres using ultrasound and illustrates the potential for delivery of both hydrophobic and hydrophilic species as well as modifying the chitosan shell to allow specific targeting

and/or magnetic manipulation. Chitosan gives a potential sustainable solution but other situations demand responsive materials and here we turned to synthetic polymers.

3.2 Poly(methacrylic acid) and block copolymer microspheres

Caruso and co-workers [20] showed that poly(methacrylic acid), PMAA could be used to form microcapsules with stability being imparted by partial functionalisation with thiol groups which could crosslink the capsule shell by forming disulfide bonds. Cavallieri *et al.* [9] demonstrated that such $PMAA_{SH}$ polymers formed hydrocarbon filled microspheres in good yield using the sonochemical method and that a range of hydrophobic materials could be encapsulated. In our previous work, we demonstrated that microspheres containing water-in-oil emulsions [11] could be encapsulated and release of hydrophilic compounds achieved. Following the sustainability theme developed above, Figure 4(a) shows that PMA_{SH} microspheres can also be made using vegetable oil or vegetable oil-based emulsions as the organic phase. The microspheres were 5 – 10 μm in diameter although they were in relatively low yield compared with other matrices that were investigated. The microspheres were however stable over several weeks. A number of different organic and fluorocarbon media were successfully used as the water-immiscible phase, extending the potential use of the methodology.

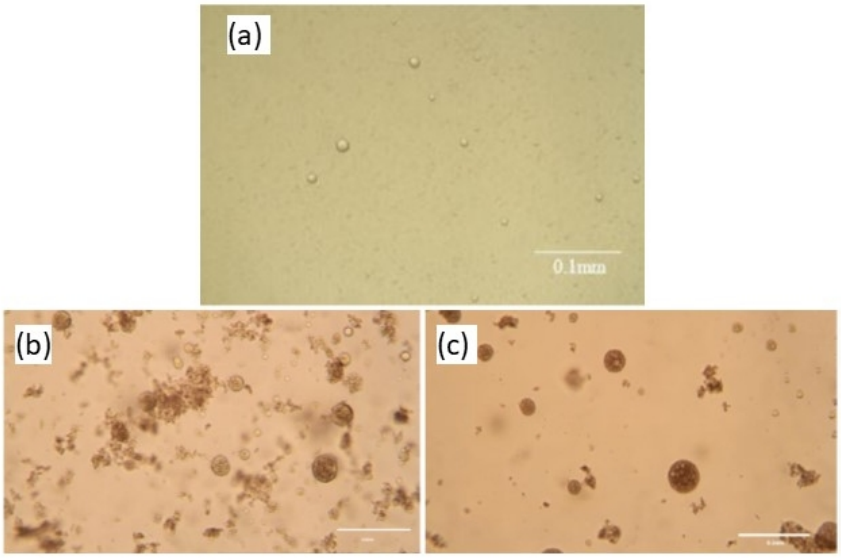


Figure 4. Sonochemically produced $PMAA_{SH}$ microspheres; (a) containing vegetable oil; (b) containing vegetable oil-water emulsion as freshly prepared; (c) containing vegetable oil-water emulsion after 1 month storage

In order to develop a targeted delivery system, the polymer must recognise and be responsive to its environment. Previous work has shown that the addition of dithiothreitol, DTT, to reduce the disulphide and hence break the crosslinks or the use of high shear conditions generated by ultrasound can be used to break the microspheres and release their contents. In the case of $PMAA_{SH}$, the organic acid (pK_a for methacrylic acid ≈ 4.7) potentially allows changes of pH to be used to modify microsphere behaviour.

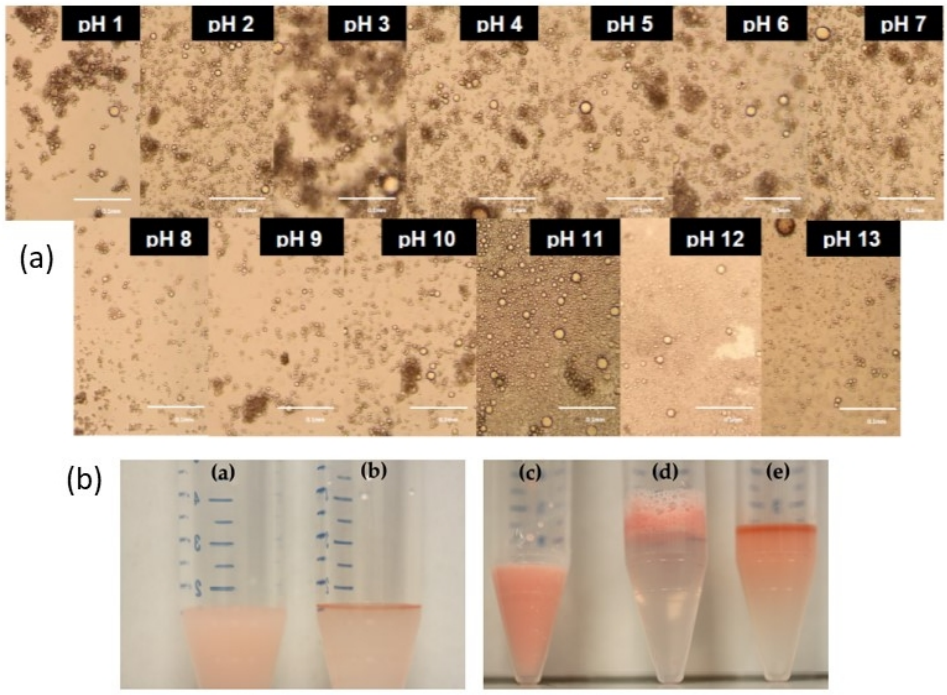


Figure 5. (a) $PMAA_{SH}$ 50% crosslinked microspheres containing tetradecane after 2hrs in pH1 – pH13 solutions (Scale bar is 100 μm); (b) 20% crosslinked microspheres containing tetradecane with Sudan III dye: (a) 30 min after preparation, diluted; (b) 30 min after addition of DTT; (c) 60 min after preparation; (d) 60 min at pH = 1; (e) 60 min at pH = 14.

Figure 5(a) shows the effect on $PMAA_{SH}$ microspheres at room temperature of pH, adjusted by adding aqueous solutions of HCl or NaOH. From the images it is evident that some microspheres remain present in all the pH environments. However, the numbers vary and there are also varying amounts of aggregated polymer visible, particularly at low pH. Some of the large droplets could be tetradecane released from microspheres although the mobility would be restricted by the cover slip on the microscope slide. The level of crosslinking in these samples was quite high at 50% so the microspheres may be fairly resistant to pH changes. That triggered release is possible is illustrated by Figure 5(b) where

the tetradecane has been dyed with Sudan III to aid visibility. The level of crosslinking here is lower at 20%. Here, the oil contents of the microspheres is clearly released at the extremes of pH whereas the as-prepared microspheres are stable in water.

The ability of $PMAA_{SH}$ to respond to other stimuli other than pH is limited. Since $PMAA_{SH}$ readily forms stable microspheres it was retained as the 'structural' part of our polymer shell material but we introduced other monomers into the materials by making a variety of block copolymers following the design principles outlined in Figure 6. $PMAA_{SH}$ would form the majority of the shell but the central blocks of the copolymer are formed from stimuli-responsive monomers. Three examples will serve to illustrate our approach.

As a first example, poly(ethylene glycol), PEG, is biocompatible polymer that undergoes slow hydrolysis, the rate of which can be accurately controlled, for example by copolymerization with propylene glycol or by variation of pH. We therefore set out to synthesise a $PMAA_{SH}$-PEG-$PMAA_{SH}$ block copolymer and to determine if microspheres can be formed using the sonochemical method. Direct synthesis of the ABA triblock copolymer is not straightforward so an indirect route, adapted from [21], was designed as shown in Scheme 1.

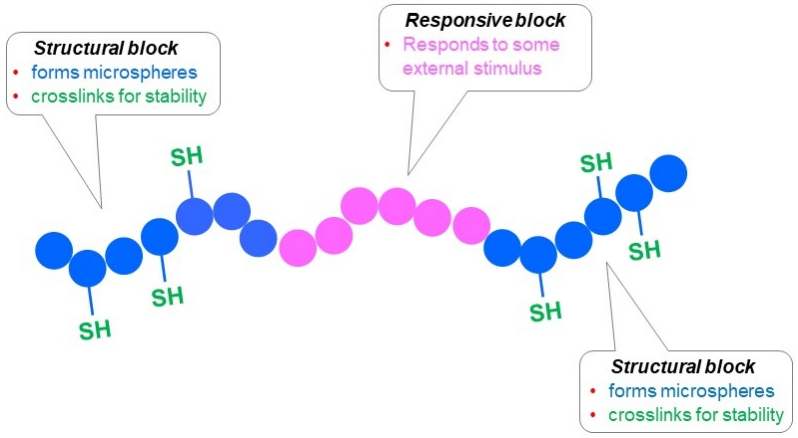
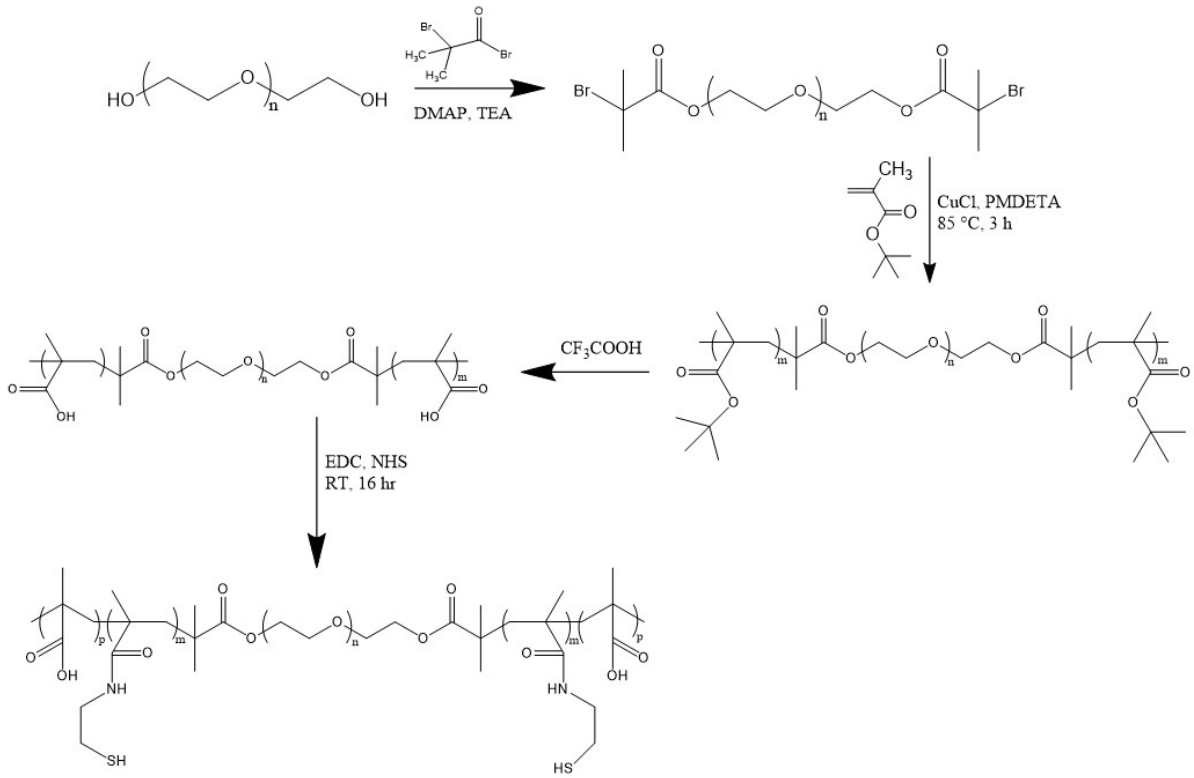


