Polymer Chemistry
c7py00455a

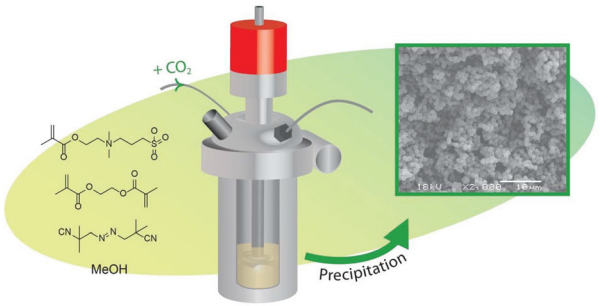
We have presented the Graphical Abstract text and image for your article below. This brief summary of your work will appear in the contents pages of the issue in which your article appears.

1

One-pot synthesis of micron-sized polybetaine particles; innovative use of supercritical carbon dioxide

Simon P. Bassett, Natasha A. Birkin, James Jennings, Emma Chapman, Rachel K. O'Reilly, Steven M. Howdle and Helen Willcock*

Well defined micron-sized polybetaine particles have been synthesised for the first time by precipitation polymerisation in a $scCO_2$/methanol mixture.



Please check this proof carefully. **Our staff will not read it in detail after you have returned it.**

Proof corrections must be returned as a single set of corrections, approved by all co-authors. No further corrections can be made after you have submitted your proof corrections as we will publish your article online as soon as possible after they are received.

Please ensure that:

- The spelling and format of all author names and affiliations are checked carefully. Names will be indexed and cited as shown on the proof, so these must be correct.
- Any funding bodies have been acknowledged appropriately.
- All of the editor's queries are answered.
- Any necessary attachments, such as updated images or ESI files, are provided.

Translation errors between word-processor files and typesetting systems can occur so the whole proof needs to be read. Please pay particular attention to: tables; equations; numerical data; figures and graphics; and references.

Please send your corrections preferably as a copy of the proof PDF with electronic notes attached or alternatively as a list of corrections – do not change the text within the PDF file or send a revised manuscript. Corrections at this stage should be minor and not involve extensive changes.

Please return your **final** corrections, where possible within **48 hours** of receipt, by e-mail to: polymers@rsc.org. If you require more time, please notify us by email.

Funder information

Providing accurate funding information will enable us to help you comply with your funders' reporting mandates. Clear acknowledgement of funder support is an important consideration in funding evaluation and can increase your chances of securing funding in the future. We work closely with Crossref to make your research discoverable through the Funding Data search tool (http://search.crossref.org/fundref).

Further information on how to acknowledge your funders can be found on our webpage (http://rsc.li/ funding-info).

What is Funding Data?

Funding Data (http://www.crossref.org/fundingdata/) provides a reliable way to track the impact of the work that funders support. We collect funding information from our authors and match this information to funders listed in the Open Funder Registry. Once an article has been matched to its funders, it is discoverable through Crossref's search interface.

PubMed Central

Accurate funder information will also help us identify articles that are mandated to be deposited in PubMed Central (PMC) and deposit these on your behalf.

Providing funder information

We have included the funder information you gave us on submission in the table below. The 'Funder name' shown and their associated 'Funder ID' number is written as listed in the Open Funder Registry. **Please check that the information in the table is correct.** The funder information should match your acknowledgements. This table will not be included in your final PDF but we will share the data with Crossref so that your article can be found via the Funding Data search tool.

Funder name	Funder ID	Award/grant/contract number
H2020 European Research Council	100010663	615142

If a funding organisation you included on submission of your article is not currently listed in the registry it will not appear in the table above. We can only deposit data if funders are already listed in the Open Funder Registry, but we will pass all funding information on to Crossref so that additional funders can be included in future.

Researcher information

If any authors have ORCID or ResearcherID details that are not listed below, please provide these with your proof corrections. Please check that the ORCID and ResearcherID details listed below have been assigned to the correct author. Please use this space to add your own unique ORCID iDs and not another researcher's, as errors will delay publication.

Please also update your account on our online manuscript submission system to add your ORCID details, which will then be automatically included in all future submissions. See here for step-by-step instructions and more information on author identifiers.

First (given) name(s)	Last (family) name(s)	ResearcherID	ORCID
Simon P.	Bassett		
Natasha A.	Birkin		
James	Jennings		
Emma	Chapman		
Rachel K.	O'Reilly		0000-0002-1043-7172
Steven M.	Howdle		0000-0001-5901-8342
Helen	Willcock		0000-0002-4316-1993

Queries for the attention of the authors

Journal: **Polymer Chemistry** Paper: **c7py00455a**

Title: **One-pot synthesis of micron-sized polybetaine particles; innovative use of supercritical carbon dioxide**

For your information: You can cite this article before you receive notification of the page numbers by using the following format: (authors), Polym. Chem., (year), DOI: 10.1039/c7py00455a.

Editor's queries are marked like this [Q1, Q2, ...], and for your convenience line numbers are indicated like this [5, 10, 15, ...].

Please ensure that all queries are answered when returning your proof corrections so that publication of your article is not delayed.

Query Reference	Query	Remarks
Q1	Please carefully check the spelling of all author names. This is important for the correct indexing and future citation of your article. No late corrections can be made.	
Q2	Text has been provided for footnote *a* in Tables 1 and 2, but there does not appear to be a corresponding citation in the tables. Please indicate a suitable location for the footnote citation.	

