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Generation of Ultra-stable Pickering Microbubbles via Poly alkylcyanoacrylates

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Abstract

A range of solution conditions (pH, surfactant concentration and type) have been tested for the polymerization of alkyl cyanoacrylates (ethyl (ECA), butyl (BCA) and octyl (OCA)) into nanoparticles (NPs) potentially capable of stabilizing highly unstable microbubbles (MBs) of air in aqueous solutions. The optimum system was butyl cyanoacrylate (BCA) polymerized into PBCA particles at pH 4 in the presence of 1 wt.% Tyloxapol surfactant. These PBCA particles were highly effective at stabilizing MBs of only a few microns in size for at least 2 months. Microscopy over a range of length scales clearly indicated that these particles were stabilized via a Pickering mechanism. Only a relatively low volume fraction (ca. 1 vol.%) of MBs could be obtained via a single aeration step of a 0.7 wt.% dispersion of PBCA particles in a high shear mixer. Although this could be increased to 2 and 3 vol.% by second and third aerations, this reflects the difficulty of obtaining and maintaining rapid enough particle coverage of small bubbles even under turbulent conditions. Similar sizes and yields of PBCA particles could be obtained in the absence of surfactants, but these particles, with or without addition of surfactant afterwards, could not stabilize MBs. We estimate that approximately one quarter of the Tyloxapol when present during polymerization is incorporated into the particles on polymerization, which somehow imparts the correct surface hydrophobicity and contact angle to the particles at the A/W interface, making such particles so very effective as Pickering MB stabilizers.

Keywords: microbubbles, nanoparticles, Pickering stabilization, alkyl cyanoacrylates

Introduction

In the late 1960's Claude R. Joyner noticed that dye injection into patients' veins improved the contrast of ultrasound signals. Later on it was found that contrast enhancement arose from the formation of very fine bubbles (a few microns in size) at the needle tip (Kremkau et al., 1970). Since this time, microbubbles (i.e., a few µm in size or less) have become an interesting topic for medical applications but also in a wide variety of other areas, ranging from foods, coatings, cosmetics and crude oil recovery, sonochemistry (Ashokkumar et al., 2007, Brotchie et al., 2010, Wood et al., 2017), plus the fabrication of ultra-light weight microporous materials for use in various medical and engineering fields (Gonzenbach et al., 2006).

Some of these other effects of microbubbles highlight their transient nature and the origin of this, at least for gases that have reasonable solubility (e.g., air in water) is the Laplace pressure, which in turn leads to rather rapid dissolution of the bubbles, known as disproportionation. For example, in the absence of any significant interfacial barrier to bubble shrinkage, the lifetime of a 1 um sized air bubble in water at room temperature is a few ms, even though the adsorbed surfactant layer makes them perfectly stable to coalescence. Therefore, generating a stable foam of bubbles of this sort of size has been the subject of many studies over the past few years (Ettelaie and Murray, 2014, 2015). Phospholipids and polymers have been used to stabilize bubbles of very insoluble gases, (e.g.,(Abou-Saleh et al., 2013), but even these bubbles only have lifetimes of several hours (Segers et al., 2016) – although this is an essential feature for intravenous applications. For lifetimes of weeks or longer and certainly for air bubbles in aqueous systems, so far the only widely known way to prevent disproportionation is via the adsorption of nanoparticles to their surfaces, i.e., Pickering foams (by analogy with Pickering emulsions). Particles of 20 nm size upwards, if they have appropriately high contact angles – generally between ca. 20 and 90 degrees (Binks and Horozov, 2006) – can have energies of displacement from the air-water (A/W) interface of 100's to even 1000's of k_BT (Aveyard et al., 2000; Du et al., 2003; Dickinson et al., 2004), (where k_B is the Boltzmann constant and T the absolute temperature). Such particles are therefore effectively irreversibly adsorbed and so the

bubbles cannot shrink. The topic has been extensively discussed in the literature (Binks and Horozov, 2006)

Most studies of Pickering bubbles have used inorganic particles as stabilizer, partly because the effects of different shape and size can be tested but also a wide variety of chemical agents exist for modifying the particle surface chemistry and therefore contact angle (Binks and Horozov, 2005). However, such particles are not biocompatible in most instances. Furthermore, the presence of small bubbles is essential to the mouth feel and texture of many foods (e.g., ice cream, breads, cakes, etc.) and there is a definite need for improved food-grade stabilizers in many cases (Dickinson, 2010). Thus, there has been increasing interest in finding organic Pickering particles for both food products and medical applications (Lam et al., 2014, Dickinson, 2010). Alternatives include polymer microgel particles (Matsumiya and Murray, 2016), certain proteins that act almost as Janus particles like fungal (Wang et al., 2013, Burke et al., 2014) and bacterial (Schor et al., 2016) hydrophobins and other polymer particles. Note that all these particles may not be strictly considered as Pickering stabilizers in that they have some degree of flexibility/deformability, though even silica particles have been shown to sinter together on adsorption, leading to so-called armoured bubbles (Abkarian et al., 2013). Of all these possible Pickering foaming agents, one of the most effective seems to be poly butyl cyanoacrylate (PBCA) particles. These were first developed by Schering AG (Berlin) to stabilize microbubbles for imaging of human and animal tissues (Harris et al., 1995).

Alkyl cyanoacrylates are a group of monomers that react rapidly with moisture and are widely used as adhesives. Shorter chain cyanoacrylate such as ethyl cyanoacrylate are used as ‘super glue’ while longer chain lengths are mainly used as medical glues to hold together ruptured skin and other tissues (King and Kinney, 1999). Couvreur and co-workers (Couvreur et al., 1979), produced a simple technique to generate alkyl cyanoacrylate nanoparticles by dropwise addition of monomer to water in the presence of surfactant solution at pH 2 to 3. The previous techniques of polymerisation usually contained harsh chemicals and subsequently required several purification steps once they had been made. However, there are many conflicting results in the literature concerning the ideal conditions for polymerisation of alkyl cyanoacrylates to particles. In addition, as pointed out by Yordanov,

Abrashev and Dushkin (2010) the emulsion polymerization route via surfactant micelles should be distinguished from the alternative dispersion polymerization route using polymeric surfactants.