Figure 6. The design principle for responsive block copolymers to form microspheres

Hydroxyl terminated PEG was reacted with bromoisobutyryl bromide to form a difunctional initiator for atom-transfer radical polymerization, ATRP [22]. This was used to grow end blocks of ^{t}butyl methacrylate which was subsequently hydrolysed to methacrylic acid with 5eq of trifluoroacetic acid. Reaction with varying amounts of cysteamine hydrochloride, [23] allowed introduction of a controlled amount of pendant thiol groups to facilitate and control the extent of the crosslinking needed for long-term stability. It is worth noting that copolymers with and without the thiol functionalisation were able to form

microspheres but that those microspheres formed from copolymers those lacking the thiol groups were not stable for more than a few hours.



Scheme 1.

Each copolymer was characterised by NMR spectroscopy while DOSY NMR was used to confirm the formation of block copolymers. The presence of a block copolymer was further confirmed using GPC chromatography in which the molecular weight of the polymer was found to be M_n = 13100 $gmol^{-1}$. Using optical microscopy, it was found that microspheres were produced in high yield using tetradecane as the organic phase as in Figure 7. The microspheres formed were smaller than when using the homopolymer and were well under 10 μm in diameter. This established the feasibility of the approach and work is underway to characterise the performance of these microspheres in triggered release applications.

The next example gives a system that is potentially chemoresponsive. Boronic acids are known to interact strongly with sugars such as glucose. Polymers containing boronic acid functionality undergo pH dependent conformation changes depending on the sugar concentrations [14, 24]. The idea here was that if the central block of the copolymer changed

conformation, it could modify the shell structure to release the microsphere contents in response to high or changing sugar levels.

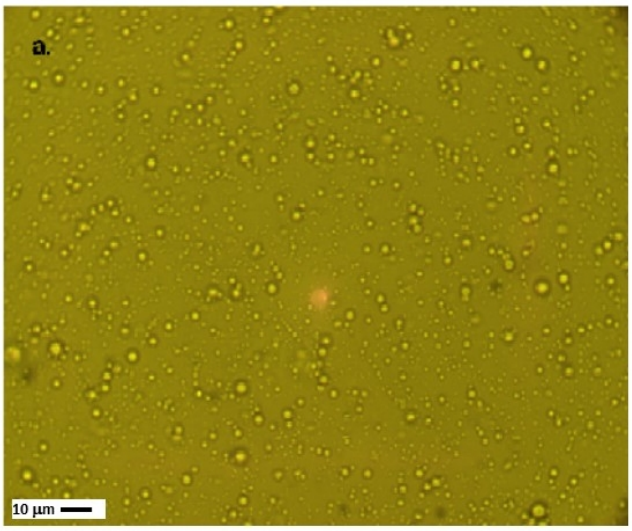
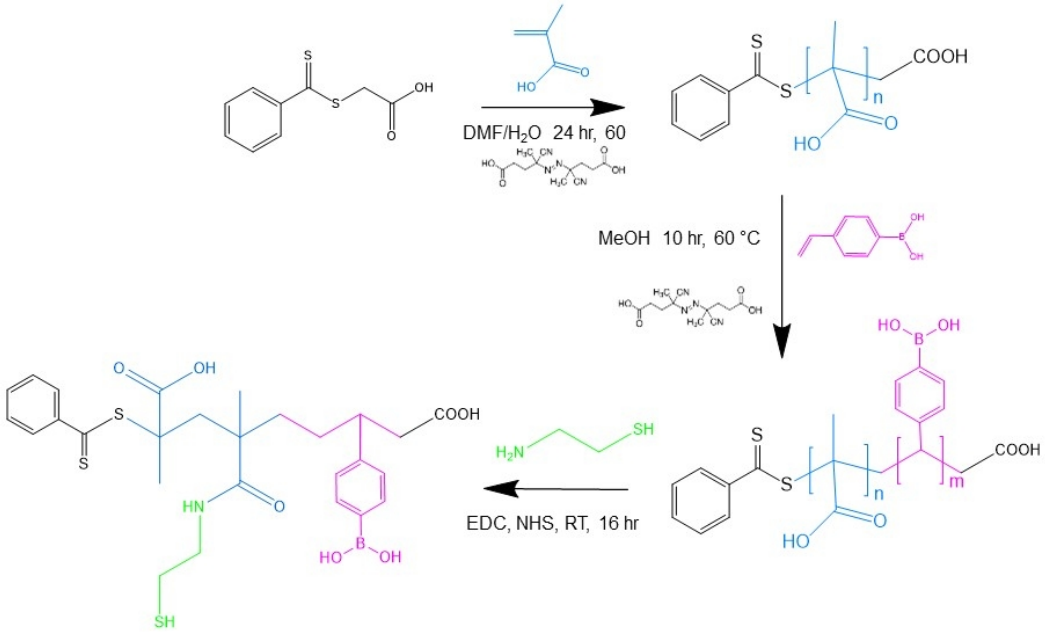


Figure 7. Sonochemically produced tetradecane microspheres using $PMAA_{SH}$-PEG-$PMAA_{SH}$ block copolymer.

Block copolymers of ($PMAA_{SH}$) can be prepared using the RAFT method of controlled radical polymerization [25]. Here, growing polymer chains are terminated by a labile thioester group which can exchange with growing radicals. This offers control over chain lengths and also, by fully reacting one portion of monomer and then adding a different monomer, allows the formation of block structures. In this case, S-(thiobenzoyl)thioglycolic acid was used as the RAFT agent to directly polymerise methacylic acid, followed by a second, responsive monomer and finally another block of methacrylic acid. As outlined above, the final reaction in the sequence was to introduce the thiol group to enable crosslinking.

Boronic acid containing block copolymers were prepared using RAFT with vinyl phenyl boronic acid, VPBA, as the monomer to comprise the central block as shown in Scheme 2. In this case, only a diblock material could be prepared. These successfully formed microspheres as illustrated in Figure 8(a) although there was a wide range of diameters, from about 2.5 µm to 8 µm with a mean value of 5 µm. Again, the thiol crosslinked microspheres were more stable than those without the thiol and were stable on cold (refridgerator) storage for a period of weeks.



Scheme 2.

Microspheres were formed successfully from both P(MAA-b-4-VPBA) and P(MAA_{SH}-*b*-4-VPBA) following the same method as above. Again, the sonication time and intensity were varied and the conditions selected gave a reasonable yield of microspheres (judged optically) with minimal degradation. To test how well the disulfide crosslinking stabilises the microspheres, a comparison between microspheres from the thiolated and non-thiolated copolymers showed that the non-thiolated microspheres were slightly smaller. Over the course of 16-days, the stability of microspheres can be assessed by the change in size over time. P(MAA-*b*-4-VPBA) microspheres decrease in size over the 16-day period whereas the P(MAA_{SH}-*b*-4-VPBA) microspheres maintain their size overall. This stability can be attributed to having the disulfide cross-linkages.

The P(MAA_{SH}-*b*-4-VPBA) microspheres were formed with the aim of being potentially pH and glucose responsive, the change in pH affecting both the carboxylic acid on MAA and the boronic acid groups. Unfortunately, only low incorporation of VPBA could be achieved and so little effect of adding glucose to the system was observed (Figure 8(b)). The small amounts of 4-VPBA present in this sample means the binding of glucose to the boronic acids present does not cause enough swelling to burst the microspheres although some perturbation to the microsphere shape is apparent. This was confirmed by a small change in mean diameter revealed by dynamic light scattering measurements (Figure 8(c)). However, these changes are much smaller than might be suggested from literature work [24] so that further work is needed to confirm whether this is a viable approach. The current levels of

incorporation of boronic acid may be too small to affect microsphere breakage and a different pH range may be needed. The presence of 4-VPBA in a diblock material may give rise to unusual phase behaviour where the short boronic acid chains lie on the surface of the microsphere shells rather than being incorporated.