Polymer Chemistry

PAPER

One-pot synthesis of micron-sized polybetaine particles; innovative use of supercritical carbon dioxide†

Cite this: DOI: 10.1039/c7py00455a

Simon P. Bassett,[a] Natasha A. Birkin,[a] James Jennings,[a] Emma Chapman,[b] Rachel K. O'Reilly,[c] Steven M. Howdle[a] and Helen Willcock*[d]

Received 16th March 2017,
Accepted 2nd July 2017

DOI: 10.1039/c7py00455a

rsc.li/polymers

Polybetaines exhibit unique properties including anti-polyelectrolyte and low protein fouling behaviour, as well as biocompatibility. We recently presented the synthesis of *ca.* 20 nm polybetaine particles by aqueous RAFT polymerisation, but the synthesis of larger particles proved to be extremely challenging with standard emulsion and dispersion techniques being unsuccessful. Here we present the first reported synthesis of micron-sized, discrete cross-linked polybetaine particles, using polymerisation in $scCO_2$ with methanol as a co-solvent. Discrete particles are produced only when the methanol is efficiently removed *in situ* using $scCO_2$ extraction. A relatively high crosslinking agent initial concentration (10 wt%) was found to result in the most well defined particles, and particle integrity reduced as the crosslinking agent initial concentration was decreased. A monomer loading of between 3.0×10^{-2} mol L^{-1} and 1.8×10^{-1} mol L^{-1} resulted in discrete micron sized particles, with significant agglomoration occuring as the monomer loading was increased further. A spherical morphology and extremely low size dispersity is observed by SEM analysis for the optimised particles. The particles are readily re-dispersed in aqueous solution and light scattering measurements confirm their low size dispersity.

Introduction

Polybetaines have found a wide range of commercial uses in recent years, from viscosifying agents in the formulation of cosmetics, to anti-fouling agents for biosensors.[1] There are several detailed reviews covering the breadth of techniques used for their synthesis as well as their applications.[2,3] The McCormick group in particular have reported extensively on the synthesis and applications of polybetaines, mainly from acrylamido based monomers and have published numerous high quality publications in this area.[4–6]

There are several examples of the use of polybetaines for both non-fouling coatings and filtration membranes. In non-fouling surface coatings the amphiphilicity of the coatings was tuned by incorporating side groups of varying hydrophobicities, with a combination of fluorinated side groups and zwitterionic moieties resulting in efficient non-fouling surfaces, whereas hydrophobic groups alone caused significant protein adsorption.[7] When used in membranes, incorporation of the polybetaines reduces the protein adsorption of these materials as well as increasing their water permeability.[8]

Since the first reported synthetic polybetaines in the 1950s, which were made using conventional free radical techniques,[9] there have been various reports on their synthesis using polymerisation techniques from conventional free radical polymerisation[10] to single electron transfer living radical polymerisation (SET LRP)[11] and more recently reversible addition fragmentation chain transfer (RAFT) polymerisation.[12] These advances in synthetic techniques have allowed for the development of block co- and ter-polymers, with ever expanding complexity of architecture. However, the synthesis of discrete particles of polybetaines has been somewhat limited by their complex solubility characteristics. Polybetaines are in general only soluble in very polar solvents such as water and fluorinated alcohols, though the monomers can also be solubilised in methanol and acetone.[13] The polymers display antipolyelectrolyte behaviour in aqueous solution, becoming more soluble upon the addition of salts.[14] Moreover, the highly charged nature of these polymers results in increased inter-

[a]School of Chemistry, University of Nottingham, University Park, Nottingham, NG7 2RD, UK. E-mail: steve.howdle@nottingham.ac.uk; Fax: +44 (0) 115 846 8459; Tel: +44 (0) 115 9513486
[b]BP Exploration Operating Company Ltd., Chertsey Road, Sunbury-on-Thames, Middlesex, TW16 7BP, UK
[c]Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK. E-mail: rachel.oreilly@warwick.ac.uk; Fax: +44 (0) 2476 524112
[d]Department of Materials, Loughborough University, LE11 3TU, UK. E-mail: H.Willcock2@lboro.ac.uk; Tel: +44 (0) 1509 223432

† Electronic supplementary information (ESI) available: Additional SEM images, details of alternative synthesis methods. See DOI: 10.1039/c7py00455a

actions between the polymer chains, and this phenomenon leads them to have an upper critical solution temperature (UCST) in water, becoming more soluble with increasing temperature.[15] It also results in an increased likelihood of aggregation of polymers with increasing molecular weight, limiting the possibility of incorporating high molecular weight polybetaines into discrete particles.

There have been very few reports detailing the incorporation of betaine monomers into large particles (*ca.* >100 nm). In 2008 Das *et al*. reported the synthesis of copolymer particles made from the temperature responsive poly(*N*-isopropylacrylamide) (pNIPAAm) and the sulfobetaine poly(3-dimethyl (methacryloyloxyethyl)ammonium propane sulfonate) (pDMAPS), in which the DMAPS was used in an attempt to infer antipolyelectrolyte behaviour to the pNIPAAm particles. They report that the size of the microgels increased with increasing DMAPS content, but incorporation of more than 7.3 wt% DMAPS resulted in precipitation of the particles.[16] The synthesis of copolymer particles of vinyl acetate (VAc) and DMAPS for use in drug delivery matrices was reported by Kostova *et al*., using an emulsifier free emulsion polymerisation. Particles of ~250 nm with 20 mol% DMAPS were formed. The amphiphilic DMAPS is thought to act as an emulsifier for the hydrophobic VAc, adsorbing onto the surface of the droplets during polymerisation. However, no detailed analysis on the particle morphology or size dispersity was reported.[17] Membranes containing betaine copolymer colloid particles (synthesised from hydroxyethyl acrylate (HEA) and DMAPS) have been reported to display tuneable selectivity, improved antifouling properties and reduced phase separation (and therefore higher membrane stability) when compared to membranes containing inorganic nanoparticles, highlighting the benefits of using such polybetaine colloids.[18] Again, little characterisation data for the particles was given in this case.

