

# Synthesis of non-aqueous fluorescent hard-sphere polymer colloids

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## Abstract

We describe the synthesis of fluorescent colloidal polymer spheres which when suspended in non-aqueous solvents behave as classical hard spheres. Monodisperse poly(methyl methacrylate) particles with sizes varying between 200 and 600 nm have been prepared by dispersion copolymerisation of methyl methacrylate, methacrylic acid and the fluorescent monomer, 7-nitrobenzo-2-oxa-1,3-diazole-methyl methacrylate. Increasing the concentration of dyed particles in suspension resulted in the rapid nucleation and growth of colloidal crystals. Measurement of the equilibrium phase behaviour confirmed that the potential between dyed particles was close to hard-sphere. The resulting polymer colloids provide an ideal model system for the study of colloidal phenomena using fluorescent confocal microscopy.

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## 1. Introduction

Understanding the structure and dynamics of colloidal suspensions is important in many areas of technology, such as paints, coatings, and ceramics. Traditionally, experimental information on suspension structure has been obtained from light, X-ray or neutron scattering measurements. However, recent work [1–4] has demonstrated that the three-dimensional structure and motion of colloidal phases can be obtained precisely in real space and time using confocal microscopy. Com-

pared with sophisticated image analysis methods [1], confocal microscopy can yield the 3D-positions of tens of thousands of individual particles within a colloidal phase. Such detailed microscopic information offers unique insights into the phase behaviour and dynamics of soft condensed matter systems. However, three-dimensional confocal microscopy places considerable demands on the colloidal system. The particles should be intensely fluorescent, resistant to photobleaching (so the fluorescent intensity does not change markedly with illumination), spherical and monodisperse.

Non-aqueous dispersions of sterically-stabilised poly(methyl methacrylate) (PMMA) spheres [5] have been studied widely as a colloidal model of atomic liquids [6]. These uncharged particles

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interact via a steeply repulsive potential and constitute probably the closest experimental realisation of the classical hard sphere system. The simplicity of the hard sphere potential means there is considerable interest in synthesising polymer colloids, which are both hard-sphere-like and fluorescent. Such particles would constitute an extremely useful model with which to study colloidal phenomena directly using confocal microscopy. Dyed polymer colloids have been synthesised by a variety of methods: pre-formed particles have been dyed using an organic solvent to swell and transport the dye [7], copolymerisation with reactive dyes [8–11] has been used to covalently bind dyes within the particles, and dyes have been dissolved in the monomer prior to polymerisation [12]. Attempts to swell pre-formed sterically-stabilised PMMA colloids have not been successful. Dinsmore et al. [1] used acetone to incorporate the fluorescent dye rhodamine into sterically-stabilised PMMA spheres. However, the process introduced electrostatic charge into the particles so that while the unlabeled particles behaved as hard spheres, the potential between the rhodamine-dyed particles was softer. In consequence, the phase behaviour of the dyed samples differed from that expected for hard spheres [1]. While the use of reactive dyes in the emulsion polymerisation of fluorescently-labelled aqueous PMMA colloids has been well documented [9–11] this approach does not seem to have been applied previously to a non-aqueous dispersion polymerisation. In this paper, we describe a synthesis of non-aqueous fluorescent colloidal PMMA spheres in which a reactive methacryloylated dye is incorporated in a modified dispersion polymerisation reaction. In contrast to similar works on aqueous systems reported to date [9,10], we concentrate on the effect of the fluorescent dye on the phase behaviour of the dyed particles. We show that using a reactive dye in the synthesis of sterically-stabilised PMMA particles does not change the nature of the interparticle potential. The potential remains hard-sphere in nature. The resulting non-aqueous fluorescent hard sphere colloids are ideally suited to confocal microscopy studies in colloid physics.

## 2. Experimental section

Fluorescent spheres were synthesised by a dispersion polymerisation of methyl methacrylate and a small quantity of the fluorescent 7-nitrobenzo-2-oxa-1,3-diazole (NBD) tagged monomer, NBD-MMA. The monomer was synthesised following the two-step reaction scheme detailed in Fig. 1.