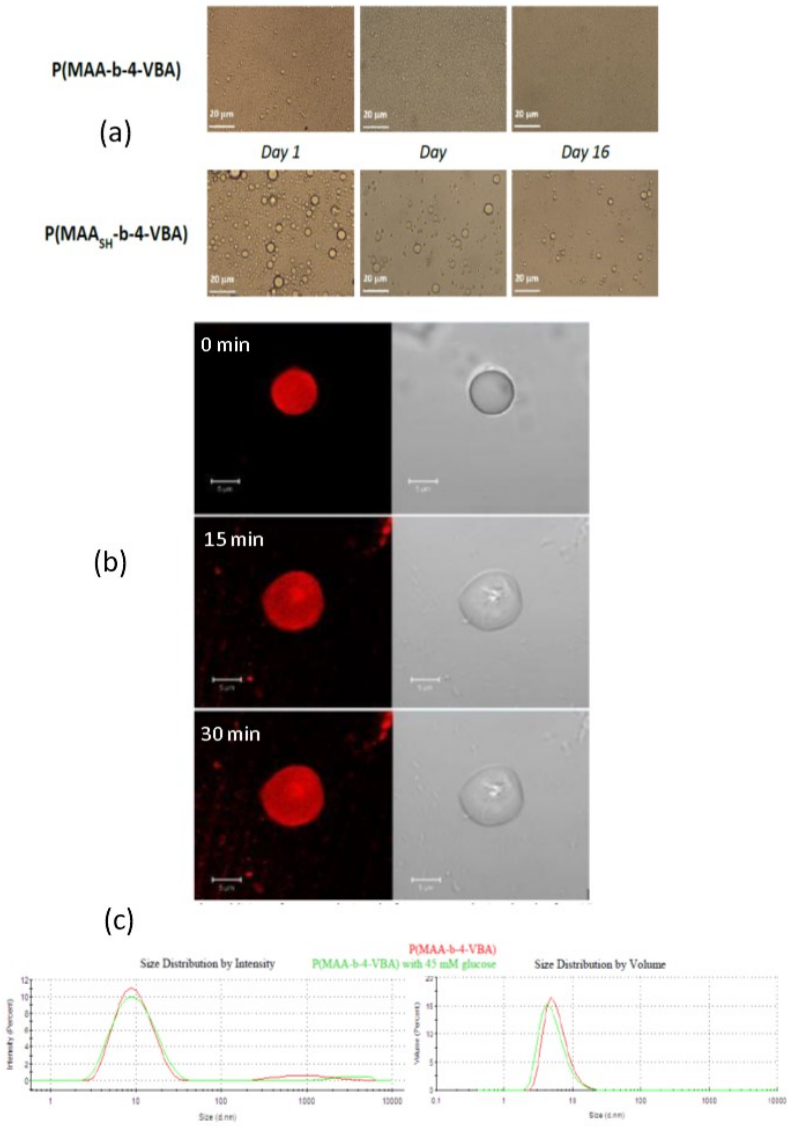
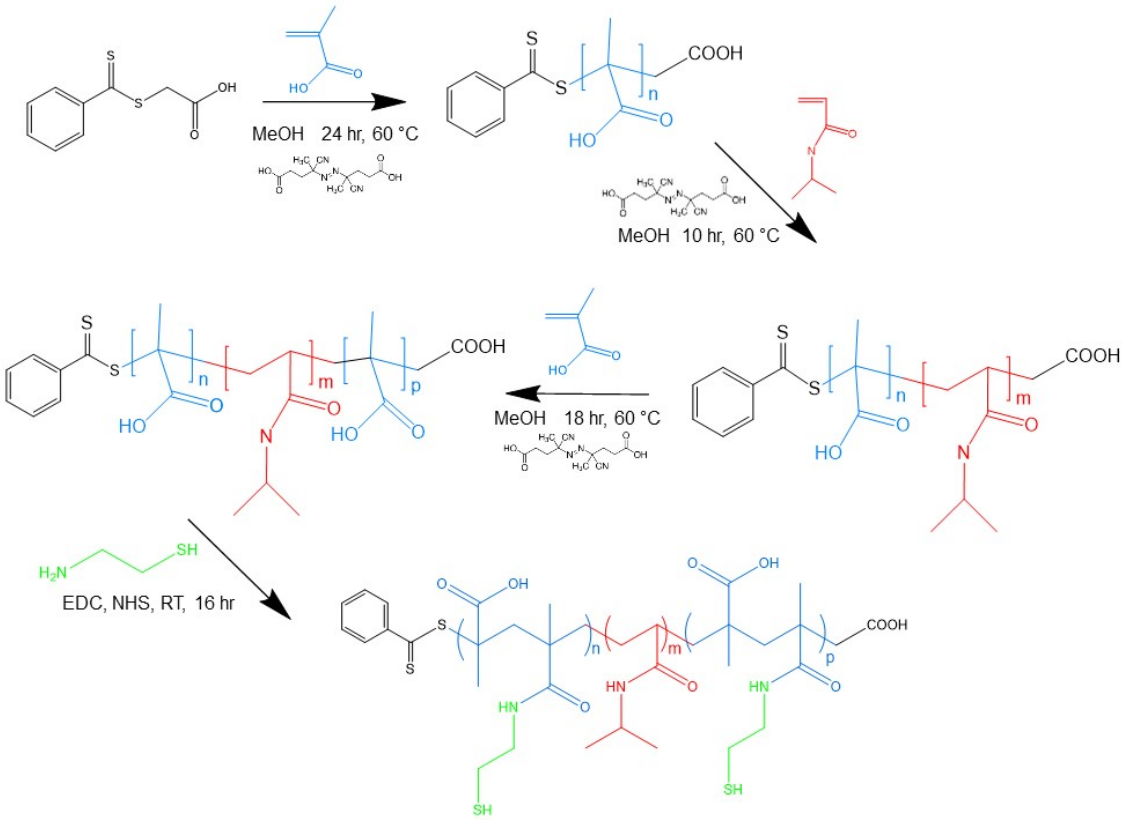


Figure 8. Sonochemically produced tetradecane microspheres from PMAA$_{SH}$-PVPBA block copolymer: (a) Formation and stability of microspheres with and without crosslinking; (b) Effect on microspheres of exposure to 0.45 M glucose solution; (c) change in size of microspheres on exposure to 0.45 M glucose solution

The final example of a responsive microsphere shell attempts to make them respond to changes in temperature. Poly(N-isopropyl acrylamide), PNIPAM, in water undergoes an

entropy driven coil to globule Lower Critical Solution Temperature (LCST) transition at ~ 31°C so that the polymer becomes insoluble with rising temperature [26]. Our hypothesis was that making the microsphere shells containing high proportions of PNIPAM would give a system that would undergo change and hence release their contents with rising temperature.

Block copolymers of $PMAA_{SH}$ and PNIPAM were prepared using the RAFT method in similar manner to the previous example as shown in Scheme 3. The copolymer was soluble in tris acetate-EDTA buffer solution and was subjected to the usual method for preparing microspheres using either tetradecane or vegetable oil as the organic phase. Figure 9(a) shows the triblock copolymer microspheres containing tetradecane are around 10 – 15 μm in diameter, rather larger in comparison to the single block, PMAASH, microspheres.



Scheme 3

The temperature responsive nature of the microspheres is shown in Figure 9. Images were recorded on a slide at room temperature and after the slide had been heated to 40ºC. The polymer denatures as the sample is heated and the microspheres are broken and so release the oil inside leading to the oil patches observed in Figure 9(b). Control experiments

showed that microspheres consisting solely of $PMAA_{SH}$ or the block copolymer microspheres held at 30 ºC for extended periods showed no change.

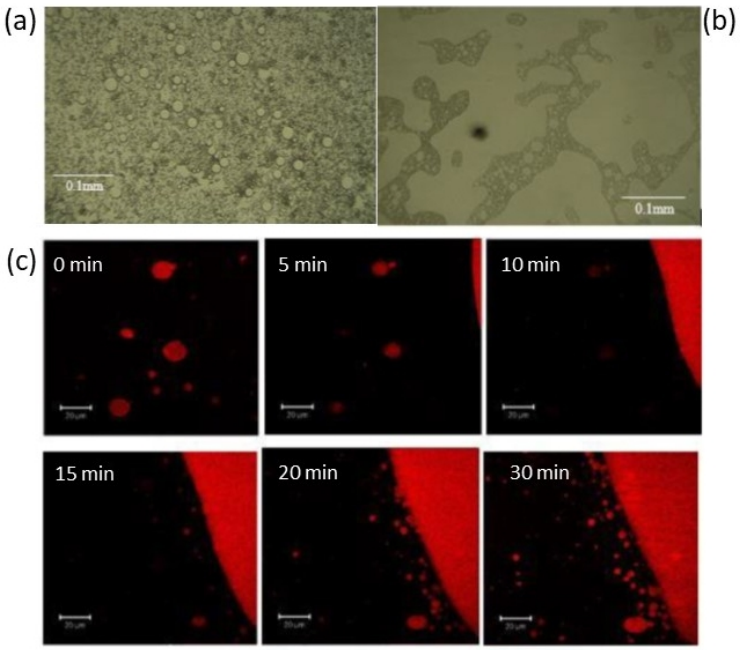


Figure 9. (a) Sonochemically produced tetradecane/nile red microspheres using $PMAA_{SH}$-PNIPAM-$PMAA_{SH}$ block copolymer; (b) as (a) but heated to ~40 °C; (c) confocal micrographs of microspheres from (a) being heated to 40 °C over the indicated time periods (Scale bar is 20 μm).

These experiments have shown that under temperature increase P(MAA_{SH} -*b*-NIPAAM-*b*-MAA_{SH}) microspheres broke apart, but $PMAA_{SH}$ microspheres lacking the temperature responsive block did not. The experiments also showed that P(MAA_{SH} -*b*-NIPAAM-*b*-MAA_{SH}) microspheres did not break down when left at room temperature. These experiments confirm that P(MAA_{SH} -*b*-NIPAAM-*b*-MAA_{SH}) microspheres do have a temperature responsive middle block, which can potentially be used for triggered release. Similar experiments were carried out using a confocal microscope as in Figure 9(c) and confirm the release of the contents of the microspheres.

3.3 Targetted delivery of gold nanoparticle catalysts

Targetted delivery using microspheres could have a range of applications as outlined above. One is in targeted catalysis so we were interested to apply our systems to this area. We have

previously [17] used gold nanoparticles, AuNPs, as catalysts in the borohydride reduction of nitrophenol to aminophenol as a model reaction.

Gold nanoparticles were synthesised by usual Turkevich method of reduction of $HAuCl_4$ using sodium citrate as the reductant. The resulting gold nanoparticles showed a UV/visible maximum absorbance at 522 nm corresponding to nanoparticles which are approximately 25 nm diameter, confirmed by electron microscopy measurements. These were used to successfully catalyse the borohydride reduction of nitrophenol to aminophenol as a model reaction as reported previously [17].

AuNPs cannot be well dispersed in organic solvents such as tetradecane so that in order to encapsulate the AuNPs inside microspheres, an aqueous solution was dispersed in tetradecane as described above and this emulsion used to form $PMAA_{SH}$ microspheres. Colloidal gold solution is observable in the droplets of aqueous phase in Figure 10(a) tetradecane confirming the emulsion formation. This emulsion was extremely stable and could be left for over two weeks without change. These microspheres containing the emulsion were subjected to a short burst of ultrasound of ~45 W cm^{-2} to destroy them and release the gold as shown in Figure 10(b) demonstrates that the microspheres have been broken as no microspheres are visible and all that can be seen are large droplets of tetradecane.

However, applying this process to the reduction of nitrophenol was not successful. No catalysis of the reaction occurred. This was due to the small amount of AuNPs available which proved too low a concentration to be effective. For a successful catalytic system, higher amounts of gold were needed. Ray *et al.* [18] demonstrated a method of forming high concentration gold colloids in the presence of a block copolymer Pluronic P85 as a stabilising agent. Solutions were prepared and it proved possible to incorporate these into a tetradecane emulsion and hence into both $PMAA_{SH}$ and chitosam microspheres using the methods outlined earlier. TEM images (Figure 10(c)) showed the sizes of the gold nanoparticles were between 10 and 15 nm.

Catalysis of the reduction of 4-nitrophenol was investigated using the pluronic stabilised gold nanoparticles to determine the minimum amount necessary for the reaction. Sufficient microspheres were synthesised to deliver this amount of AuNPs. The emulsion formed was not as stable as that above. When the ultrasonic horn was used to form the emulsion it caused gel formation as the heat generated during acoustic cavitation pushed the temperature of the Pluronic above its gel temperature. An emulsion could be prepared using a

vortex shaker and was stable for ~ 20 minutes. This was sufficient to allow microspheres to be prepared.