Whilst there have various studies of PBCA particles as foam stabilizing agents for medical applications (Palmowski et al., 2008, Fokong et al., 2011, Pirker et al., 1996, Mørch et al., 2015) there seems to have been no systematic investigation as to why the particles are such good foam stabilizers. Part of the confusion possibly arises from different workers using different monomers, different sources of the same monomer (Behan et al., 2001, Müller et al., 1992) with different purities, differences in pH and other polymerisation conditions. Different monomers appear to produce nanoparticles with different physiochemical properties. Douglas et al. (1984) reported that monomer concentration and pH are the main factors governing properties of PBCA nanoparticles – an increase in particle size occurring as the pH of polymerization was raised. Schmidt and Roessling (2006) established a two step technique to produce microbubbles stabilized by PBCA nanoparticles. The first step involved polymerisation of monomer in an aqueous phase at low pH in the presence of Triton X-100 as surfactant. They studied the effect of temperature, monomer concentration and stirring speed on microbubble formation and size and by optimizing these conditions reported nanoparticle yields of up to 97%. Increasing monomer concentration led to increasing nanoparticle (NP) size, whilst increasing the aeration time gave higher bubble volume fractions and higher stirring speeds gave smaller microbubbles. Temperature had little effect. Despite these previous studies it is still not clear how monomer type and concentration, surfactant type and concentration, plus other solution variables determine the adsorption and bubble stabilizing properties of poly alkylcyanoacrylate particles. In this study we aim to throw more light on the nature of these unique Pickering bubble stabilizers. Since there is a vast range of possible surfactants that could be used, we have concentrated on the main types used so far by other workers: the nonionics Triton X-100 and its polymeric equivalent, Tyloxapol.

Materials and methods

Materials

De-ionized Milli-Q water (Millipore, Watford, UK) was used. Glycerol (purity 99.5%) was purchased from Fischer Scientific (Loughborough, UK). Butyl cyanoacrylate (BCA) monomer was supplied by Chemence (Corby, UK). Ethyl cyanoacrylate (ECA) monomer was purchased from Loctite (Henkel, Slough, UK) and octyl cyanoacrylate (OCA) monomer was supplied by Liquid Skin (Chemence, Corby, UK). Tyloxapol®, Triton X-100®, and AnalR HCl (37%), used to adjust the pH, were supplied by Sigma Aldrich (Gillingham, Dorset, UK). An Amicon® stirred cell (EMD Millipore Corporation, USA) fitted with 30 kDa Ultracel® filter were purchased from EMD Millipore Corporation (Hertfordshire, UK). Glass specimen tubes (100 x 26mm) used to store the samples, were obtained from Scientific Laboratory Supplies Ltd (Nottingham, UK). Nile Red was purchased from Sigma-Aldrich (Steiheim, Germany). Xanthan gum, supplied by Sigma-Aldrich (Poole, UK), SP701PA clear casting resin, containing 1% catalyst was purchased from Trylon Ltd (Northants, UK).

Nanoparticle (NP) and microbubble (MB) formation

Butyl cyanoacrylate (BCA), ethyl cyanoacrylate (ECA) and octyl cyanoacrylate (OCA) were polymerised by anionic polymerisation to produce poly butyl cyanoacrylate (PBCA), poly ethyl cyanoacrylate (PECA) and octyl cyanoacrylate (POCA) NPs, respectively. The optimum monomer concentration according to Schmidt and Roessling (2006) is 1.4 wt.%. Monomer was therefore added drop-wise up this concentration, in preliminary experiments to 1% Triton X-100 in deionized water, at pH 2.2 under mild stirring (at 500 – 700 rpm) using a magnetic stirrer and magnetic followers (PTFE covered, 25 mm long). i.e., following their procedure. (In subsequent experiments Triton X-100 was replaced with Tyloxapol and polymerization at different pH was also tested – see later). Under these conditions the typical polymerization time, taken as the time when there appeared to be no further change in NP size (within experimental error, measured as described below) was 30 min. In order to determine the NP yield, a sample of polymerized material was filtered through a Whatman No. 3 filter paper (pore size 6 μm) and the material retained was weighed, after drying. The polymer yield was defined as the wt.% fraction of monomer transformed into the material retained on the filter.

For preparation of microbubble (MB) dispersions, a 25 ml sample of NP dispersion was aerated for 2 min via an Ultra-Turrax T25 mixer at room temperature at 24000 rpm. To some dispersions glycerol was added to give 76% glycerol prior to aeration as above. These samples were also then subjected to multiple aeration at different time intervals. The density and therefore air content (volume faction) of aerated samples was measured via a high precision oscillating U-tube densitometer (DMA 4500, Anton-Paar, Graz, Austria) at 25°C. The glycerol served to increase the viscosity sufficiently to reduce any coalescence of bubbles in manipulation of the sample into the densitometer. In all cases of density measurement, a 1wt% Tyloxapol in 76% glycerol was used as a blank.

In order to separate excess surfactant from PBCA NPs a sample of the dispersion was centrifuged in a Universal 320 centrifuge (Hettich, Tuttlingen, Germany) at 2370g for 35 min. The supernatant was removed carefully and sedimented particles were transferred to a clean beaker and deionized water (pH 4) was added, stirred gently by a spatula for 10 min, followed by 1 minute sonication in a Kerry Ultrasonic Bath (Hitchin, UK) of 1 litre capacity at 60 Hz.

Microscopy

Images used for MBs size analysis were obtained via an optical light microscope, using 10, 20 and 40 x objective lens and fitted with a Nikon SMZ-2T (Tokyo, Japan) digital camera. For each measurement around 1 ml of diluted MB dispersion were transferred to a welled slide (diameter 19 mm and depth 3 mm) and sealed with a coverslip (0.17 mm thickness). Samples were diluted as appropriate, i.e., to give as far as possible a single layer of bubbles clearly visible just below the coverslip. Digital images of the bubbles were analysed via ImageJ software (version 1.51s, USA). At long times a small proportion of the larger bubbles appeared distorted and non-spherical: the diameter of such objects was taken as the mean of the longest and shortest axis. A Leica TCS SP2 confocal laser scanning microscope was also used in fluorescent mode to obtain images of bubbles stabilized by PBCA NPs. In order to increase the viscosity of the MB dispersions and immobilize bubbles so as to more clearly image the adsorbed particles, xanthan gum solution (0.1 wt%) was added. Then 1 droplet of Nile red was stirred gently into the dispersion to stain the PBCA NPs. Dyed samples were

scanned at room temperature using a 40 x objective lens, exciting the dye with the 488 nm Ar laser line and emission wavelength 525 nm Images were recorded at a resolution 1024 x 1024 pixels.

Selected samples were also imaged via scanning electron microscopy (SEM) via an FEI Nova450 SEM (Eindhoven, The Netherlands). Approximately 5 droplets of MB dispersion were stirred inside silicone moulds filled with the clear casting resin and allowed to set for at least 24 hours. The solid samples were fractured using a sharp blade. Samples were coated with a thin layer of iridium (2 nm) via a Cressington 208HR sputter coater. Samples were imaged at 3 kV.

Particle size distributions *and ζ potential*s

The particle size distribution (PSD) and ζ- potential of the NP dispersions were measured by dynamic light scattering at 25 °C using a Zetasizer Nano- ZS (Malvern instruments, Worcestershire, UK). Samples were diluted 1:10 (wt/wt) in MilliQ water (pH 6 -7) prior to measurement and transferred into disposable PMMA cells for PSD measurement. The refractive index of water and PBCA were set at 1.33 and 1.46 respectively. For ζ- potential measurements the diluted dispersions transferred to DTS 1070 measurement cells.