### 2.1. Materials

The monomers methyl methacrylate (MMA), methacrylic acid, and methacryloyl chloride were obtained from Aldrich. Prior to use they were distilled under a reduced pressure of nitrogen and stored at 4 °C. The poly(12-hydroxy stearic acid) stabiliser (PHSA) was synthesised according to the method described elsewhere [5]. Tetrahydrofuran (Aldrich) was dried with a 5 Å molecular sieve before use. The initiator  $\alpha,\alpha'$  azo-bis-isobutyronitrile (BDH) was recrystallised from acetone prior to use. All other materials were used as supplied by Aldrich.

### 2.2. Preparation of fluorescent monomer

Sodium hydrogen carbonate (2.52 g, 30 mmol) and 0.74 g (9.9 mmol) of 2-(methylamino)ethanol were dissolved in 20 cm<sup>3</sup> of water in a round bottomed flask. 1.99 g (10 mmol) of NBD-chloride was dissolved in 80 g of methanol which was added to the flask. The reagents were warmed for 3 h at 55 °C whilst being stirred continuously. The methanol was then removed by rotary evaporation and the remainder left to cool to room temperature. The mixture was acidified to pH 1.5 with 28 cm<sup>3</sup> of 1 M hydrochloric acid. The bright orange product precipitated on cooling. It was then filtered and washed with distilled water before being dried, under a reduced pressure, at 40–45 °C. Elemental analysis gave the proportion of carbon, nitrogen and hydrogen in the product as 45.3, 23.7, and 4.26%, in close agreement with the proportions (45.4, 23.5, and 4.23%) expected for NBD-MAE. The successful attachment of the 2-(methylamino) ethanol chain was confirmed by <sup>13</sup>C-NMR in deuterated dimethylsulphoxide which

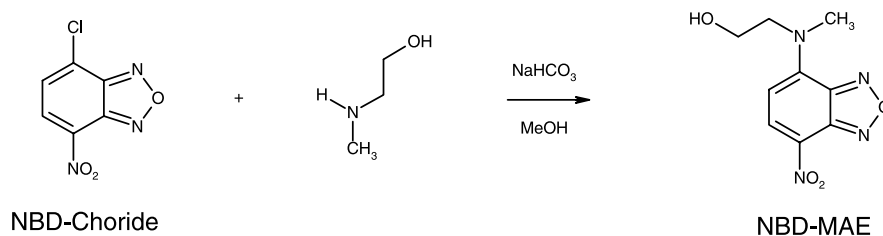
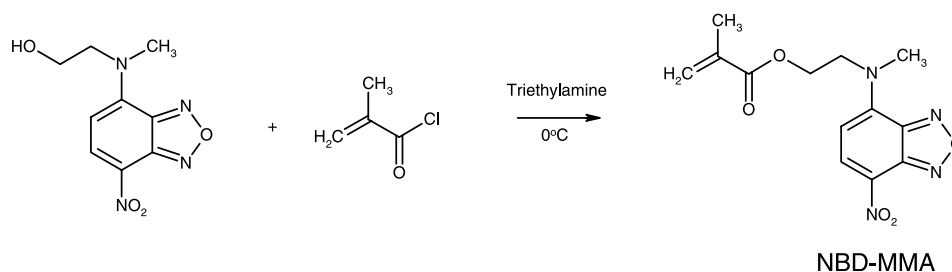
**Step 1****Step 2**

Fig. 1. Reaction scheme for the synthesis of fluorescent monomer NBD-MMA.

showed resonances at 58.5 ppm (–NCH<sub>3</sub>–CH<sub>2</sub>CH<sub>2</sub>OH), 57.3 ppm (–NCH<sub>3</sub>–CH<sub>2</sub>CH<sub>2</sub>OH), and 30.7 ppm (–NCH<sub>3</sub>–CH<sub>2</sub>CH<sub>2</sub>OH) as well as peaks characteristic of the six aromatic ring carbons in the range 102–146 ppm.