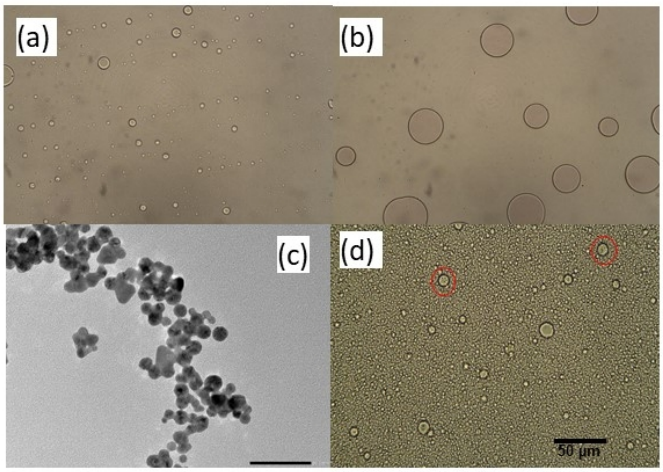


Figure 10. (a) $PMAA_{SH}$ microspheres containing tetradecane-AuNP solution; (b) as (a) after application of ultrasound to break the microspheres; (c) TEM images of AuNPs stabilised with pluronic P85 scale is 50 nm; (d) Chitosan microspheres containing tetradecane-AuNP solution stabilised with pluronic P85

The reactions were followed by changes in the UV-visible spectrum but the occurrence of a reaction is readily observable since 4 nitrophenol is yellow but the product is colourless. Addition of microspheres with $PMAA_{SH}$ shells yielded no reaction under any conditions. A series of control experiments showed that the AuNPs were being released but the $PMAA_{SH}$ polymer was inhibiting the catalysis. A likely reason for the inhibition of the catalysis is a strong interaction between the thiol groups in the polymer (assuming that not all are involved in crosslinking during microsphere synthesis) and the gold nanoparticle [27].

We therefore attempted the same experiments but using chitosan as the microsphere shell material. Chitosan microspheres were successfully synthesised using a 50:50 emulsion of tetradecane and pluronic stabilised gold nanoparticle solution as the organic phase, shown circled in red in Figure 10(d). Although further experiments are needed to confirm, using chitosan appeared to form larger microspheres than $PMAA_{SH}$ so that greater volumes of emulsion could be encapsulated. Chitosan microspheres also gave a much higher yield compared with the $PMAA_{SH}$ microspheres.

The microspheres containing pluronic gold were added to the reaction mixture as shown in Figure 11. Five samples were prepared. One had nothing added to it to serve as a blank; one had a small amount of the AuNPs added while another had AuNPs added in the

form of the pluronic stabilised emulsion. Both of these should give a reaction to show the activity of the AuNPs. Two other samples had portions of the chitosan microspheres added. Of these one remained unbroken while the other was exposed to a short 10 s burst of ultrasound to release the microsphere contents. A separate experiment showed that this burst of ultrasound did not influence the reaction.

The results demonstrate that gold nanoparticles have been encapsulated in chitosan microspheres and subsequently used in catalysis of the model reaction for 3 hr. The colour changes seen in Figure 11(b) indicate that simple addition of the microspheres has no effect on the reaction while breaking them to release the contents catalyses the reaction in the same way as simple addition of gold.

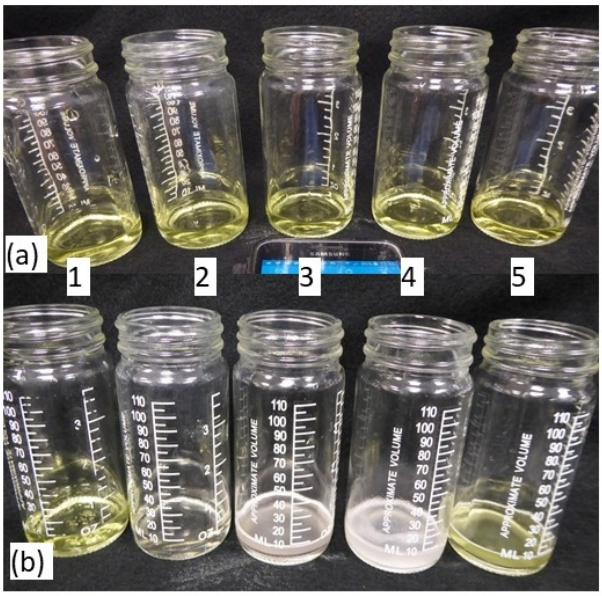


Figure 11 AuNP catalysis of borohydride reduction of 4-nitrophenol: (a) before reaction; (b) after 3 hr. 1. Nothing added; 2. AuNPs alone added; 3. AuNP-tetradecane emulsion added; 4. AuNPs in chitosan microspheres added and broken; 5. AuNPs in chitosan microspheres added and left intact. The amount of AuNPs added was the same in 2 – 4.

4 Conclusions

This work extends previous studies and further reinforces the usefulness of the sonochemical method for forming microspheres. It builds on our previous description of a method for encapsulating and delivering hydrophilic species by demonstrating that the 'double emulsion' method can be used with chitosan or thiolated poly(methacrylic acid) based systems. The sustainability and potentially lower environmental impact is improved by substituting commercial vegetable oil for the hydrocarbons used previously.

We also report for the first time the use of responsive block copolymers to form microspheres. Our systems include copolymers that respond to pH, sugar concentrations or temperature and the work illustrates the potential for a new range of responsive microspheres.

Coupling the microsphere systems with sonochemical nanoparticle synthesis enables an aqueous based gold nanoparticle catalyst system to be developed and encapsulated. Here, using ultrasound to break the microspheres to release the catalyst acts as a trigger for the reaction. Combining the various aspects of work reported here offers great potential for the sonochemical method to greatly enhance the tools available to the materials chemist.