Determination of free Tyloxapol concentration

Tyloxapol concentrations in water can be determined by spectrophotometry, the phenol ring giving strong absorbance at 270 nm. However, PBCA particles also absorb at this wavelength, so care had to be taken to separate NPs from Tyloxapol, via filtration. Serial dilution of Tyloxapol in water was used to obtain a standard curve. The same solutions were also re-measured after passing through a 30 kDa Ultracel® filter in the Amicon stirred cell fitted. NPs dispersions that also contained Tyloxapol were diluted (1:500) and passed through the same filter to remove the NPs. Tests showed that this filter had a pore size sufficiently small to retain NPs of all the sizes produced but allow the surfactant to pass through. The absorbance of all solutions was measured at 270 nm at 25°C in a 3 ml quartz cuvette.

All experiments described above in the Methods section were performed in triplicate and mean values are presented with the standard deviation about the mean as the error bar.

Results and discussion

Effect preparation conditions on NP properties

There appears to be no consensus in the literature over the optimum surfactant concentration, type of surfactant or pH for the polymerization on cyanoacrylates into NPs. Table 1 summarizes the results for most of the systems we have studied. For ease of comparison we have given the systems numbers, from 1 to 11.

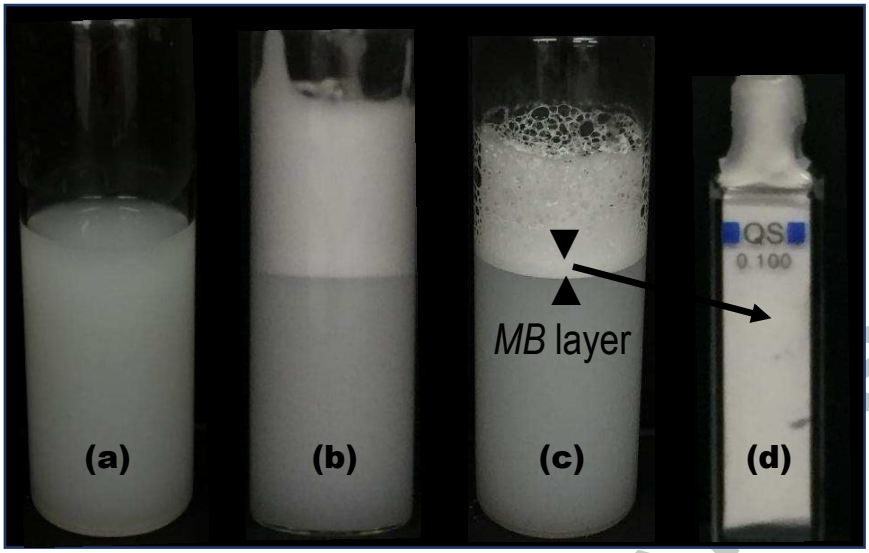
System No.	Surfactant and concentration	pH	NP Yield/ %	d/ nm	ζ /mv	MB stability
1	0.1 wt.% Triton-X100	2.2	≤ 15 ± 5	140 ± 7	−20	None
2	1 wt.% Triton-X100	2.2	≤ 30 ± 3	110 ± 2	−20	≥ 2 weeks
3	10 wt.% Triton-X100	2.2	≤ 10 ± 2	140 ± 5	−21	None
4	1 wt.% Triton-X100	4	≤ 30 ± 3	100 ± 10	−20	≤ 2 months
5	1 wt.% Tyloxapol	4	≥ 50 ± 5 %	150 ± 30	−24	≥ 2 months
6	No surfactant	4	≥ 70 ± 2 %	90 ± 5	−61	None
7	1 wt.% Tyloxapol (added after particle formation)	4	≥ 70 ± 2 %	90 ± 5	−34	None
8	1 wt.% Tyloxapol	2.2	≤ 20 ± 3 %	105 ± 10	−19	≥ 2 months
9	1 wt.% Tyloxapol	6	≥ 40 ± 3%	131 ± 5	−28	≥ 2 months
10	1 wt.% Tyloxapol*	4	40 ± 6 %	90 ± 10	−33	2-5 days
11	1 wt.% Tyloxapol#	4	50 ± 10 %	25-151	−30	2-5 days

Table 1: Yield, size (d) and zeta potential of NPs formed under different conditions and the stability of the corresponding microbubbles (MBs) formed on aeration. All systems used BCA monomer except * = ECA, # = OCA.

Triton-X100 was used first as surfactant and the effect of its concentration on polymerization of BCA studied. The pH and monomer concentration were kept constant at 2.2 and 1.4 wt.%,

respectively. Table 1, systems 1 to 3, shows the effect of Triton-X100 concentration on the yield, mean diameter (d) and zeta potential of the NPs formed. (Note that the pH of the diluted dispersions during these measurements was pH 4 to 6). It is seen that zeta potential was largely unaffected across 0.1 to 10 wt.% Triton-X100, at around −20 mV. Triton-X100 is a non-ionic surfactant and so in itself is not expected to impart any charge if adsorbed to the NPs, whilst the monomer is anionic. The monomers are neutral (and fairly insoluble) in these pH ranges so that the negative zeta potential is mostly likely due to adsorbed impurities (possibly some traces of de-esterified acrylates). The NP diameter was also not greatly affected by the Triton-X100 concentration during the polymerization, although 1 wt.% gave slightly lower d (110 nm) than 0.1 and 10 wt.% (140 nm). Overall then, it seems that although the BCA polymerization is supposed to proceed via an emulsion polymerization route and initiation within the surfactant micelles (Nicolas and Couvreur, 2009, Behan et al., 2001), the final NP properties do not seem very sensitive to this surfactant concentration.

Other workers (Budde et al., 2003) have used Tyloxapol as the surfactant rather than Triton-X100. Tyloxapol is an oligomer of Triton-X100 and as such is expected to have a lower critical



micelle concentration and higher affinity for the interface (Regev and Zana, 1999). It is reported to consist of 3 to 8 (average 7) Triton X-100 molecules with their head groups linked together via a single methylene group (Dharaiya et al., 2015); with a molecular weight of 1709 to 5263 Daltons. Polymerization of BCA in the presence of 1 wt.% of either surfactant was therefore carried out for comparison of NP properties. Polymerization was carried out at pH 4 in line with other studies (e.g., (Budde et al., 2003) and polymerization was also attempted in the absence of any surfactant as a control. Table 1, systems 4 to 7 show the properties of the NPs produced and also an indication of the stability of the MBs produced by aerating these dispersions, as described above. Figure 1 shows typical images of a system that gives stable MBs (system 5, discussed below). A MB layer starts to appear in the first few h of storage – an extremely optically dense layer with a coherent, 'creamy' texture – that can be removed, as shown, for subsequent analysis of MB sizes (as described in the Materials and Methods section). Individual bubbles are too small to be perceived by the naked eye.