Supercritical carbon dioxide ($scCO_2$) has attracted significant attention as an alternative "green" reaction medium for polymerisations owing to its readily accessible critical point (T_c = 31.1 °C and p_c = 7.38 MPa), its unique combination of liquid-like density and gas-like diffusivity and the fact that it is inert to radical reactions. Whilst monomers are generally soluble, polymers tend to be insoluble, making $scCO_2$ an ideal solvent for performing heterogeneous polymerisations.[19] There are several well-established routes for producing particles using supercritical carbon dioxide, either as a reaction solvent or *via* a range of polymer processing methods.[20] Dispersion polymerisation in $scCO_2$ has been used for the synthesis of well-defined block copolymers,[21] and is also generally the method employed to produce well-defined spherical particles; their formation often aided by the addition of a polymeric stabiliser and allowing production of particles in the size range of 0.1–15 μm.[22] Another common approach is to use precipitation polymerisation in $scCO_2$. For example, acrylic acid (AA) has been polymerised in both batch[23] and semi-continuous[24] systems, to prepare polymer particles. Generally, the particles produced in these systems are irregular and often highly agglomerated, with reaction temperature an important factor in determining particulate morphology, especially since the $scCO_2$ can plasticise and significantly lower the polymer T_g. For example, in $scCO_2$ the T_g of PAA is depressed to *ca*. 75 °C and it was observed that working above this temperature yielded agglomerated morphologies whereas more discrete primary particles were produced below this. Partially neutralised AA (*i.e.* a mixture of the acid and sodium salt) has been polymerised in a suspension process, using a water/CO_2 solvent mixture. Water was required because sodium acrylate is insoluble in both CO_2 and AA, and a PDMS-*b*-PEO stabiliser was used to prevent particle coagulation.[25]

The addition of a cross-linker can have a significant influence on the particle morphology in heterogeneous polymerisations, as the initial particle nucleation and growth, and overall colloidal stability are very sensitive to cross-linker addition.[26] A major advantage of $scCO_2$ here is that its low viscosity and high diffusivity swells polymers, improving monomer and cross-linker diffusion into the particles. Cooper first demonstrated success with divinyl benzene (DVB) and ethylvinylbenzene (EVB) in $scCO_2$.[27,28] Commercial grades of DVB/EVB were polymerised to form well-defined cross-linked spherical particles in $scCO_2$ to high yields (90%), both with and without a fluorinated stabiliser. The surprising observation of spherical particles without stabiliser was rationalised by formation of rigid cross-linked surfaces which were unable to aggregate when collisions occurred. Similar systems of DVB polymerisation in $scCO_2$ have since been studied, with different surfactants,[29] controlled using RAFT polymerisation,[30] and using acetone as a co-solvent.[31] Interestingly, in pure $scCO_2$ the particles were highly agglomerated, but with increasing acetone concentration the particles became more discrete and also more uniform in size (around 2 μm). This was attributed to the enhanced solubility of the initial oligomers that would otherwise precipitate out in the pure $scCO_2$ system, thus demonstrating that in some cases a co-solvent may be required to aid solubility and subsequent particle formation.

Thermoresponsive cross-linked pNIPAAm particles have been synthesised by several groups using $scCO_2$ precipitation polymerisation. The first report by Temtem *et al.* used *N*,*N*-methylenebisacrylamide (MBAc) as the cross-linker up to 4.5 wt%.[32] As shown earlier by Cooper,[27,28] higher cross-linker concentrations led to more discrete particles, with the rigid surfaces apparently overcoming agglomeration. Others have also shown very high cross-linker concentrations to be advantageous in precipitation polymerisations of PNIPAAm. For example, Cao *et al.*[33] used MBAM at concentrations up to 20 wt%, and Hu and co-workers utilised ethylene glycol dimethacrylate (EGDMA) at 26.4 wt%.[34]

We have previously reported the synthesis of small *ca.* 20 nm particles of the polysulfobetaine pDMAPS and copolymers with polyethylene glycol methacrylate (PEGMA) by RAFT polymerisation directly in aqueous solution. These branched polymers were shown to be discrete, well defined particles, which could be readily dispersed in aqueous solution, showing high salt tolerance and significantly lower upper

critical solution temperature (UCST) cloud points compared to their linear counterparts.[35] We have also shown that pDMAPS can be incorporated into micellar structures with controlled disassembly[36] and swelling.[37] However, except for the example of carboxybetaine particles (~100 nm) by Jiang *et al.*, made in an inverse emulsion system, the synthesis of larger (>100 nm) well-defined polybetaine particles has not been widely reported. The method used by Jiang requires very low monomer concentration (115 mg in 20.5 mL of solution) thus limiting its commercial scalability, and the redispersion behaviour of the particles is not described in detail.[38,39]

Here we describe the simple, one-pot synthesis of well-defined polybetaine particles on the micron scale. We demonstrate how the unique solvent properties of $scCO_2$ and use of a cosolvent can overcome process limitations and provide a new route to access cross-linked pDMAPS particles in a larger size regime than has previously been reported. Such materials may find applications as stabilisers, delivery vehicles or in non-protein fouling membranes and surface coatings.

Experimental

Materials

2,2′-Azobis(isobutryonitrile) (AIBN, Sigma-Aldrich, 97%) was used as initiator and purified by recrystallization from methanol prior to use. 3-Dimethyl(methacryloyloxyethyl) ammonium propane sulfonate (DMAPS, Sigma-Aldrich, 97%), ethylene glycol dimethacrylate (EGDMA, Sigma-Aldrich, 98%), polyethylene glycol dimethacrylate M_n 330 (pEGDMA, Sigma-Aldrich), methylene bisacrylamide (MBAc, Sigma Aldrich, 99%), sodium dodecyl sulfate (SDS, Sigma Aldrich, 98%) methanol (VWR, reagent grade) and dry CO_2 (BOC Gases, 99.99%) were used as received.

Equipment

Mastersizer. A Malvern Mastersizer 2000 with a Hydro 2000S accessory, using full power agitation and sonication was used to obtain particle size in solution.

SEM. A Zeiss Supra55VP was used to acquire the SEM images, operated at an accelerating voltage of 5 kV. The samples were prepared by drop deposition on glass (followed by sputter coating with gold).

Synthetic procedures

General procedure for aqueous inverse emulsion polymerisation. Surfactant, DMAPS monomer, polyethylene glycol dimethacrylate (pEGDMA – M_n 330) and initiator (for amounts see ESI – Table S1†) were dissolved by stirring into the aqueous phase. The oil phase was added to this and the mixture was sonicated in an ice bath for 10 minutes. The resultant emulsion was purged with nitrogen for 30 minutes and heated in an oil bath with stirring (600 rpm) at 65 °C for 16 hours.