Acknowledgements

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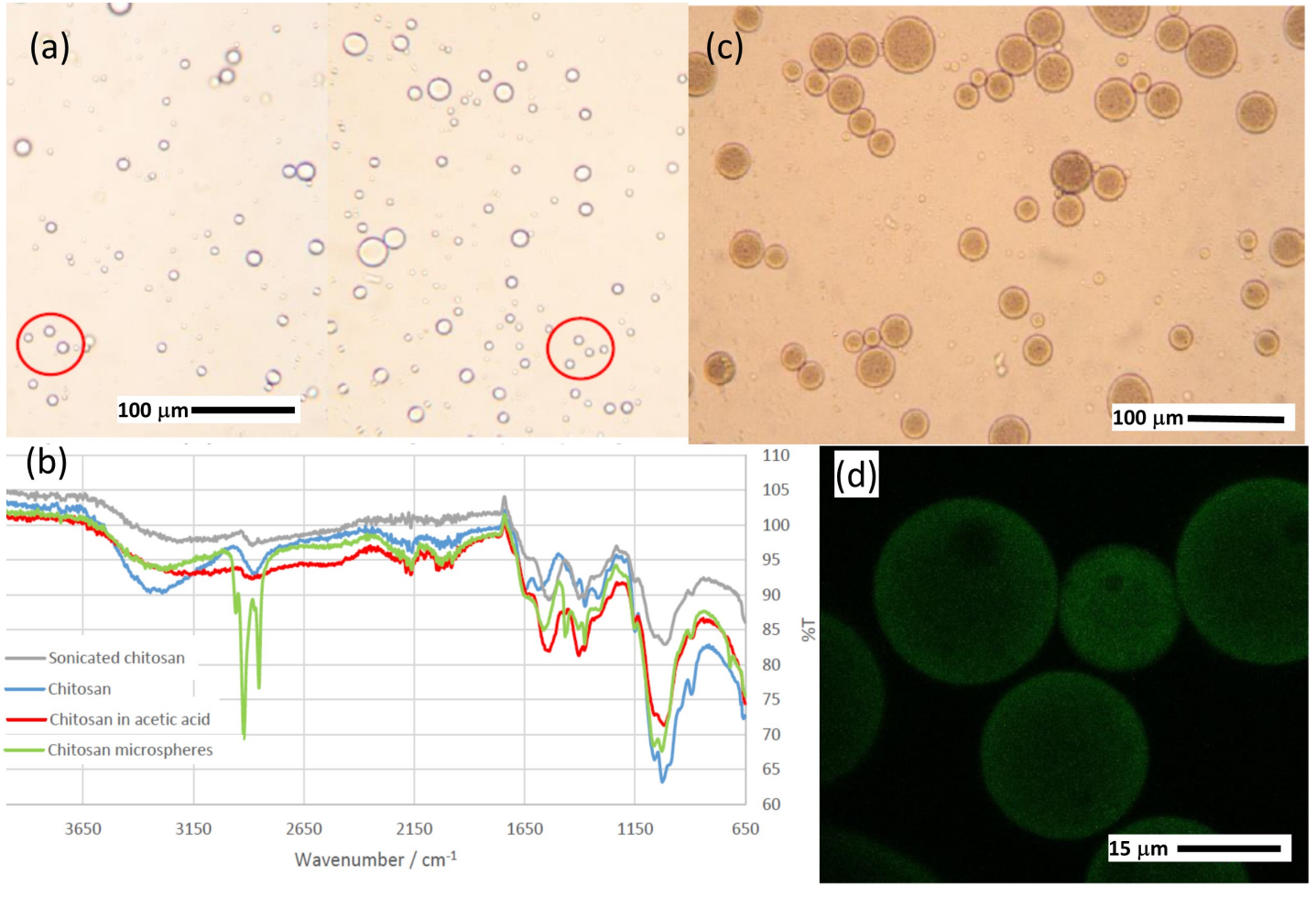
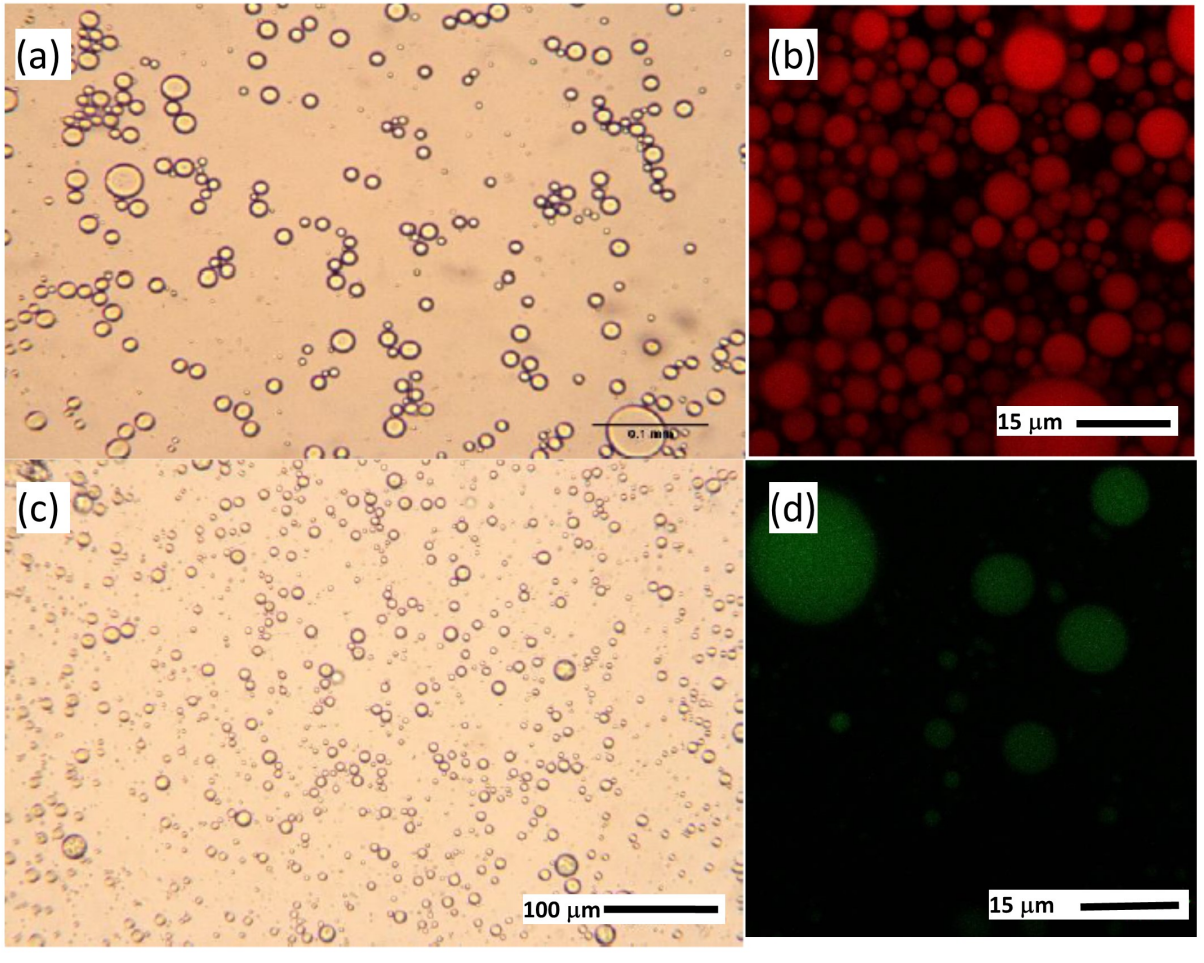
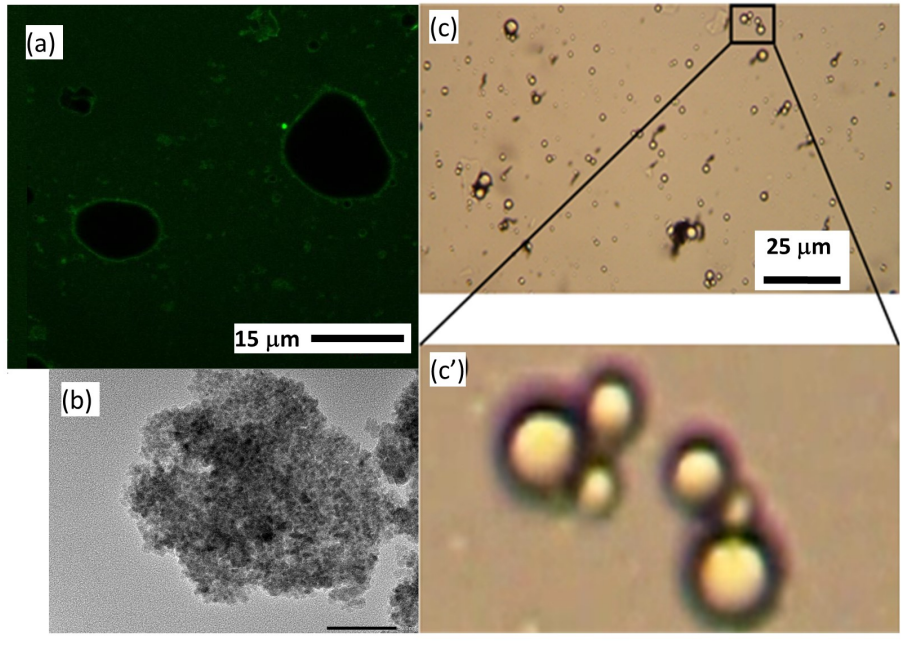
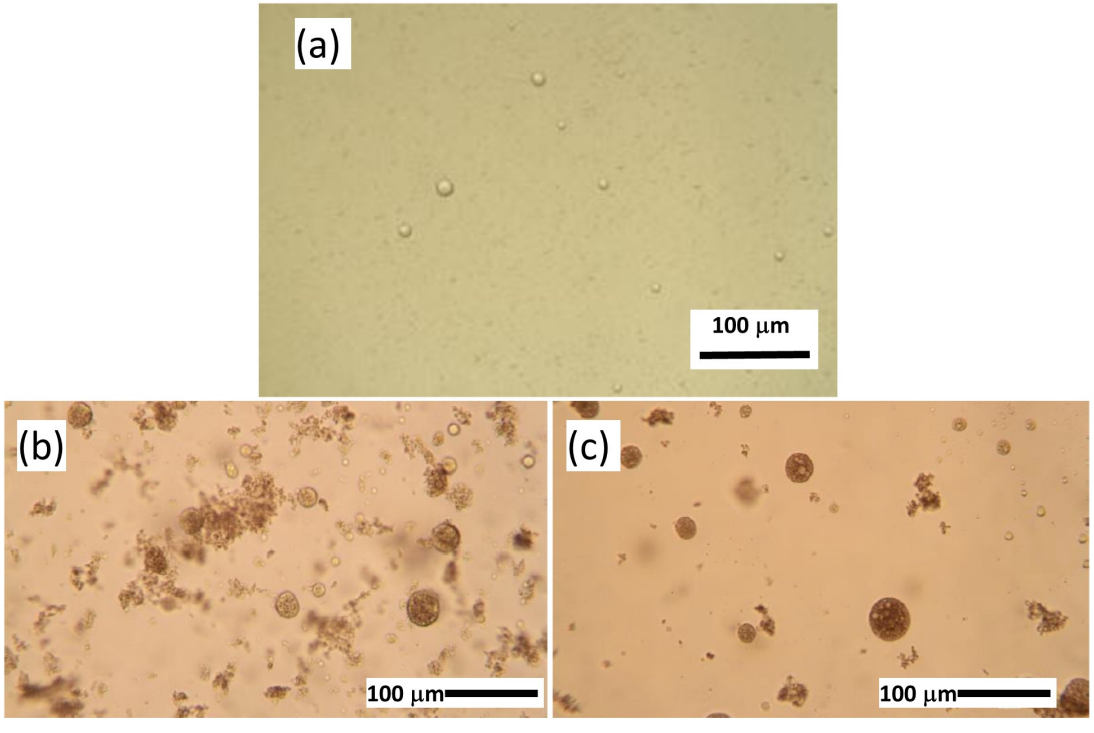
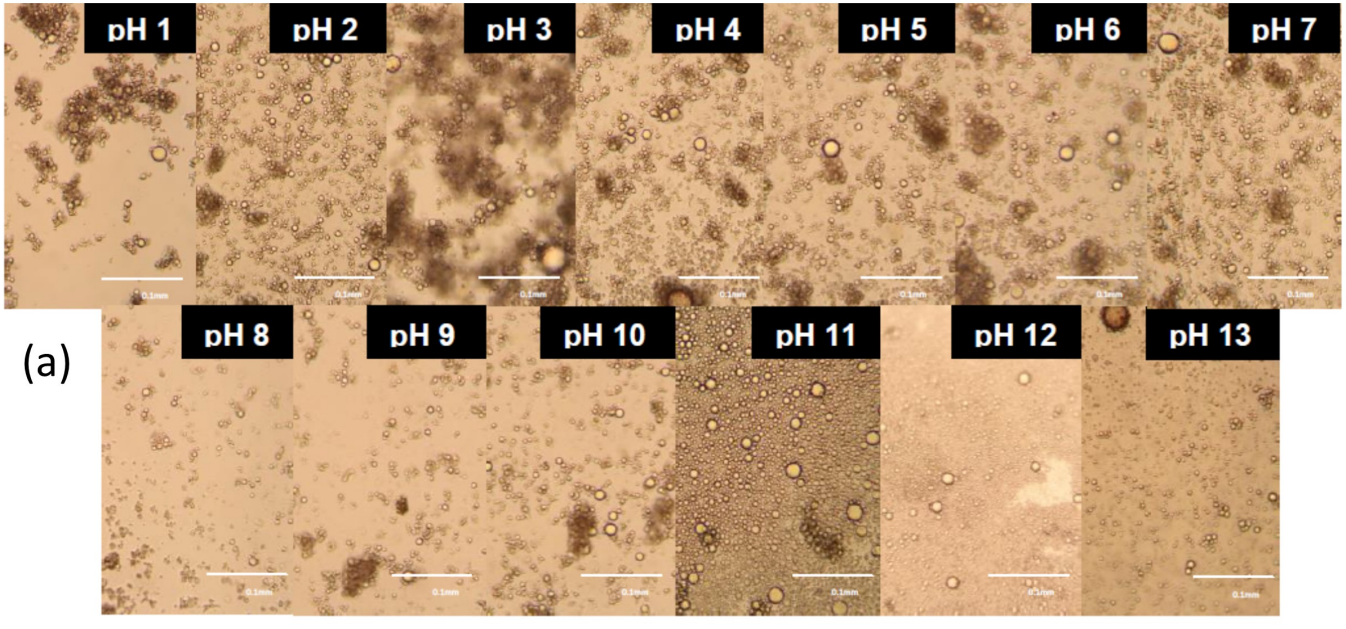

(a)
100 µm
(c)
100 µm
(b)
Sonicated chitosan
Chitosan
Chitosan in acetic acid
Chitosan microspheres
110
105
100
95
90
85
80
75
70
65
60
%T
3650
3150
2650
2150
1650
1150
650
Wavenumber / cm-1
(d)
15 µm


(a)
(b)
15 μm
(c)
100 μm
(d)
15 μm

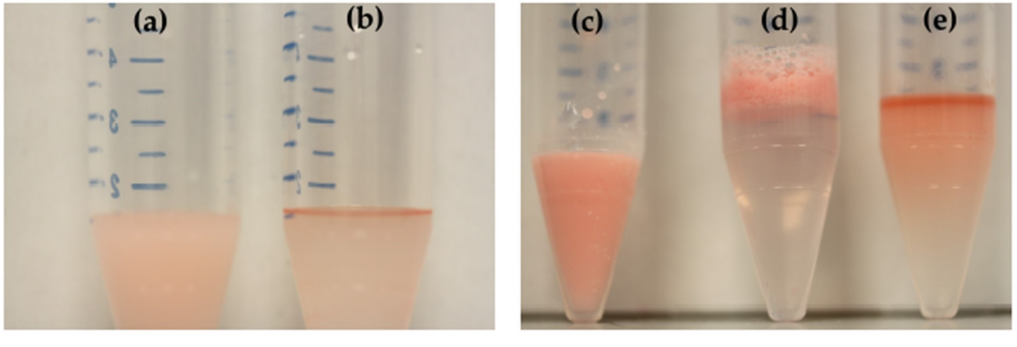

(a)
15 μm
(b)
(c)
25 μm
(c')