Figure 1. Images of PBCA NP dispersions: (a) before, (b) 5 min after, and (c) 1 h after aeration, respectively. The width of the sample tubes is 26 mm. Image (d) is an example of samples of the MB layer indicated, pipetted from tubes after 3 months into a 10 mm wide cuvette.

Tyloxapol (system 5) gave slightly larger NP diameter (d) than TritonX-100 (system 4) but a higher NP yield (50 % cf. 30% for TritonX-100). At first sight this higher yield is probably explained by the Tyloxapol giving greater NP stabilization due to (i) its higher molecular weight and therefore more effective steric stabilization, and/or (ii) greater coverage due its higher molecular weight and affinity for the interfaces and/or (iii) the slightly higher (more negative) zeta potential imparted by Tyloxapol (−24 mV) versus Triton-X100 (−20 mV). However, very interestingly, no surfactant at all (system 6) gave a similar d (90 ± 5 nm), a higher yield than either surfactant and NPs with a zeta potential almost twice as negative (− 61 mV) as with surfactant. Possibly some sort of dispersion polymerization mechanism is responsible, or the polymerizing monomer acts as some sort of surfactant or stabilizer. However, these NPs were no use whatsoever at stabilizing MBs, possibly due to their higher negative charge, making them too hydrophilic for adsorption to the surface of the bubbles. This suggests that the surfactant is not essential to BCA NP formation but plays some other role in controlling the NP surface active properties at the A/W interface. Excess surfactant is of course present when the NP dispersions are aerated, but control experiments aerating 1% Tyloxapol or Triton-X100 on their own did not give any stable MBs – any bubbles formed appeared to have completely disappeared with less than 3 h. This is expected, since only particles are able to prevent disproportionation of MBs over long periods of time (Du et al., 2003). Furthermore, adding 1 % Tyloxapol to the NP dispersion formed in the absence of surfactant, after NP formation, gave a system (system 7) where the magnitude of the zeta potential was reduced from −61 to −34 mV, but these particles were still not able to stabilize MBs. The lowering of the zeta potential by the non-ionic surfactant must be due to its adsorption and shifting of the plane of shear further away from the surface of the particles where the charge density due to surface polymer chains is lower. The magnitude of this final zeta potential was not quite as low as that of the NPs formed in the presence of Tyloxapol (−24 mV), which suggest there might be a critical value of zeta potential that determines the required surface activity (contact angle) of the NPs, but certainly simple Tyloxapol adsorption to the NPs cannot create this condition: Tyloxapol only imparts good MB-stabilizing properties when it is present during BCA polymerization.

We measured the free Tyloxapol concentration in the NP dispersions via absorbance measurements (see Figure 1, Supplementary Material) by carefully filtering off the NP particles from the aqueous phase through the 30 kDa Ultracel membrane in the stirred cell, taking care to dilute solutions enough so that surfactant micelles were also not retained by the membrane. From this data the calculated free Tyloxapol concentration in the final dispersion was 0.75 wt.%, i.e., 25% of the Tyloxapol in the system before polymerization either ended up adsorbed to the surface of the NPs and/or was incorporated into the particles on polymerization. Based on the total surface area of a typical NP dispersion of d = 160 nm, this equates to a surface load of Tyloxapol of 10.6 mg m^{-2}. This is fairly high, considering typical monolayer coverage of surfactants is 2 - 3 mg m^{-2}. Since addition of Tyloxapol after particle formation does not result in surface active NPs, we conclude that at least a significant amount of this 'lost' surfactant must be incorporated into the particles on polymerization, which somehow imparts the necessary surface hydrophobicity.

Samples of the optimum system 5 were also subjected to centrifugation (see Methods section) to separate the PBCA particles from the remaining bulk Tyloxapol solution present during their formation. From the mass of the pellet it was estimated that ≥ 90% (0.158 g out of 0.175g) of the PBCA particles present in the sample as a whole were separated from the supernatant. This pellet was then re-dispersed in MilliQ water at pH 4 (using a spatula and stirring by hand for 10 min, followed by 1 minute sonication) and the mean size and zeta potential of the dispersed material measured as d = 360 ± 10 nm and $\zeta = -34 \pm 1$ mV, i.e., different to the pre-centrifuged particles. However, when this dispersion was aerated no stable MBs formed. This shows that the excess surfactant is still necessary to lower the surface tension and help stabilize bubbles until sufficient NP coverage is achieved so as to inhibit their further shrinkage, i.e., some co-adsorption of NPs and surfactant must take place during the early stages of bubble formation.

Since both the BCA monomer and the polymerized NPs had a negative charge, it was expected that the pH of polymerization might affect the size and surface charge of the resultant NPs − and both of these factors would affect the NP contact angle and surface activity. Various workers seem to recommend different pH values. BCA was therefore also polymerized at pH 2.2 (system 8)

and pH 6 (system 9), where the charge on the monomer is expect to be significantly different. Since Tyloxapol seemed to give the best particles for MB stabilization, 1 wt.% Tyloxapol was used as surfactant. Comparison of systems 8 and 9 with system 5 (pH 4) in Table 1 shows that pH had a significant effect on the yield of PBCA NPs, but not their size or MB-stabilizing capabilities. The yield increased when the pH was increased from 2.2 to 4 and decreased again slightly at pH 6, whilst the MBs stabilized by all 3 systems appeared equally stable – for at least 2 months there was very little change in mean bubble size (see later). The optimum pH, i.e., for highest PBCA NP yield, was therefore taken as pH 4.

Finally in this section, although polymerization in the presence of 1 % Tyloxapol at pH 4 seemed to give the optimum NPs for MB stabilization in the case of BCA (across all the conditions investigated thus far), it was thought worthwhile to investigate if any other monomers were advantageous, since there is no obvious reason why polymerization of the butyl derivative should give better NPs for MB stabilization than other alkyl cyanoacrylates. Two relatively easily available monomers are ethyl cyanoacrylate (ECA) and octyl cyanoacrylate (OCA), that have also been investigated by others (Couvreur et al., 1979, Nicolas and Couvreur, 2009, Sulheim et al., 2016) although no details of NP yields were given. Polymerization of both was attempted under the optimum conditions for BCA, i.e., 1.4 wt% monomer, pH 4 with 1 wt% Tyloxapol and the results (systems 10 and 11) are also shown in Table 1. Comparison with BCA (system 5) shows that all 3 monomers gave similar yields under these conditions, although ECA gave slightly lower yields than BCA or OCA. ECA gave similar NP sizes to BCA (slightly smaller in fact) but the OCA particle size was very irreproducible, with most of the material present as very large aggregates and a representative d for POCA could not be determined. Most significantly, only the PBCA particles were able to give high MB stability, PECA and POCA material gave bubbles that disappeared completely via coalescence and/or dissolution in less than 2 to 5 days, whereas PBCA-stabilized MBs were stable for at least 2 months. It is noteworthy that the size and zeta potential of the PECA particles were $d = 90 \pm 10$ nm and $\zeta = -33$ mV, very close to the values (90 ± 5 nm and -34 mV) of the PBCA particles formed without surfactant but when Tyloxapol was added after NP formation

(system 7). The latter particles also did not give good MB stability, again suggesting that such zeta potentials are too high in magnitude, making the particles too hydrophilic for a high enough contact angle and energy of desorption at the A/W interface.