General procedure for aqueous dispersion polymerisation. Surfactant, DMAPS monomer, MBAc and initiator (for amounts see ESI – Table S3†) were dissolved in water (in the order listed) by stirring. The mixture was purged with nitrogen for 30 minutes and heated in an oil bath with stirring (600 rpm) at 65 °C for 16 hours. High conversion (>90%) was confirmed by ^{1}H NMR spectroscopy (remaining monomer concentration was compared to that of a standard – dimethylformamide (DMF)).

General procedure for solubility measurements in high pressure view cell. Solubility measurements of monomer in $scCO_2$ were visually determined using a 100 mL static volume view cell equipped with two sapphire windows[40] and an overhead stirrer. A known amount of DMAPS was added into the view cell body, and CO_2 was pumped in until a pressure of 5 MPa was reached. The vessel was then heated to the reaction temperature of 65 °C, and the pressure increased to 27.6 MPa through further addition of CO_2. This was repeated with the addition of methanol, 2,2′-azobis(2-methylpropionitrile) (AIBN) and EGDMA, maintaining concentrations equal to those used during the synthesis in the 60 mL vessels.

General procedure for precipitation polymerisation in $scCO_2$. Polymerisations were performed in a 60 mL autoclave equipped with a magnetically driven overhead stirrer (maximum operating temperature 150 °C, maximum operating pressure 30.0 MPa).[41] DMAPS monomer (0.5 g) and methanol (4.5 mL) were separately degassed by purging with argon for 15 minutes. AIBN (0.025 g, 5 wt% with respect to monomer) and EGDMA (47.5 μL, 10 wt% with respect to monomer) were introduced into the autoclave and oxygen removed by purging the vessel with CO_2 at 0.2 MPa for 15 minutes. DMAPS monomer was dissolved in methanol and transferred into the autoclave, which was sealed and the pressure raised to 5 MPa through CO_2 addition. The vessel was then heated to 65 °C, and the pressure raised to 27.6 MPa through further CO_2 addition. The polymerisations were conducted for 2 hours. Upon completion, the autoclave was cooled to 45 °C, and CO_2 flowed through the vessel at 27.6 MPa for 30 minutes to remove the methanol (no filter used on exit line). Finally, the vessel was cooled to ambient temperature before being vented slowly. The product was recovered as a white, free-flowing powder.

General procedure for the redispersion of particles in water. PDMAPs synthesised by $scCO_2$ precipitation polymerisation (75 mg) was added to stirred DI water (50 mL) with SDS (75 mg) if required. Three cycles of stirring (500 rpm) and sonication (15 minutes each) were performed to achieve a cloudy dispersion. These were kept stirring to avoid sedimentation.

General procedure for the casting of films. One drop of PDMAPS dispersed in water was added to a glass slide and allowed to dry in ambient conditions.

Results and discussion

Attempted synthesis using traditional techniques

Firstly, two conventional methods – inverse emulsion polymerisation and dispersion polymerisation – were used in an

attempt to synthesise large (*ca.* >100 nm) PDMAPS particles. A range of inverse emulsion polymerisation conditions were explored and in all cases resulted in destabilisation or reversal of the inverse emulsion (see ESI – Fig. S2† top left). Polybetaines are highly soluble in aqueous salt solutions, and their temperature responsive behaviour has been shown to be both molecular weight and concentration dependent.[14] However, they can also be swollen by organic solvents due to their hydrophobic backbone (see ESI – Fig. S2† top right). Whilst this complicated amphiphilic behaviour allows polybetaines to find use as stabilisers of polymer colloids[42] and nano-objects in polymerisation induced self-assembly (PISA),[43] it also means that they do not reside within a single phase of the inverse emulsion systems, causes extensive aggregation occurring during the attempted dispersion polymerisation, and therefore ill-defined particles (see ESI – Fig. S2† bottom). Because the standard synthesis attempts were unsuccessful, a new route was sought for the synthesis of PDMAPS particles.

Synthesis in supercritical carbon dioxide

Whilst precipitation polymerisations in $scCO_2$ have proven effective in the production of cross-linked microparticles of other monomers, the synthesis of polybetaine particles using $scCO_2$ as a solvent has not previously been investigated. Initial attempts to polymerise DMAPS in pure $scCO_2$ failed, as the DMAPS was found to be completely insoluble at the conditions tested (up to 65 °C, 27.6 MPa, Fig. 1). Adamsky and Beckman showed that another poorly $scCO_2$ soluble monomer (acrylamide) could be polymerised in a $scCO_2$/water inverse-emulsion system.[44] We first attempted to replicate this approach for the synthesis of polybetaine particles using DMAPS and EGDMA. Polymer was certainly formed, but swelled within the reaction vessel causing blockages in the pressure release outlets. In addition, the end product after removing CO_2 was obtained as a water-swollen gel with no evidence of particle morphology (see ESI – Fig. S4†).

As DMAPS is known to exhibit solubility in highly polar solvents such as acetone, methanol, water and fluorinated alcohols,[13] we therefore introduced methanol as a $scCO_2$ miscible co-solvent to aid dissolution. CO_2/methanol mixtures have been studied in the literature by a number of groups, showing good miscibility at a range of concentrations.[40,45] The phase behaviour studies of DMAPS, methanol and the DMAPS/methanol mixture (Fig. 1) shows that DMAPS is insoluble in $scCO_2$ (a) and methanol is completely miscible at the reaction conditions of 65 °C and 27.6 MPa (b). A mixture of 3.33 g DMAPS with 7.5 mL methanol in the 100 mL volume view cell reactor at 65 °C and 27.6 MPa (c) results in a two phase system, but it was clear that a significant portion of the DMAPS is dissolved in the CO_2-rich upper phase. Ideally a single phase system is required for an efficient precipitation polymerisation, but our experiment demonstrated that to achieve this would require a very low DMAPS concentration (too low to allow effective polymerisation) and a much higher pressure. Thus, experiments were performed in the two phase regime at 65 °C and 27.6 MPa.