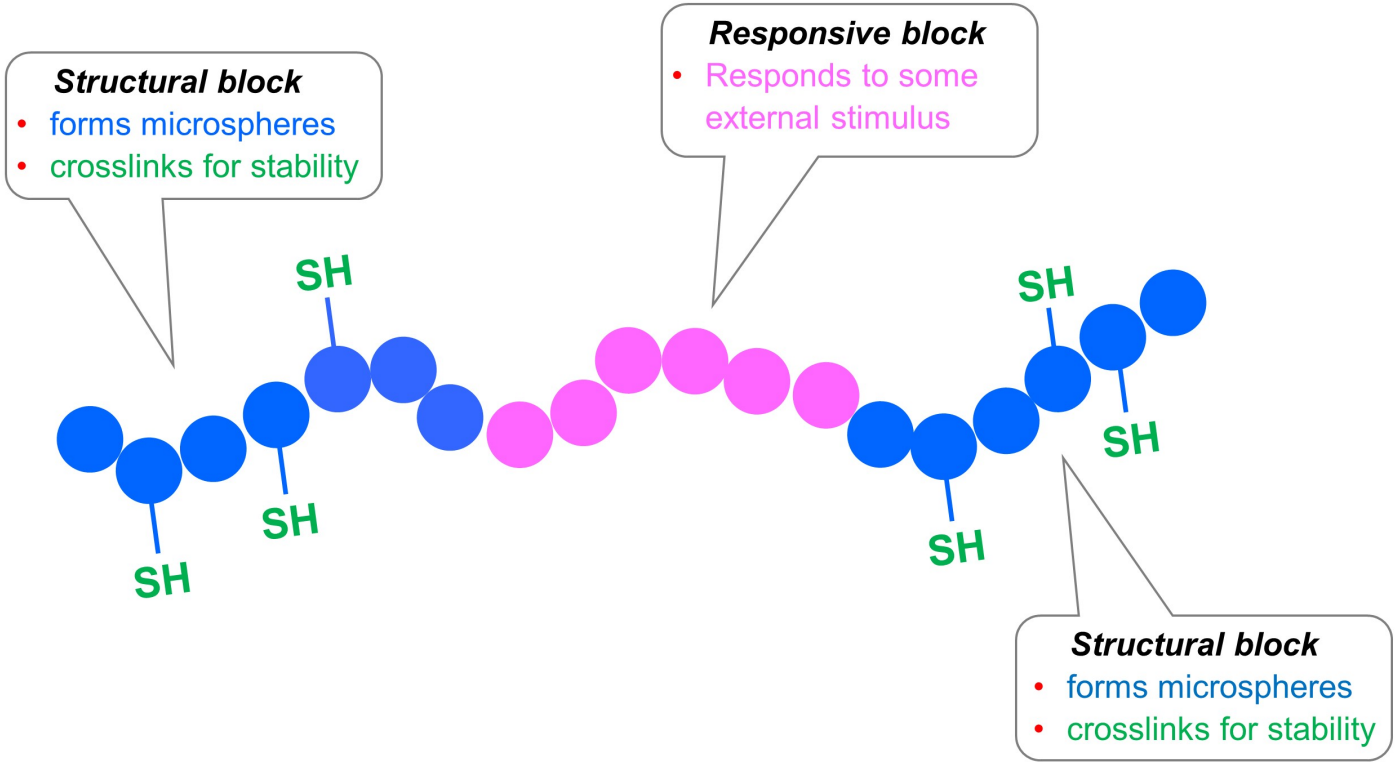

(a)
100 μm
(b)
100 μm
(c)
100 μm

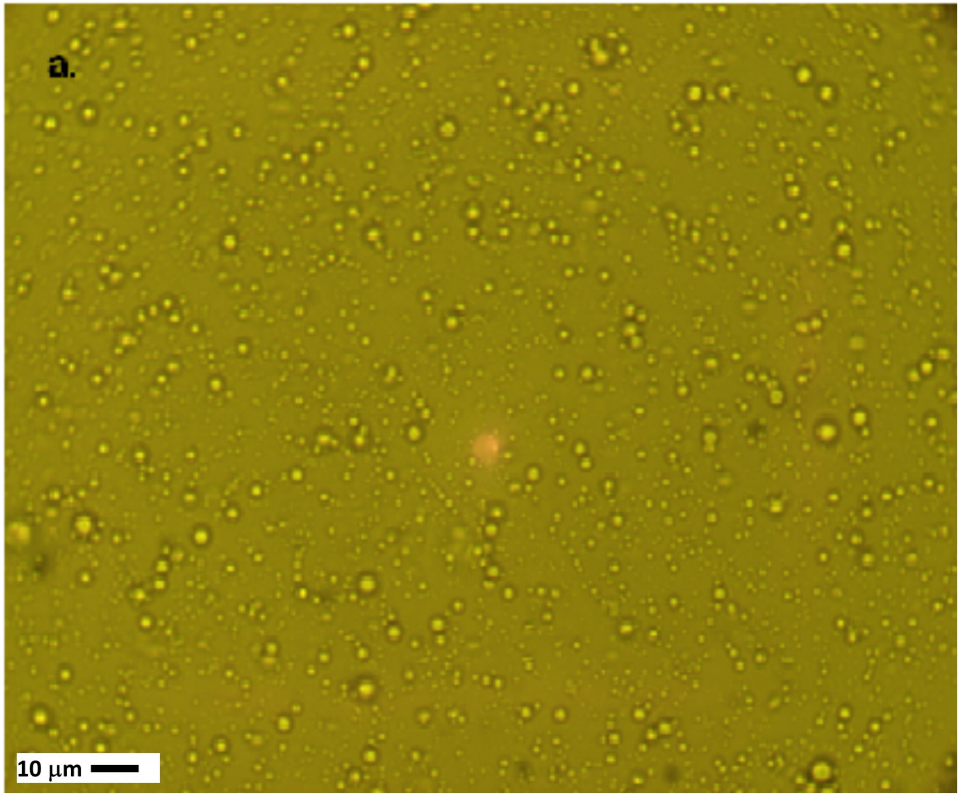


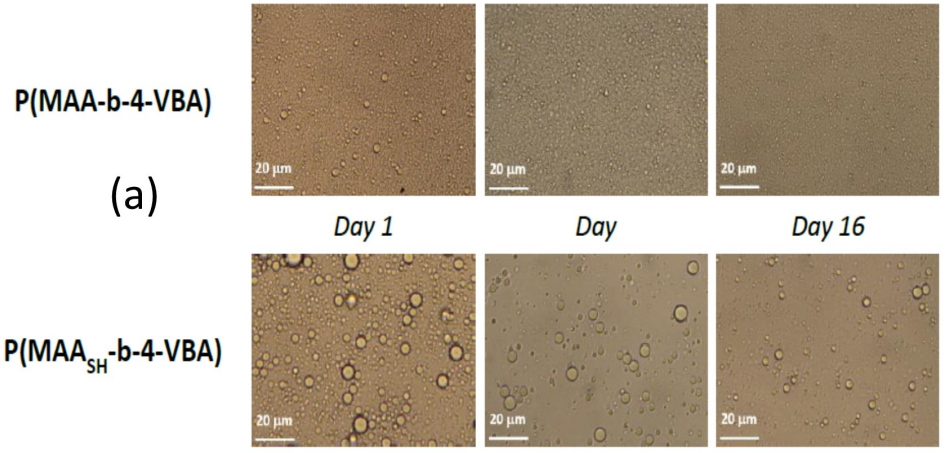
(a)

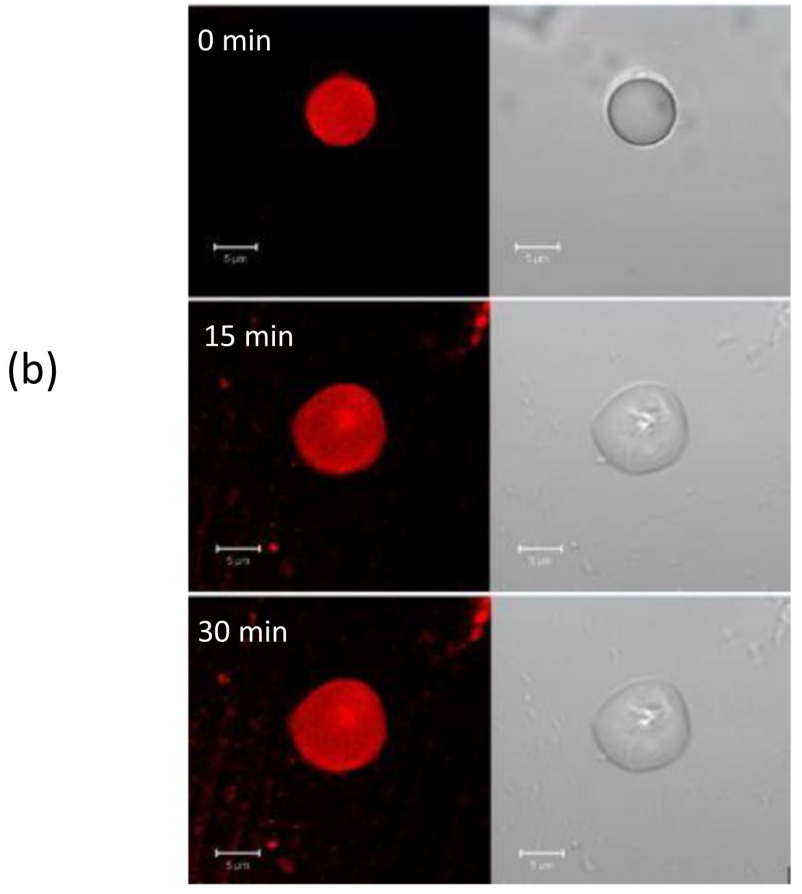
(b)