The micrographs in Figure 2 illustrate the appearance and different stabilities of the MBs formed with some of these systems.

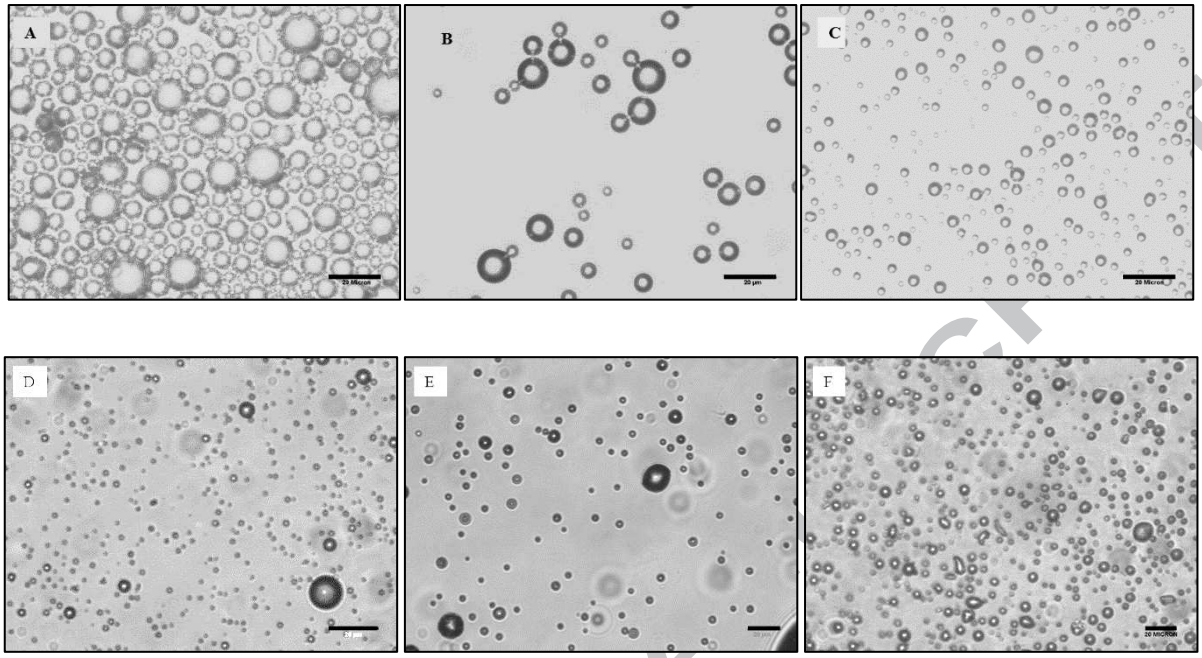


Figure 2. Representative micrographs of MBs stabilized by different NPs at pH 4, 24 h after aeration. Scale bar = 20 µm. (A): BCA, Tyloxapol, pH 4 (B): ECA, Tyloxapol, pH 4 (C): BCA, TritonX-100, pH 4, (D): OCA, Tyloxapol, pH 4 (E): BCA, Tyloxapol, pH 2.2 (F): BCA, Tyloxapol, pH 6

Detailed comparison of MB properties and their stability

To follow any changes in the size distribution of the MBs and their stability, optical microscopy images of MBs were taken at regular time intervals, typically 1 h, 3 h, 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 8 weeks and 10 weeks (70 days). At least 5 images were taken and the diameters of at least 250 bubbles were highlighted and measured via ImageJ for each time. Figure 3 gives examples of images analysed and Figure 4 gives typical histograms of MB size distributions obtained with PBCA and PECA NPs at different times.

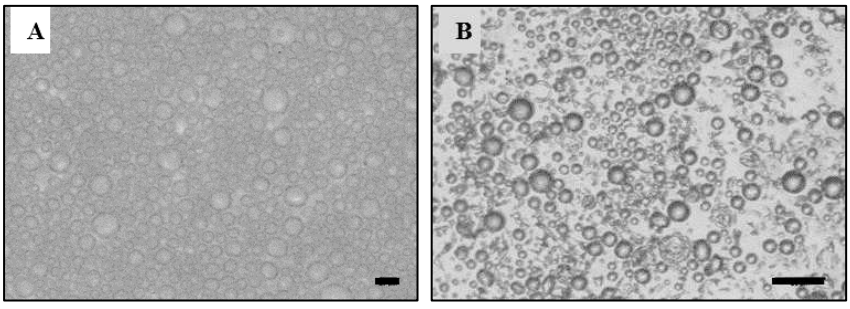


Figure 3. Micrographs of MBs stabilized by PBCA NPs with 1wt.% Tyloxapol at pH 4. Scale bar = 20 µm. (A): 1 hour (B): 70 days

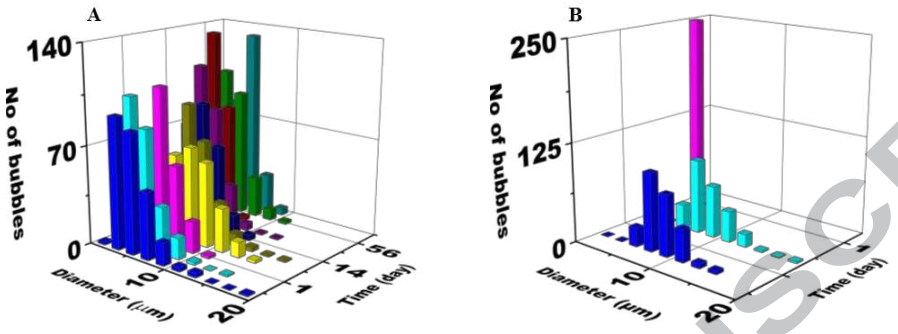


Figure 4. Histogram of MB size distributions at different storage times stabilized by PBCA (A) and PECA (B) NPs formed with 1wt.% Tyloxapol at pH 4

The histograms in Figure 4(A) show the MBs for system 5 (PBCA NPs formed in presence of 1 wt.% Tyloxapol at pH 4) were stable for the period of this study (70 days), i.e., the size distribution did not change significantly with time, shifting only slightly to larger sizes. For example, the Sauter mean diameter ($d_{32} = \frac{\sum_{i=1}^{n} n_i d_i^3}{\sum_{i=1}^{n} n_i d_i^2}$) was calculated at each specific time and d_{32} after 1 h was 5.3 µm and 4.60 µm after 70 days, with a standard deviation 1.3 µm. In other words, during 70 days the MBs showed an outstanding stability with a less than 1 µm change in diameter. In contrast, MBs stabilized with PECA NPs were not stable for a long time. Their initial size distribution was in a larger size range compared to MBs stabilized by the PBCA NPs and they started to shrink relatively quickly so the d_{32} changed from 8.35 µm to 1.25 µm in only 24 h. After this time there were not enough (< 250) MBs visible to continue the measurement satisfactorily.