Having established these parameters we repeated the experiment in the 60 mL volume reaction autoclave, and reactant amounts were scaled down from the 100 mL view cell, to 1 g DMAPS and 9 mL methanol. Initiator AIBN (5 wt% wrt DMAPS), which has good solubility in $scCO_2$, and cross-linker EGDMA (10 wt% wrt DMAPS) were also added to the autoclave. AIBN was used at a relatively high loading to compensate for its slower decomposition in $scCO_2$ compared to conventional solvents, so to achieve high crosslink densities.[46] After 2 hours of polymerisation at 65 °C and 27.6 MPa the autoclave was cooled to room temperature and the CO_2 released. The product was obtained as a methanol soaked wet solid. The methanol could then be removed *in vacuo*, to give a high yield (87%) of powdered product, but further inspection of the polymer using SEM (Fig. 2) showed the majority of the sample consisted of highly agglomerated particles. However, there were small regions of the sample where discrete spherical microparticles were evident. These observations strongly suggest that particles were formed, but after venting the $scCO_2$, the residual methanol caused agglomeration. The

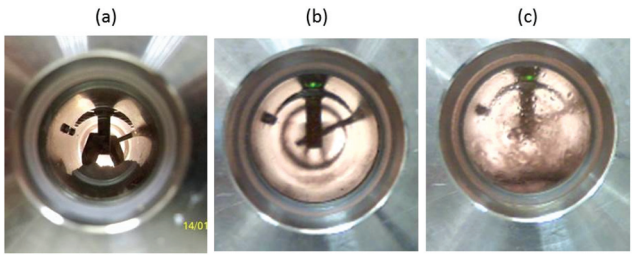


Fig. 1 View cell images of reactants at reaction conditions of 65 °C and 27.6 MPa CO_2. (a) Solid DMAPS monomer is not soluble in $scCO_2$, (b) methanol and $scCO_2$ are miscible (c) DMAPS/methanol mixture – a small lower phase containing some DMAPS is clearly visible.

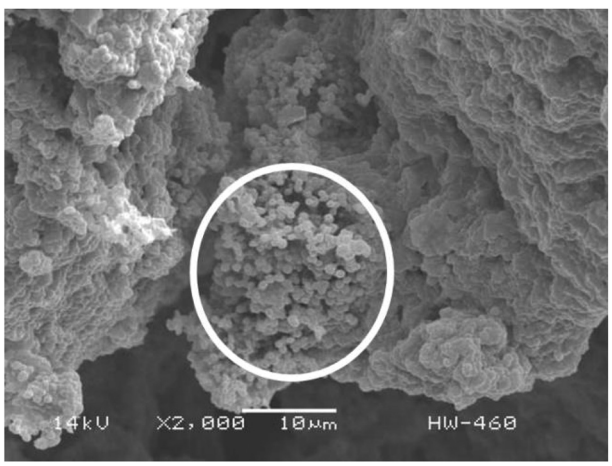


Fig. 2 SEM image of the reaction performed with DMAPS (1 g), AIBN (50 mg, 5 wt% with respect to monomer) and 9 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h. Methanol was removed *in vacuo* post-reaction after venting and removal of reaction mixture from the autoclave. Spherical particles highlighted.

methanol penetrates into the cross-linked network, swells the polymer aided by the $scCO_2$, and leads to agglomeration of the particles as $scCO_2$ is vented (or immediately after venting) and the particles "collapse".

Supercritical fluid extraction. In order to prevent the softening, swelling and subsequent agglomeration of the particles, it is necessary to remove the MeOH quickly from the reaction system. We realised that $scCO_2$ extraction (a step typically used to remove residual monomer post-reaction)[24,47] could be employed immediately after the end of the polymerisation reaction to flush the methanol before depressurisation. Through optimisation, we found that lowering the temperature to 45 °C (and maintaining constant pressure of 27.6 MPa) before the extraction, which increased the CO_2 density, allowed enhanced removal. This methodology proved highly effective, and products were obtained as free flowing white powders with yields typically above 80% when using an extraction time of 30 minutes. Any residual monomer was also flushed out of the reaction mixture due to its high solubility in MeOH. ^{1}H NMR spectroscopy of the products after flushing showed no evidence of residual monomer. The SEM image (Fig. 3 top left) shows well-defined spherical particles, around 1 μm in size, throughout the sample, with minimal agglomeration. These particles were readily redispersed in water (1.5 mg mL^{-1}), with and without SDS (1.5 mg mL^{-1}) as a stabiliser, by repeated cycles of stirring and sonication to form cloudy solutions (Fig. 3 top right). Resettling does occur over time (periods of hours to days); however this can be avoided by stirring or agitation of the dispersions. Light scattering analysis of the dispersed samples obtained with a Mastersizer instrument (using both stirring and sonication) reveals their low size dispersity and the narrow particle size distribution (PSD, Fig. 3 bottom) highlights the lack of aggregation.

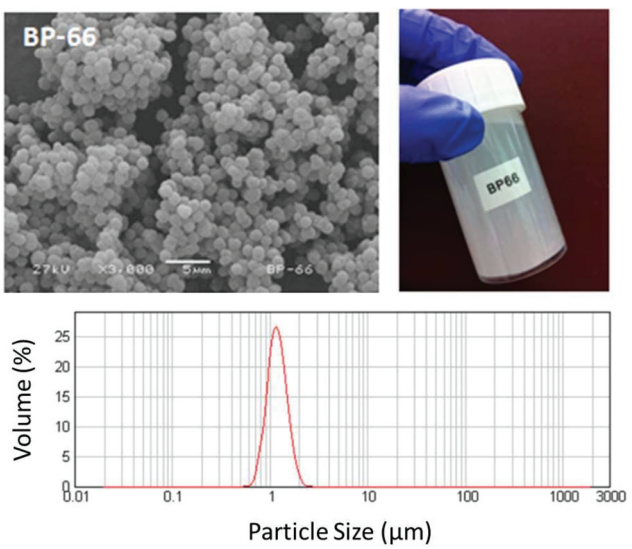


Fig. 3 SEM image (top left), photograph of dispersion in water (1.5 mg mL^{-1} with SDS 1.5 mg mL^{-1} – top right) and narrow PSD of a representative sample (d(0.5)volume = 1.17 μm. bottom) (sample produced under same conditions as **Sample 6** – Table 1).