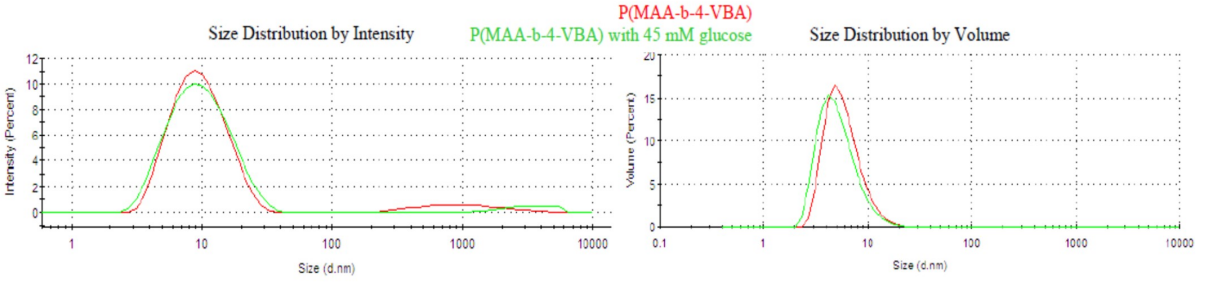


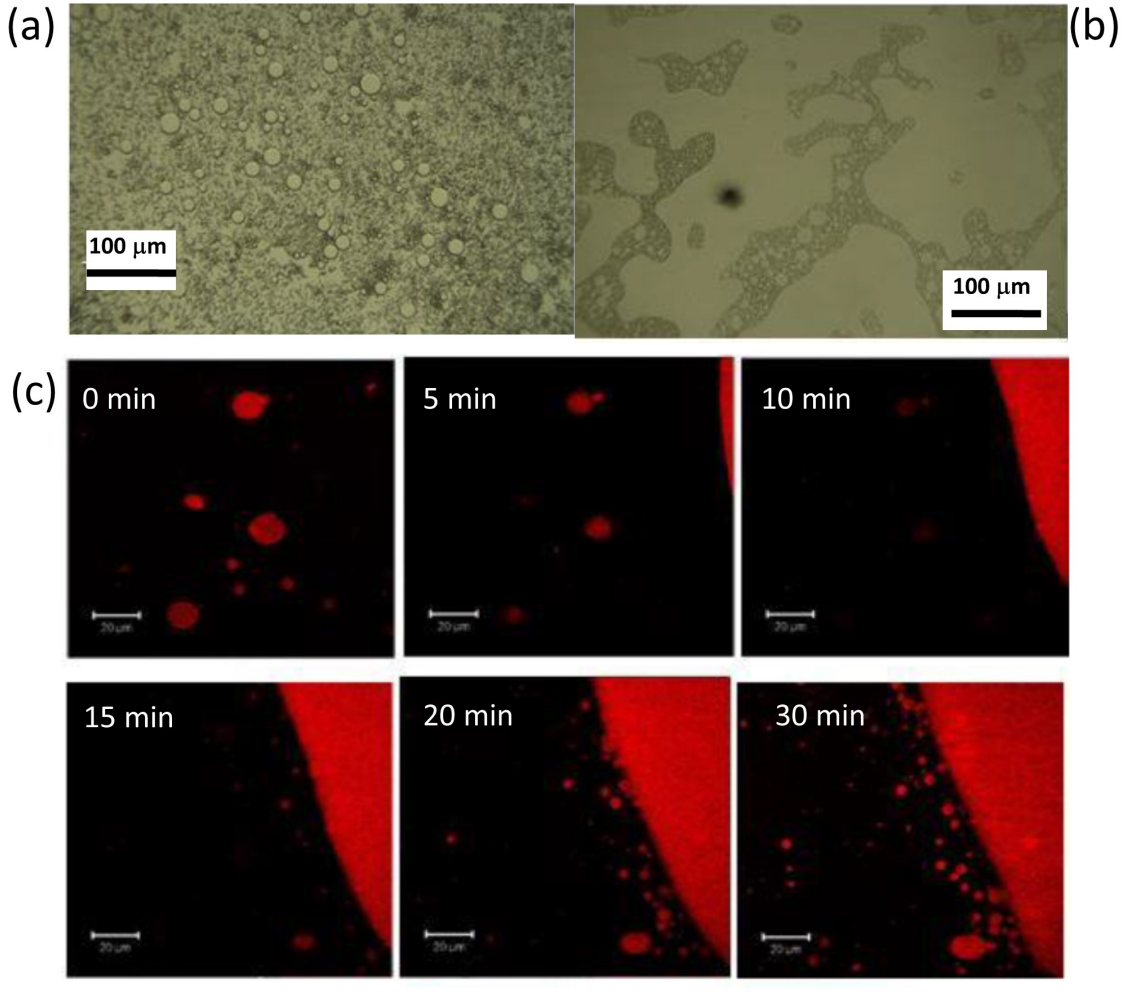

Structural block
• forms microspheres
• crosslinks for stability
Responsive block
• Responds to some external stimulus
SH
SH
SH
SH
SH
Structural block
• forms microspheres
• crosslinks for stability