Another measure of the extreme stability of the MBs stabilized by system 5 was illustrated by subjecting them to a freeze-thaw cycle. A sample of system 5 was placed in a domestic freezer at –18 °C for 22 h then thawed at room temperature. No visible loss of foam volume was noticeable and d_{32} showed only a small increase from 5.9 ± 1.1 µm to = 8.5 ± 3.3 µm, indicating that some coalescence

may have taken place, although a slightly greater proportion of distorted non-spherical bubbles was observed which may also account for this apparent increase.

The volume fraction of MBs formed in system 5 were calculated from the density measurements of the aerated systems, as described in the Methods section. Densities were measured 15 min after each of 3 aerations of the same system, to see if repeated aeration increased the volume fraction of stable MBs. This is important because MBs formed after one aeration might well be stable to subsequent re-shearing or coalesce on re-shearing, thus releasing NPs back into the aqueous phase to give essentially the same dispersion as before the first aeration. Samples were also taken for density measurement from each aerated system 48h after each aeration stage ceased, to examine longer term stability. Figure 5 shows the results of these multiple aerations.

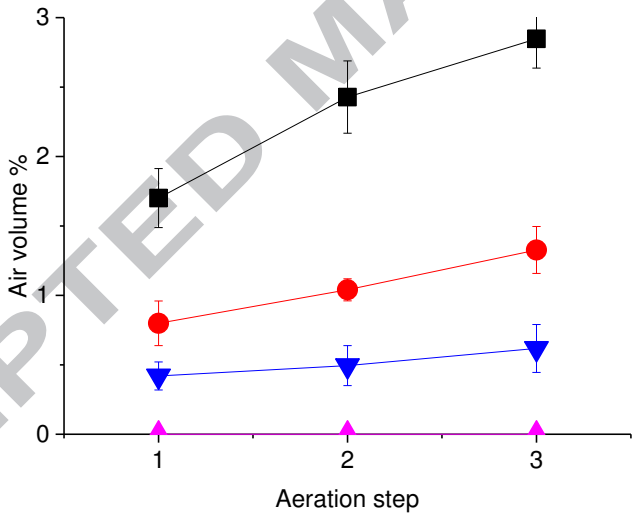


Figure 5. Air volume% of MBs stabilized by PBCA NPs 15 min after aeration (■), and 48 h after aeration (▲), [Tyloxapol blank in 76% glycerol 15 min after aeration (●) and 48 hours after aeration (▼)]

The d_{32} of these bubbles was typically 4 - 5 µm, which is so small that they must be NP-stabilized to exist for even as short a time as a few seconds. However, it is seen that the volume fraction of MBs formed was not high, e.g., 1.7 vol.% after the first aeration. This probably reflects the difficulty (Ettelaie and Murray, 2015) of coating bubbles fast enough with sufficient particles to stop them shrinking and dissolving and/or coalescing, even under the very high shear rates of the UltraTurrax

mixer. Repeating the aeration 15 min after the first aeration did increase the volume fraction further, and further still after the third aeration, reaching almost 3 vol.%. This suggests that a fair proportion of the MBs formed in one aeration can survive subsequent aerations, at least at these time intervals, opening up the possibility of producing higher and higher volume fractions of MBs from the same dispersion. We note that Schmidt and Roessling (2006) obtained 10 vol.% in a continuous aeration system, but over 3 hours. However, the densities measured 48 h after each aeration all fell back to 0.4 vol.%, suggesting that there is still some shrinkage/dissolution and or coalescence and loss of bubbles over this time-scale. Nevertheless, considering the extreme instability to disproportionation of air bubbles of this size in water (lifetimes typically of a few 10's of ms), the achievement of several vol.% in 3 steps (in less than 1 h) could be of technological significance, particularly if such MBs only need to be stable for a few hours or could be fairly quickly immobilized in a truly solid phase.

In the normal light microscopy images used to size the MBs, such as Figure 3, the resolution is obviously not good enough to visualize the NPs at the interface. CLSM was therefore used to confirm the presence of a layer of NPs at the air bubble surfaces. As explained in the Methods section, the PBCA particles appeared to pick up the hydrophobic fluorescent stain Nile Red, which enabled their imaging. Representative images are shown in Figure 6, clearly indicating the presence of PBCA particles mainly at A/W interface, i.e., relatively few in the bulk aqueous phase, although it is still hard to discern whether some of the non-spherical objects are NP aggregates or shrunken air bubbles surrounded by NPs. Non-spherical bubbles are a sure sign of Pickering stabilization, the strong driving force for bubble dissolution causing the adsorbed particle layer to crumple under the stress of shrinkage (Bala Subramaniam et al., 2005).

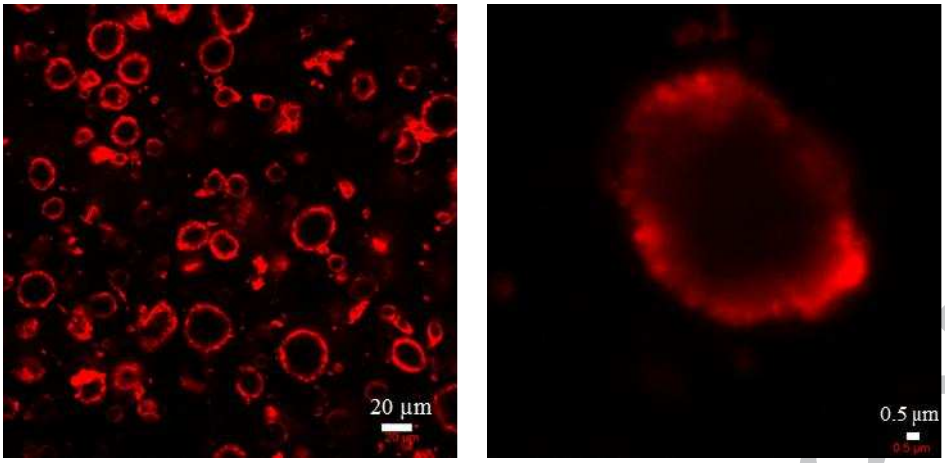


Figure 6. Representative CLSM micrographs of PBCA-stabilized MBs. Red colour due to Nile red = PBCA.