Cross-linker concentration. It has been reported that in precipitation polymerisations in $scCO_2$, the cross-linking density is vital to the successful formation of discrete particles, with crosslinking agent initial concentrations up to 20 wt% often required.[32] In precipitation polymerisations, all reactants should be soluble at the beginning of the reaction, with the polymer chains precipitating once they reach a critical molecular weight. Usually these particles would agglomerate, but through the addition of a crosslinking agent the particle surfaces become harder and if collisions occur, agglomeration is prevented. The effect of increasing the crosslinking agent initial concentration is shown in Table 1, with associated SEM images in Fig. 4. A clear trend is seen, with low crosslinking agent initial concentrations (below 5 wt%) leading to highly agglomerated morphologies. As crosslinking agent initial concentration increases, the particles become more discrete, until at 10 wt% the SEM image shows very uniform micron-sized particles (Fig. 4). When redispersed in water particles with lower than 5 wt% crosslinking agent initial concentration formed clear solutions, and particle sizes were not able to be measured by light scattering (see ESI Fig. S5†). The crosslinking agent chemistry was also varied; when using a short chain hydrophilic crosslinking agent (MBAc) – well-defined spherical particles were obtained, however when using a long chain hydrophilic crosslinking agent (pEGDMA), poorly-defined particles with a high degree of agglomeration were observed (see ESI – Fig. S6†). When redispersed in water these poorly-defined particles formed swollen gel-like masses rather than cloudy solutions, and particle sizes were again not able to be measured by light scattering.

Table 1 Effect of varying the concentration of cross-linker in the polymerisation of DMAPS in $scCO_2$ Q2

Sample	MeOH/ mL	EGDMA[b]/ wt%	Obtained yield[c]/%	Morphology and particle size[d]/μm
1	4.5	0.0	64	Highly agglomerated microparticles
2	9	0.5	85	Highly agglomerated microparticles
3	9	1.0	90	Highly agglomerated microparticles
4	9	2.5	87	Agglomerated microparticles
5	9	5.0	86	Agglomerated microparticles
6	9	10.0	84	0.92

[a] Reactions performed with DMAPS (1 g), AIBN (50 mg, 5 wt% with respect to monomer) and 9 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h, followed by supercritical fluid extraction of methanol at 45 °C and 27.6 MPa. [b] Cross-linker concentration with respect to monomer. [c] Yield determined gravimetrically after drying *in vacuo*. [d] Determined by SEM, average particle sized based on measurement of 100 microparticles. n.b. It was noted that the efficiency of the removal of methanol was variable, and residual amounts often remained in the polymer. For this reason the amount of methanol was reduced from 9 to 4.5 mL for the remaining reactions, which did not affect the phase behaviour significantly, but provided the benefit of easier removal post reaction.

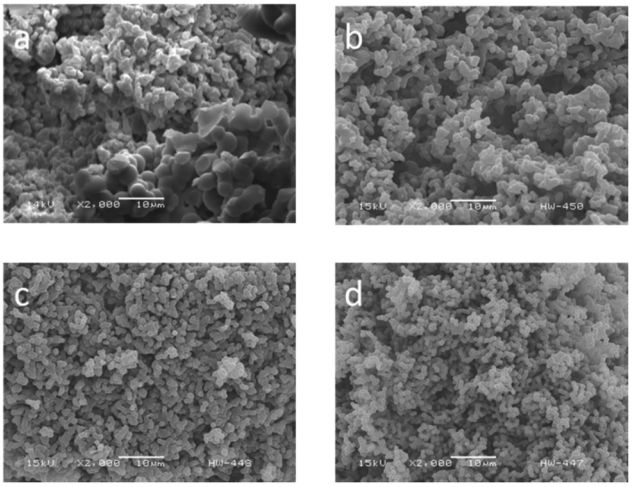


Fig. 4 SEM images showing the effect of varying the amount of cross-linker for the polymerisation of DMAPS in scCO2. (a) **Sample 1** – 0 wt%, (b) **Sample 2** – 0.5 wt%, (c) **Sample 5** – 5 wt%, (d) **Sample 6** – 10 wt% (see Table 1 for details of each experiment).

Initiator concentration. Another variable affecting the cross-linking efficiency and particle formation of the polymers is the initiator concentration; for the previous examples 5 wt% AIBN was used. Variations between 1–10 wt% showed that lower concentrations were less successful, while 5 wt% was optimal for achieving discrete crosslinked particles (see ESI – Table S7 and Fig. S8†). The crosslinking must occur rapidly, as once the particles precipitate they must be hard enough to not agglomerate during collisions. At low AIBN concentrations, the radical concentration will be lower, thus resulting in a slower reaction rate, meaning the particles may be softer and more likely to agglomerate. At higher than 10 wt% AIBN, the reaction will proceed too quickly, potentially consuming the EGDMA too early in the reaction; therefore not all the particles will be evenly crosslinked, again leading to agglomeration. Hence, for the remaining polymerisations, 5 wt% AIBN was used, combined with an EGDMA initial concentration of 10 wt%.

Monomer loading. Whilst the view cell experiments showed evidence of two phases at all monomer loadings, to probe the effect of this on the polymerisation, a range of loadings were studied at constant methanol concentrations (Table 2). Highly agglomerated particles were obtained at low monomer concentrations; too low for efficient cross-linking/polymerisation to occur quickly enough. Micron-sized particles were obtained at loadings between 3.0×10^{-2} mol L^{-1} and 1.8×10^{-1} mol L^{-1} monomer (**Sample 8** to **Sample 10**), with little difference in the particle sizes between samples (Fig. 5). Above this loading, the products began to agglomerate. In this case, the polymer will precipitate at lower conversions/reaction durations, and the cross-linking may not fully occur prior to this. Another issue was that the removal of methanol became more difficult at the higher monomer loadings, as the cross-linked networks retained more of the co-solvent. This is evident from **Sample 12**, where the obtained yield was calculated to be 112% and the product appeared wet and clumped together, likely due to residual methanol.