NBD-MAE product (0.45 g, 1.8 mmol) was added to 10 cm<sup>3</sup> of dry tetrahydrofuran, followed by 0.6 cm<sup>3</sup> (4.3 mmol) of triethylamine. The reaction mixture was stirred at 0 °C while 0.5 cm<sup>3</sup> (5.1 mmol) of methacryloyl chloride was added slowly dropwise. The mixture was stirred continuously at 0 °C for 2 h before being left overnight to react completely. Tetrahydrofuran was then removed by vacuum distillation, and the remaining solid dried under vacuum at 40 °C. The orange solid was recrystallised from a mixture of THF–EtOH, 80/20 (v/v) and washed with ether. Although the product yield was low at 36%, <sup>13</sup>C-NMR confirmed the attachment of the methacrylate unit with additional peaks evident in the spectrum at 173.5 ppm (–O–CO–C(CH<sub>3</sub>)=CH<sub>2</sub>), 120.7 ppm (–O–CO–C(CH<sub>3</sub>)=CH<sub>2</sub>) and 17.4 ppm (–O–CO–C(CH<sub>3</sub>)=CH<sub>2</sub>).

### 2.3. Particle synthesis and characterisation

The particles were prepared following the methods described by Antl et al. [5]. The proportion of the fluorescent monomer in the total mass of monomer was varied from 0.2 to 2% and is detailed in Table 1. Hydrodynamic radii ( $R_h$ ) and polydispersities ( $\sigma$ ) of the particles were determined from multi-angle dynamic light scattering (DLS) measurements [13]. A krypton gas laser operating at 647.1 nm was used as a light source. Fluorescent emission was separated from the scattered light by placing a laser interference filter in front of the detector. Excitation and emission spectra were recorded from a dilute dispersion of the dyed latex in *cis*-decalin with a Perkin–Elmer 555 fluorescence spectrophotometer. Emission measurements were made at an excitation wavelength of 488 nm and the excitation spectrum was recorded at an emission wavelength of 525 nm. Confocal images were collected on a Leica laser scanning microscope using an argon-ion laser ( $\lambda = 488$  nm) for excitation.

Table 1  
Details of labelled particles

Code	Monomer (wt.%)	NBD-MMA (wt.%)	$R_h \pm 5$ (nm)	$\sigma \pm 0.02$
RSJC3	46.54	0.00	160	0.10
RSJF3	46.42	2.02	163	0.09
RSJF4	46.90	0.91	119	0.13
RSJF2	47.38	0.23	203	0.10
RSJF1	47.47	0.22	175	0.07
RSJF5	49.50	1.01	306	0.07

The hydrodynamic radii ( $R_h$ ) and polydispersities ( $\sigma$ ) were determined from DLS measurements [13]. The weight of non-fluorescent monomer (98% methyl methacrylate and 2% methacrylic acid) is given as a percentage of the total weight of the reaction mixture. The proportion of fluorescent monomer is expressed as a percentage of the total monomer weight. The weight of PHSA-stabiliser was maintained at 5% of the total weight of monomer while the initiator concentration was fixed at 0.4% by weight of the total reaction mixture. The unlabeled particle RSJC3 is included as a reference.

#### 2.4. Determination of phase equilibria

The phase equilibria of the fluorescent particles was determined in a mixture of carbon disulphide ( $\text{CS}_2$ ) and *cis*-decalin.  $\text{CS}_2$  was added to approximately match the refractive index of the suspension medium to the particles. The weight fraction of  $\text{CS}_2$  in the solvent was typically about 0.32. The index-matched sample was concentrated by centrifugation at  $100 \times g$  for 2 h and a weighed amount of the supernatant removed to create samples with concentrations which spanned the anticipated freezing and melting transitions.

### 3. Results and discussion

The hydrodynamic radii and polydispersities of the fluorescent PMMA particles synthesised are collected together in Table 1. RSJC3 is a non-fluorescent particle which is included as a reference point. Electron micrographs of the dyed PMMA particles confirmed that the particles were spherical and essentially uniform in diameter. All of the dyed latices formed free-flowing, bright yellow-coloured dispersions in decalin. The particles were colloidal stable with no aggregation visible by light scattering after more than a year.