Although the CLSM images are good proof of Pickering stabilizations of the MBs, the resolution is still not high enough to discern individual NPs at the interface. Obtaining such images is difficult but important, because it can confirm the contact angles of particles at the interface and therefore substantiate explanations of the different stabilizing properties of the different NP systems. Figure 7 shows some representative SEM images of PBCA NPs at the A/W interface of MBs immobilized and embedded in resin and imaged as described in the Methods. Not surprisingly, it only seemed possible to obtain images of MBs and NPs in those systems that had reasonably good stability – the preparation steps up to imaging presumably making these other systems less stable. In Figure 7, the presence of spherical NPs at the interface is clearly visible. High resolution images of selected regions where the coverage is not so crowded (such as Figure 7(B)) even allowed estimation of the particle contact angle, θ. Measurement of up to 20 such particles gave $\theta = 77 \pm 10°$.

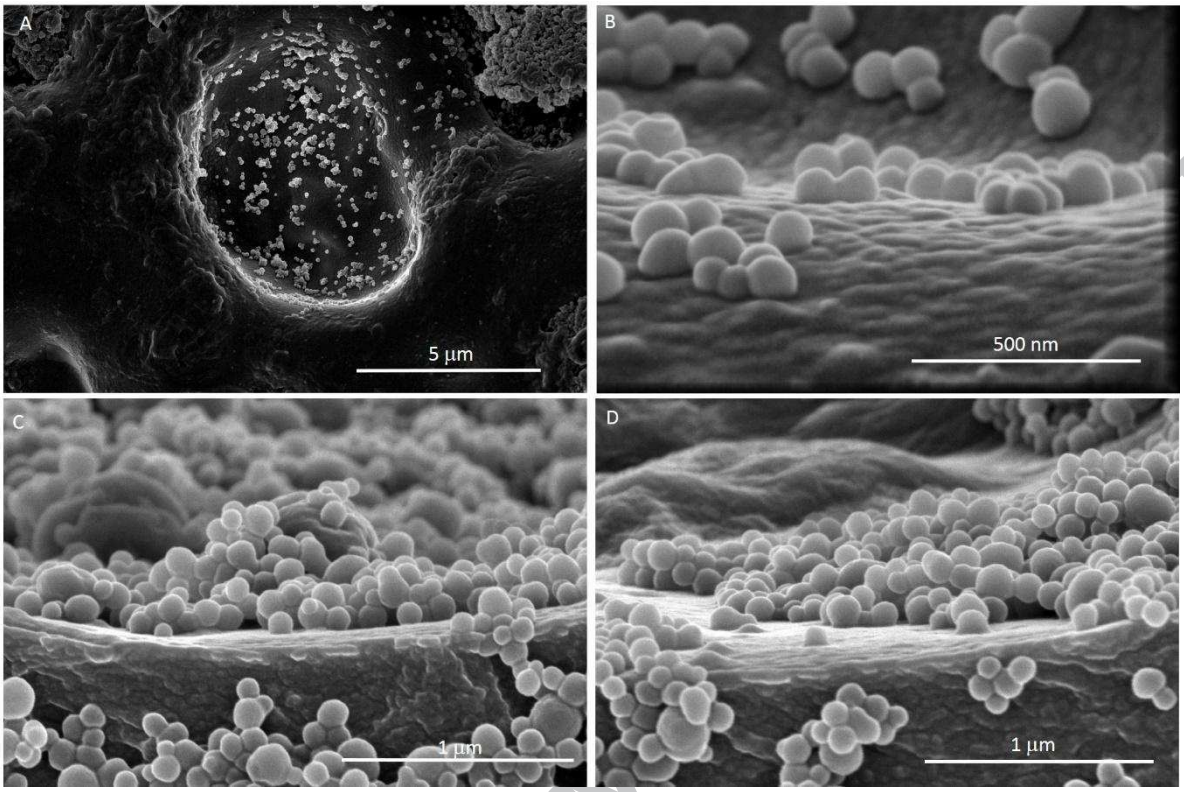
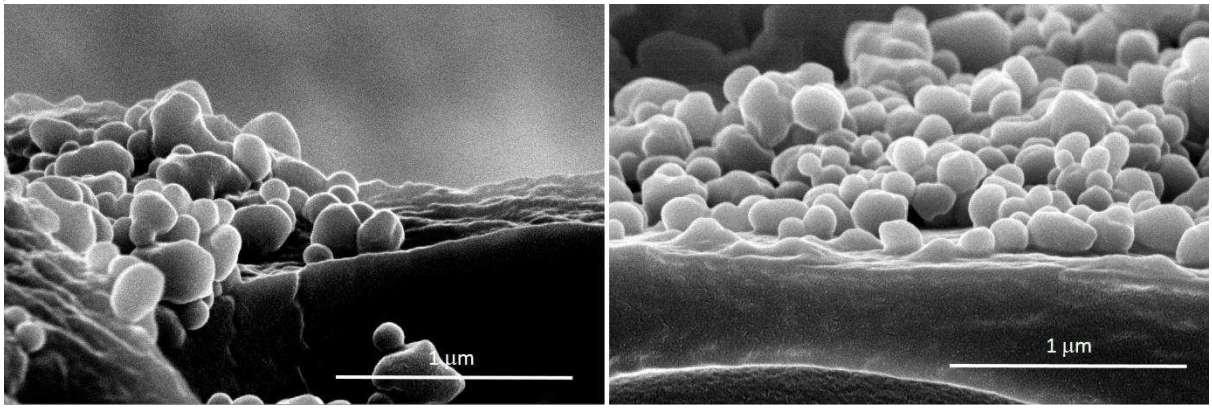


Figure 7. Representative SEM micrographs of PBCA NPs at the surface of MBs. In (B), the particle contact angle is measurable.

Figure 8 shows SEM micrographs obtained of PECA NPs, that were less effective at stabilizing MBs (see Table 1, system 10). The images show that these NPs were much less spherical than the PBCA NPs (Figure 7) and also that at the A/W interface they seemed to have aggregated or even merged together. Because of this, it was impossible to measure θ. Some of these differences could be due to electron beam damage, though care was taken to try and expose the sample to exactly



the same imaging conditions as in Figure 7.

Fig 8: SEM micrograph of PECA NPs at the surface of MBs.

Finally, it was difficult but just possible to obtain some images (Figure 9) of the surface of larger bubbles formed on attempting to aerate the PBCA NP dispersion formed in the absence of surfactant (Table 1, system 6). These NPs were very poor MB stabilizers. Figure 9 suggests that the NPs are located somewhat deeper in what was the aqueous phase compared to the PBCA NPs prepared with Tyloxapol (Figure 7). This supports the idea put forward earlier that PBCA NPs formed without surfactant are too hydrophilic to be effective as MB-stabilizers.