Upon attempted redispersion, the agglomerated particles formed cloudy solutions that displayed wide PSDs as measured by LS (see Fig. S9†).

Casting of films. The monodisperse nature of the discrete particles allows the simple casting of uniform films (see ESI – Fig. S10†), giving them the potential to be used as antifouling coatings with unique nanostructured surfaces.

Table 2 Effect of varying the monomer concentration on the polymerisation of DMAPS in $scCO_2$/MeOH

Sample	Mass monomer/g	[Monomer]/ mol L^{-1}	Yield[b]/%	Morphology and particle size[c]/μm
7	0.1	6.0×10^{-3}	N/A	Highly agglomerated microparticles
8	0.5	3.0×10^{-2}	85	1.05
9[d]	1.0	6.0×10^{-2}	84	0.92
10	2.0	1.2×10^{-1}	84	0.94
11	3.0	1.8×10^{-1}	90	Agglomerated microparticles
12	4.5	2.7×10^{-1}	112	Agglomerated microparticles

[a] Reactions performed with DMAPS, AIBN (5 wt% with respect to monomer), EGDMA (10 wt% with respect to monomer), 4.5 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h, followed by supercritical fluid extraction of methanol at 45 °C and 27.6 MPa. [b] Yield determined gravimetrically after drying *in vacuo*. [c] Determined by SEM, average particle size based on measurement of 100 particles. [d] 4.5 mL methanol used except for **Sample 9** (9 mL used).

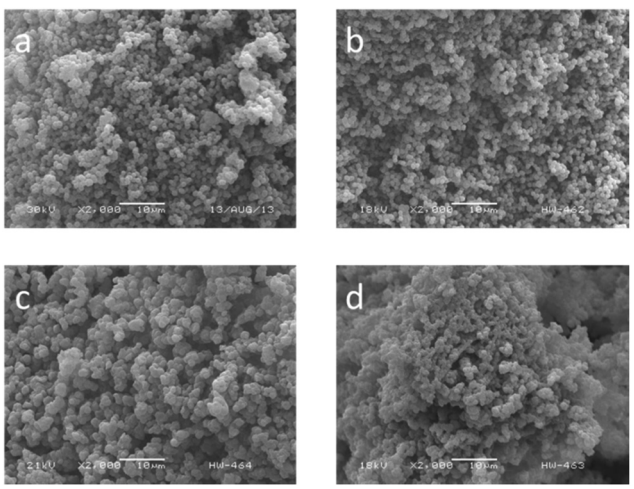


Fig. 5 SEM images showing the effect of varying the concentration of DMAPS (60 mL autoclave with 4.5 mL of methanol as co-solvent) (a) 8 (3.0×10^{-2} mol L^{-1}), (b) 10 (1.2×10^{-1} mol L^{-1}), (c) 11 (1.8×10^{-1} mol L^{-1}), (d) 12 (2.7×10^{-1} mol L^{-1}).

Conclusions

We present here the first example of the synthesis of micron-sized, discrete, cross-linked particles made entirely from poly-

betaines. The particles were synthesised by $scCO_2$ precipitation polymerisation using methanol as a co-solvent.

Observations using a view cell revealed that the polymerisation does take place in a two-phase system, and minimising the second phase results in well-defined spherical particles. Variation of the initiator and monomer concentration, as well as crosslinking agent initial concentration, revealed the optimum conditions for the particle synthesis. Monomer concentrations less than 3.0×10^{-2} mol L^{-1} result in poorly defined particles, whereas greater than 1.8×10^{-1} mol L^{-1} cause the methanol removal to be inefficient leading to aggregation. Crosslinking agent initial concentrations below 5 wt% result in ill-defined particles that swell in water to form gel-like masses rather than dispersing as discrete particles, whereas at 10 wt% well-defined spherical particles of ~1 micron in size can be observed by dry state SEM and light scattering in aqueous solution. *In situ* removal of the methanol by $scCO_2$ extraction was shown to be an important step, and whilst there is clearly opportunity for further optimisation, this method could be used for the industrially scalable synthesis of well-defined polybetaine particles. This opens up countless possibilities for their use and applications, which were previously unachievable by standard emulsion and dispersion techniques due to the complex amphiphilic behaviour of polybetaines.

Acknowledgements

HW was funded by BP Exploration Operating Company Ltd, and this collaboration was started using the University of Warwick Enterprise and Entrepreneurship fund. ROR holds an ERC consolidator grant (#615142). SPB, NAB, JJ and SMH are indebted to the very high quality technical and high pressure safety support at Nottingham from Peter Fields, Richard Wilson and Martin Dellar.