A potential disadvantage of the use of volume modified particles is the presence of dye attached directly to the particle surface which could modify the interaction potential. Indeed previous studies have observed significant differences between the

phase behaviour of labelled and unlabeled particles. For example, van Blaaderen et al. [14] observed a phase separation in suspensions of fluorescein-labelled silicas whereas suspensions of unlabeled particles under similar conditions, remained stable. They attributed this difference to an attraction generated by a hydrogen bonding interaction between dye molecules on the surfaces of different particles. Analogous effects have also been reported [1,2] for volume-labelled sterically-stabilised PMMA particles. Dinsmore et al. dyed PMMA particles by swelling pre-formed particles with a solution of rhodamine perchlorate in a mixture of cyclohexanone and acetone. Although the undyed PMMA particles behaved, essentially, as hard spheres the rhodamine-labelled particles showed, by contrast, a long-ranged repulsion which was attributed to electrostatic effects. Given such findings it is important to establish the phase behaviour of the NBD-labelled particles. Fig. 2 shows the percentage of crystals observed in suspensions of RSJF1, corrected for gravitational sedimentation [15], as a function of the core volume fraction  $\eta$ . Extrapolation to 0 and 100% crystal gave the core volume fractions at freezing and melting, as  $\eta_f = 0.412$  and  $\eta_m = 0.461$ . To compare the phase behaviour with that expected for hard spheres, we calculated the fractional width of the fluid-crystal transition,  $\Delta = (\eta_m - \eta_f) / \eta_f$ . Computer simulation studies have shown that the transition width  $\Delta$  is sensitive to the nature of the pair potential with a value of  $\Delta_{\text{hs}} = 0.103$  expected for hard spheres [16]. Increasing the

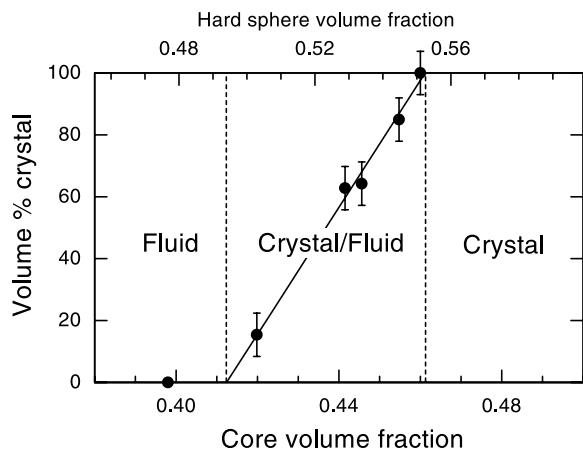


Fig. 2. The percentage by the volume of crystals observed in suspensions of the dyed latex RSJF1 as a function of the core volume fraction  $\eta$  (bottom axis). The dashed lines indicate the positions of the freezing and melting transitions. The equivalent hard sphere volume fraction ( $\phi$ ) is given on the top axis.

range of the repulsive forces increases  $\Delta$  while adding attractive terms to the potential reduces the width. The transition width is accordingly a useful measure of the strength of interparticle forces. Fig. 3 shows the transition width, for a variety of PMMA latices, determined as a function of size. Results are included for both fluorescent and non-fluorescent particles. It is clear that there is no systematic difference between labelled and unla-

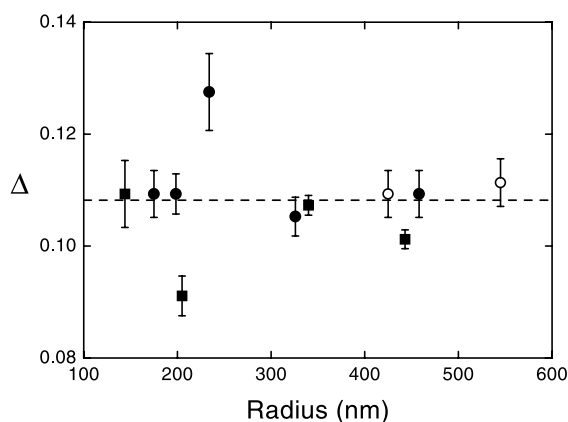


Fig. 3. The fractional width  $\Delta$  of the fluid-crystal phase transition as a function of particle radius. Data is shown for dyed (open circles) and undyed PMMA spheres (filled circles). The filled squares give equivalent measurements for undyed PMMA spheres from Underwood et al. [18].

beled particles confirming that the labelled PMMA particles behave as hard spheres. Averaging the values together, gives a mean width of  $\Delta = 0.108 \pm 0.003$  (the dashed line in Fig. 3) which is in excellent agreement with the value of 0.103 obtained for hard spheres from simulation [16].