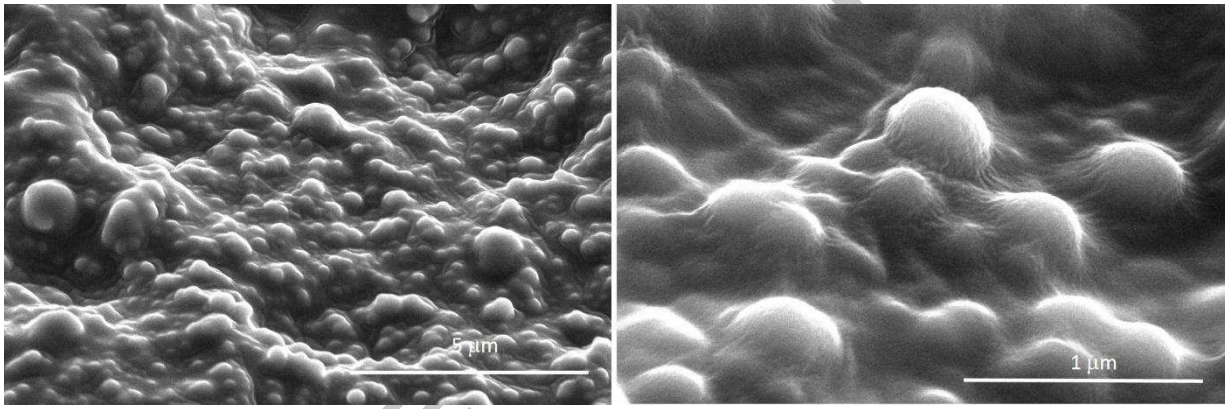


Fig 9: SEM micrograph of PBCA made in absence of surfactant at the air-water interface

Concluding Remarks

We have surveyed a range of conditions of polymerization of alkyl cyanoacrylates leading to nanoparticles (NPs) potentially capable of stabilizing highly unstable microbubbles (MBs) of air in aqueous solutions. This has improved our understanding of the optimum conditions for preparation of surface active NPs compared to previous work. Figure 10 illustrates the main areas covered.

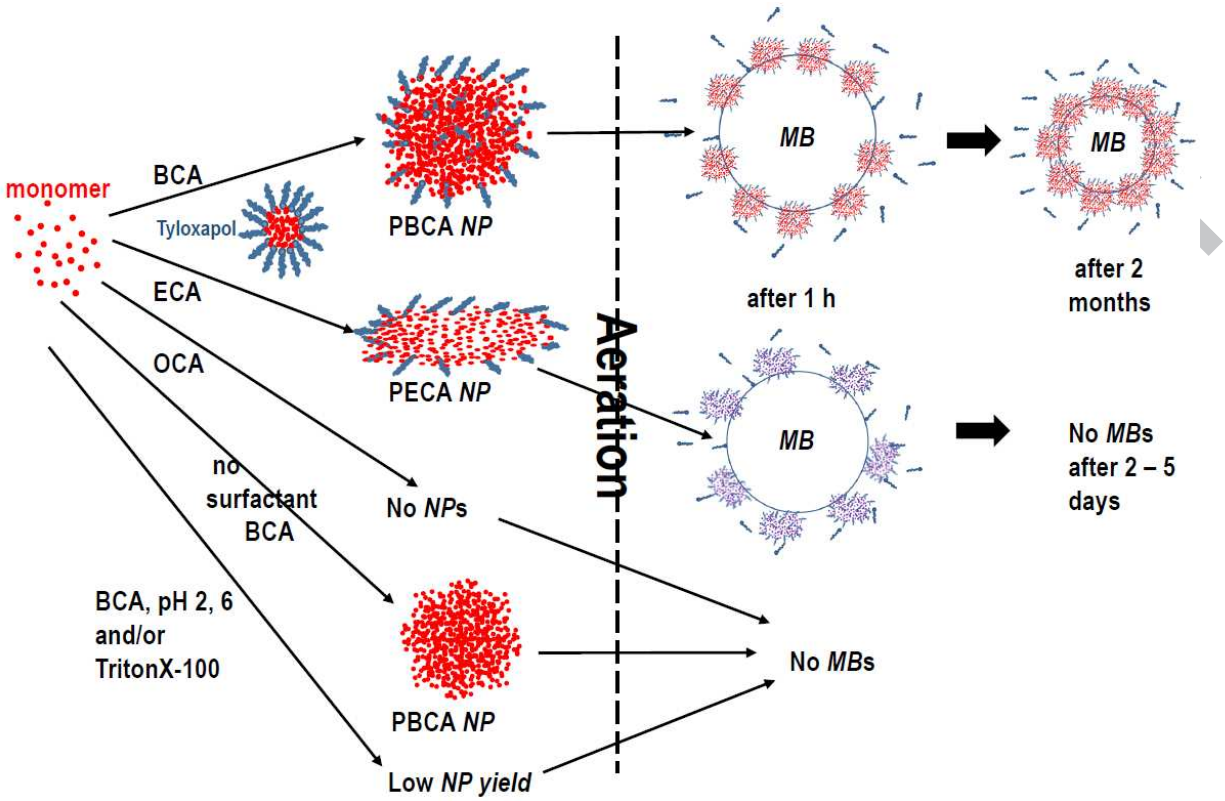


Figure 10. Schematic illustration of the main areas covered by this study, showing the effect of different monomers and polymerization conditions on the ability of the corresponding NPs to stabilize MBs.

A summary of our key findings is as follows.

- The optimum system for microbubble stabilization is butyl cyanoacrylate (BCA) polymerized into PBCA nanoparticles at pH 4 in the presence of 1 wt.% Tyloxapol surfactant.
- These PBCA particles are highly effective at stabilizing MBs of only a few microns in size for at least 2 months and these MBs are stabilized via a Pickering mechanism, as revealed conclusively by microscopy over a range of length scales.
- Only a low volume fraction (ca. 1 vol.%) of MBs can be obtained in simple, single aeration steps in a high shear mixer, reflecting the difficulty of obtaining rapid enough particle coverage of bubbles and/or its maintenance, even under turbulent conditions – a hypothesis previously put forward in other work (Ettelaie and Murray, 2014).
- Only PBCA particles formed (polymerized from BCA) in the presence of Tyloxapol act as MB stabilizers. PBCA particles of similar size and charge but with the surfactant added afterwards do not work as stabilizers.

This last point leads to the new concept that that the surfactant itself may have to become incorporated into the surface of the PBCA NPs in order to impart to the NPs the correct contact angle/wetting properties at the A/W interface. This complication probably explains the conflicting results in some of the literature on the effects of different surfactants and polymerization conditions. However, the exact way in which surfactants become incorporated into the NP surface, their orientation and surface loading, is as yet unknown and requires further investigation. It would be interesting to study a range of pure surfactants of specific but varying hydrophilic versus hydrophobic groups in this respect. Future work also suggested by this study is the production of bubbles under more controlled conditions that will allow more detailed comparison of experimental data with mathematical predictions (Ettelaie and Murray, 2014, 2015) of how NP size versus initial bubble size, plus the dynamics of particle adsorption versus bubble shrinkage, affect the final stable MB size distribution. In addition, we propose investigating more controlled methods of continuous MB production, stabilized by PBCA NPs, building up higher and higher volume fraction for practical usage.

Acknowledgements

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Graphical abstract