References

1 Z. Zhang, S. Chen and S. Jiang, *Biomacromolecules*, 2006, **7**, 3311–3315.
2 A. B. Lowe and C. L. McCormick, *Chem. Rev.*, 2002, **102**, 4177–4189.
3 N. Tarannum and M. Singh, *Rev. Adv. Sci. Eng.*, 2013, **2**, 90–111.
4 C. L. McCormick and L. C. Salazar, *Polymer*, 1992, **33**, 4617–4624.
5 K. M. Johnson, M. J. Fevola and C. L. McCormick, *J. Appl. Polym. Sci.*, 2004, **92**, 647–657.
6 M. S. Donovan, B. S. Sumerlin, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2002, **35**, 8663–8666.
7 S. Colak and G. N. Tew, *Biomacromolecules*, 2012, **13**, 1233–1239.
8 Y. Ji, Q. An, Q. Zhao, W. Sun, K. Lee, H. Chen and C. Gao, *J. Membr. Sci.*, 2012, **390**, 243–253.
9 R. Hart and D. Timmerman, *J. Polym. Sci.*, 1958, **28**, 638–640.
10 H. Wang, T. Hirano, M. Seno and T. Sato, *Eur. Polym. J.*, 2003, **39**, 2107–2114.
11 U. Edlund, C. Rodriguez-Emmenegger, E. Brynda and A. Albersson, *Polym. Chem.*, 2012, **3**, 2920–2927.
12 C. Rodriguez-Emmenegger, B. V. K. J. Schmidt, Z. Sedlakova, V. Šubr, A. B. Alles, E. Brynda and C. Barner-Kowollik, *Macromol. Rapid Commun.*, 2011, **32**, 958–965.
13 W. F. Lee and C. C. Tsai, *Polymer*, 1994, **35**, 2210–2217.
14 D. N. Schulz, D. G. Peiffer, P. K. Agarwal, J. Larabee, J. J. Kaladas, L. Soni, B. Handwerker and R. T. Garner, *Polymer*, 1986, **27**, 1734–1742.
15 J. Seuring and S. Agarwal, *Macromol. Rapid Commun.*, 2012, **33**, 1898–1920.
16 M. Das, N. Sanson and E. Kumacheva, *Chem. Mater.*, 2008, **20**, 7157–7163.
17 E. Kamenska, B. Kostova, I. Ivanov, D. Rachev and G. Georgiev, *J. Biomater. Sci., Polym. Ed.*, 2009, **20**, 181–197.
18 Y. Ji, Q. Zhao, Q. An, L. Shao, K. Lee, Z. Xu and C. Gao, *J. Mater. Chem. A*, 2013, **1**, 12213–12220.
19 J. L. Kendall, D. A. Canelas, J. L. Young and J. M. DeSimone, *Chem. Rev.*, 1999, **99**, 543–563.
20 O. R. Davies, A. L. Lewis, M. J. Whitaker, H. Tai, K. M. Shakesheff and S. M. Howdle, *Adv. Drug Delivery Rev.*, 2008, **60**, 373–387.
21 J. Jennings, M. Beija, J. T. Kennon, H. Willcock, R. K. O'Reilly, S. Rimmer and S. M. Howdle, *Macromolecules*, 2013, **46**, 6843–6851.
22 T. D. McAllister, L. D. Farrand and S. M. Howdle, *Macromol. Chem. Phys.*, 2016, **217**, 2294–2301.
23 T. Liu, J. M. DeSimone and G. W. Roberts, *Polymer*, 2006, **47**, 4276–4281.
24 T. Liu, P. Garner and J. M. DeSimone, *Macromolecules*, 2006, **39**, 6489–6494.
25 Y. A. Hussain, T. Liu and G. W. Roberts, *Ind. Eng. Chem. Res.*, 2012, **51**, 11401–11408.
26 J. Tan, X. Rao, J. Yang and Z. Zeng, *RSC Adv.*, 2015, **5**, 18922–18931.
27 A. I. Cooper, W. P. Hems and A. B. Holmes, *Macromol. Rapid Commun.*, 1998, **19**, 353–357.
28 A. I. Cooper, W. P. Hems and A. B. Holmes, *Macromolecules*, 1999, **32**, 2156–2166.
29 P. R. Garca-Moran, G. Jaramillo-Soto, M. E. Albores-Velasco and E. Vivaldo-Lima, *Macromol. React. Eng.*, 2009, **3**, 58–70.
30 G. Jaramillo-Soto and E. Vivaldo-Lima, *Aust. J. Chem.*, 2012, **65**, 1177–1185.
31 C. S. Li, J. C. Liang, X. L. Zhu and X. Z. Kong, *Colloid Polym. Sci.*, 2010, **288**, 1571–1580.
32 M. Temtem, T. Casimiro, J. F. Mano and A. Aguiar-Ricardo, *Green Chem.*, 2007, **9**, 75–79.
33 L. Cao, L. Chen, J. Jiao, S. Zhang and W. Gao, *Colloid Polym. Sci.*, 2007, **285**, 1229–1236.

34 Y. D. Hu, L. Q. Cao, F. Xiao and J. D. Wang, *Polym. Adv. Technol.*, 2010, **21**, 386–391.
35 H. Willcock, A. Lu, C. F. Hansell, E. Chapman, I. R. Collins and R. K. O'Reilly, *Polym. Chem.*, 2014, **5**, 1023–1030.
36 K. E. B. Doncom, A. Pitto-Barry, H. Willcock, A. Lu, B. E. McKenzie, N. Kirby and R. K. O'Reilly, *Soft Matter*, 2015, **11**, 3666–3676.
37 K. E. B. Doncom, H. Willcock and R. K. O'Reilly, *Eur. Polym. J.*, 2016, **87**, 497–507.
38 G. Cheng, L. Mi, Z. Cao, H. Xue, Q. Yu, L. Carr and S. Jiang, *Langmuir*, 2010, **26**, 6883–6886.
39 L. Zhang, H. Xue, Z. Cao, A. Keefe, J. Wang and S. Jiang, *Biomaterials*, 2011, **32**, 4604–4608.
40 P. Licence, M. P. Dellar, R. G. M. Wilson, P. A. Fields, D. Litchfield, H. M. Woods, M. Poliakoff and S. M. Howdle, *Rev. Sci. Instrum.*, 2004, **75**, 3233–3236.
41 J. Jennings, S. P. Bassett, D. Hermida-Merino, G. Portale, W. Bras, L. Knight, J. J. Titman, T. Higuchi, H. Jinnai and S. M. Howdle, *Polym. Chem.*, 2016, **7**, 905–916.
42 J. Wieboldt, R. Zimehl, J. Ahrens and G. Lagaly, *Prog. Colloid Polym. Sci.*, 1998, **109**, 260–269.
43 K. Doncom, N. J. Warren and S. P. Armes, *Polym. Chem.*, 2015, **6**, 7264–7273.
44 F. A. Adamsky and E. J. Beckman, *Macromolecules*, 1994, **27**, 312–314.
45 J. Ke, R. M. Oag, P. J. King, M. W. George and M. Poliakoff, *Angew. Chem., Int. Ed.*, 2004, **43**, 5192–5195.
46 Z. Guan, J. R. Combes, Y. Z. Menceloglu and J. M. DeSimone, *Macromolecules*, 1993, **26**, 2663–2669.
47 W. X. Wang, M. R. Giles, D. Bratton, D. J. Irvine, S. P. Armes, J. V. W. Weaver and S. M. Howdle, *Polymer*, 2003, **44**, 3803–3809.