The synthesised particles were intensely fluorescent with excitation and emission maxima at  $474 \pm 2$  and  $542 \pm 2$  nm. Fluorescence was readily excited by the 488 nm line of an argon laser. This is illustrated by the confocal micrograph shown in Fig. 4. The sample was a glassy mixture of fluorescent small PMMA spheres (RSJF5) and large non-fluorescent PMMA spheres. The binary dispersion had a size ratio of  $R_S/R_L = 0.58$  where  $R_L$  and  $R_S$  are the radii of the large (L) and small (S) species, a composition of  $LS_{15}$  and a total volume fraction of 0.49. The intense fluorescence of the NBD-labelled particles ensures that individual particles are readily distinguishable in Fig. 4.

Although the initial fluorescence of the NBD-labelled particles is intense the NBD-fluorophore is bleached by repeated laser excitation. The sensitivity to bleaching was assessed by repeatedly imaging, in the confocal microscope, a fixed area within a dense glassy sediment of dyed particles. The total intensity of fluorescence collected from

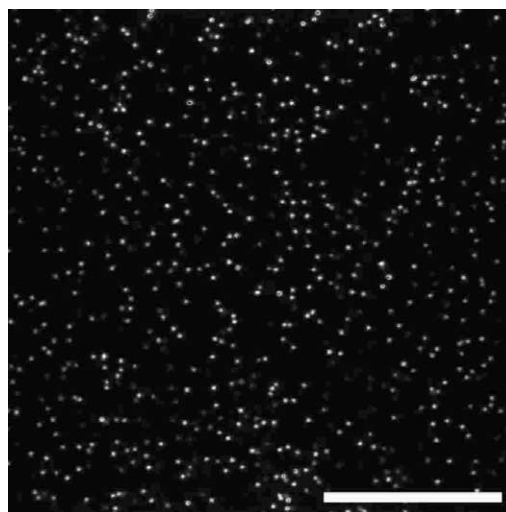


Fig. 4. Confocal image of a dense binary mixture of small fluorescent and large non-fluorescent PMMA spheres showing single particle resolution. The bar length is 18  $\mu\text{m}$ .

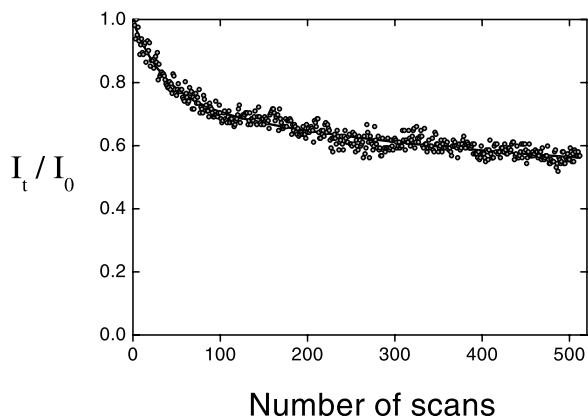


Fig. 5. The normalised fluorescent intensity as a function of exposure time for a glassy sample of NBD-labelled particles. The solid line is a double-exponential fit.

the region was followed as a function of time. In Fig. 5 the normalised fluorescence intensity is plotted as a function of the number of image scans. It is clear that although there is a rapid initial reduction in fluorescence, the rate of bleaching slows considerably after about 100 scans with 60% of the initial intensity still remaining after 500 scans. While this is a sizeable reduction in fluorescence yield, the fluorescence intensity is sufficient to ensure that the dyed particles can be detected, although with a reduced contrast, for several hundred scans.

#### 4. Conclusions

The present work details the preparation of fluorescent monodisperse poly (methyl methacrylate) spherical particles stabilised by a grafted PHSA layer. The particles were prepared using the fluorescent monomer NBD-MMA, methyl methacrylate and methacrylic acid in a modification of a dispersion polymerisation procedure, described originally by Antl et al. [5]. The main advantage of these new particles compared with other systems [1,14] is that the interaction potential is steeply repulsive and closely approximated by a classical hard sphere interaction. Because of their simple interaction potential, intense fluorescence and monodispersity the particles described

here are of particular interest as a model colloidal system for confocal studies of crystal nucleation [17] and glass formation.

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