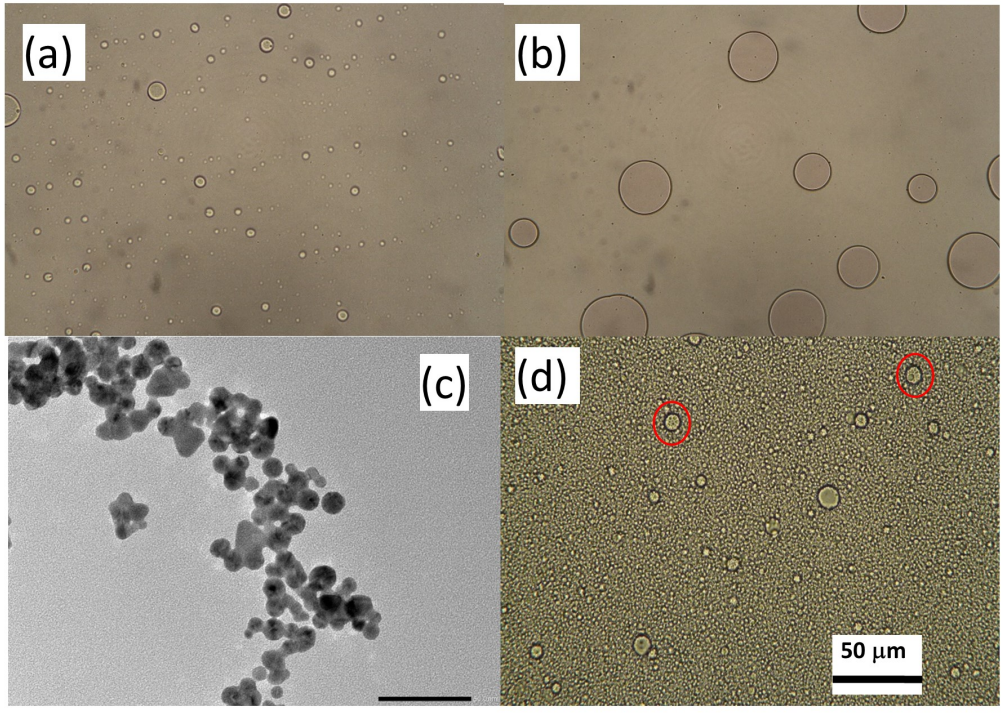

a.
10 μm

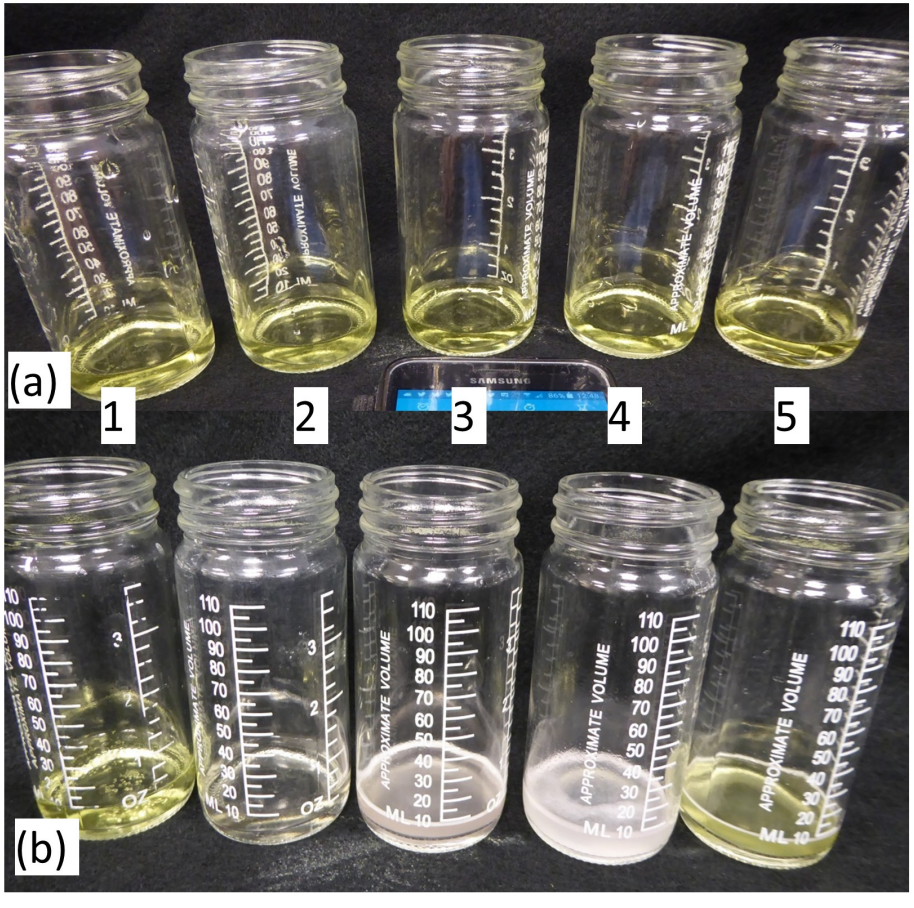

P(MAA-b-4-VBA)
(a)
Day 1
Day
Day 16
P(MAA$_{SH}$-b-4-VBA)
20 µm

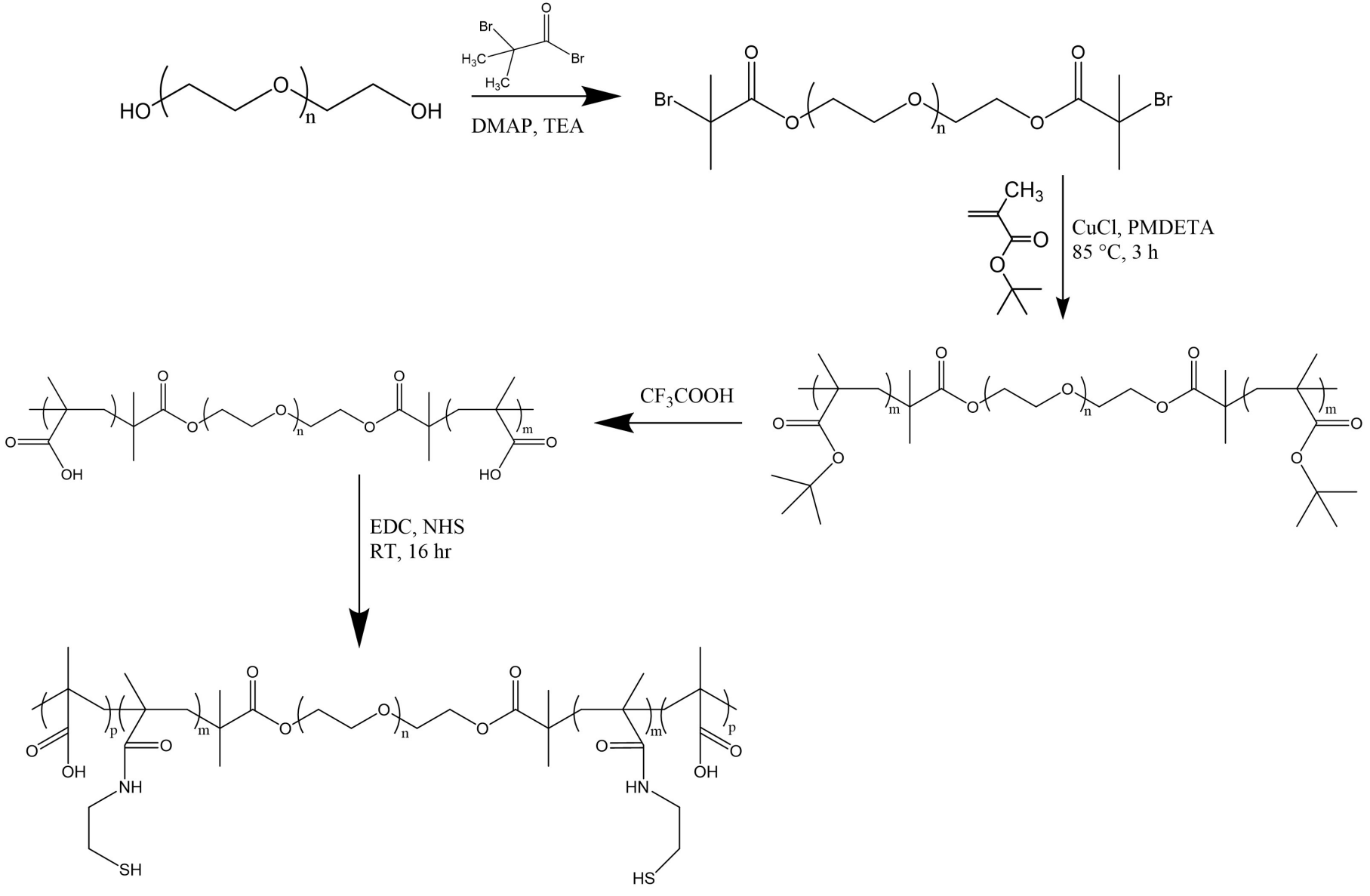

(b)
0 min
15 min
30 min

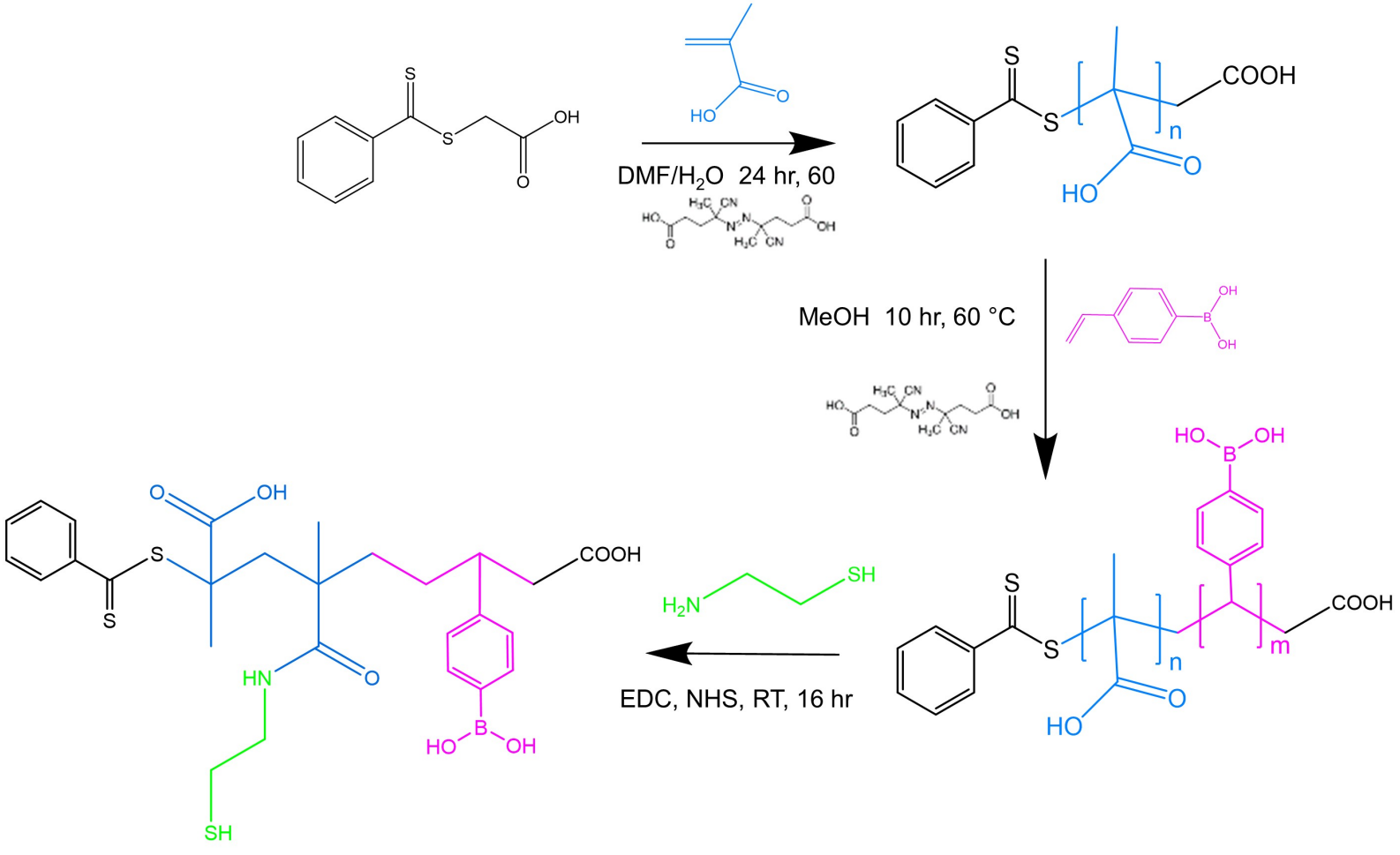

(c)
P(MAA-b-4-VBA)
P(MAA-b-4-VBA) with 45 mM glucose
Size Distribution by Intensity
Size Distribution by Volume
Intensity (Percent)
Volume (Percent)
Size (d.nm)

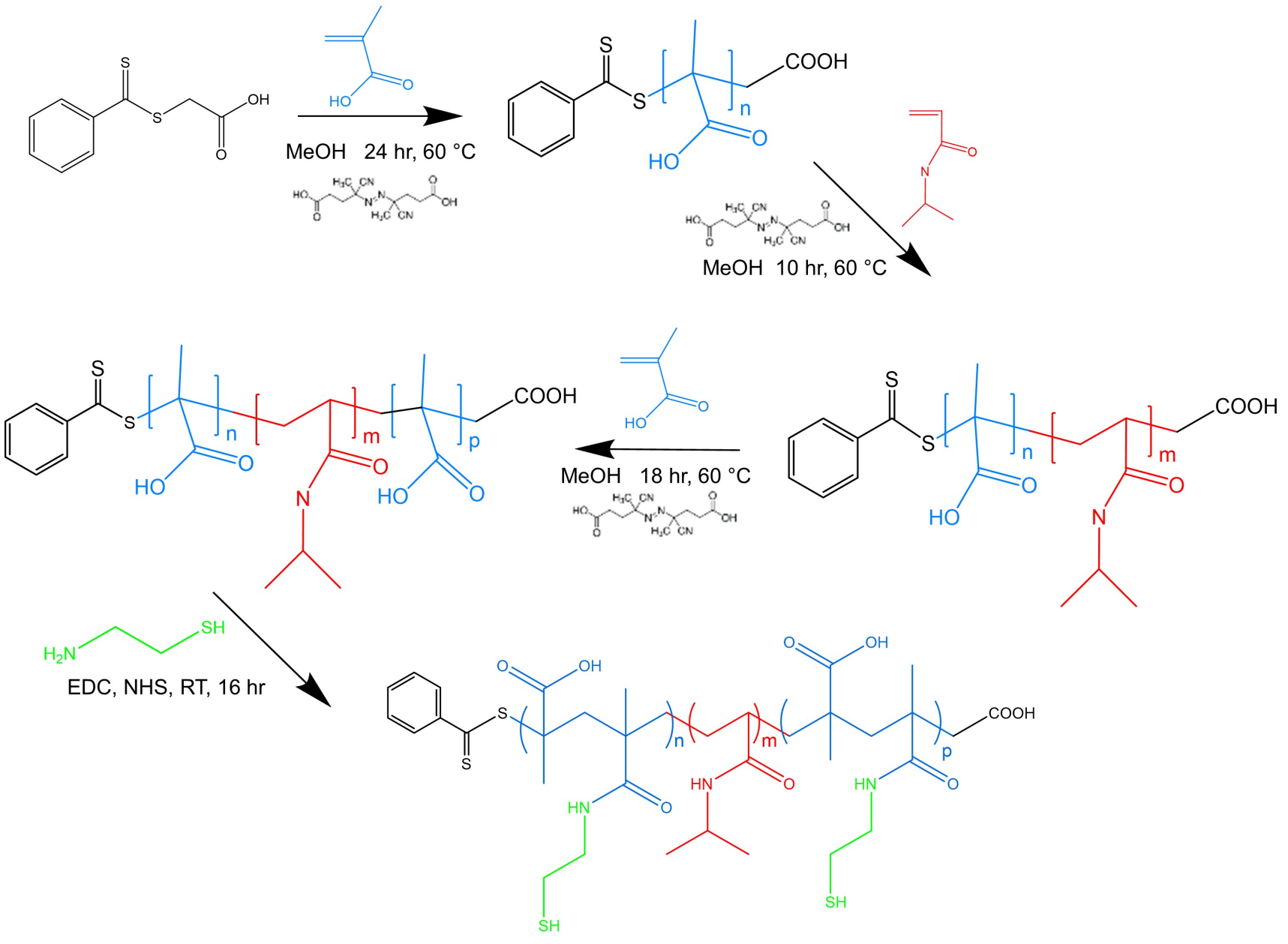

(a)
100 μm
(b)
100 μm
(c)
0 min
5 min
10 min
15 min
20 min
30 min


(a)
(b)
(c)
(d)
50 μm


(a)
1
2
3
4
5
(b)


DMAP, TEA
CuCl, PMDETA
85 °C, 3 h
CF3COOH
EDC, NHS
RT, 16 hr


DMF/H2O 24 hr, 60
MeOH 10 hr, 60 °C
EDC, NHS, RT, 16 hr


MeOH 24 hr, 60 °C
MeOH 10 hr, 60 °C
MeOH 18 hr, 60 °C
EDC, NHS, RT, 16 hr