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# 1 **Synthesis of Sub 100 nm Glycosylated-Nanoparticles via a One Step, Free Radical and Surfactant Free** 2 **Emulsion Polymerisation**

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## 13 **Abstract**

14 We present the facile synthesis of sub 100 nm glyco-nanoparticles via a one-step, free radical and surfactant  
15 free emulsion polymerisation. We show that by using sterically large, hydrophilic glycomonomers such as a  
16 lactose acrylamide with the charged azo initiator 4,4'-azobis(4-cyanovaleric acid) (ACVA), growing particles  
17 are stabilised enough to reproducibly produce well defined ( $PDI \leq 0.1$ ) glycoparticles with diameters below  
18 100 nm.

19

20 **Keywords:** Emulsion polymerisation, glyco-nanoparticle, surfactant free

21

22

## 23 Introduction

24 Emulsion polymerisation is an industrially favoured technique for synthesising many types of polymers due  
25 to its favourable kinetics, the high specific heat capacity of water and the low viscosity of the final polymer  
26 product<sup>[1]</sup>. One major use of emulsion polymerisation is the facile synthesis of functional polymer  
27 nanoparticles.<sup>[2-6]</sup> A classic emulsion polymerisation uses a surfactant, such as sodium dodecyl sulphate, to  
28 provide nucleation sites for growing “z-mer” polymer chains and particle stabilisation. Without the addition  
29 of surfactant to the system, a “self-nucleating” emulsion polymerisation, which will produce both latex  
30 particles and linear hydrophilic polymer in solution, typically produces particles in the hundreds of nanometres  
31 to micron diameters.<sup>[7-13]</sup> Latexes with particles under 100 nm in diameter are desirable however for  
32 applications such as drug delivery, to, for example, take advantage of the tumour targeting enhanced  
33 permeability and retention (EPR) effect.<sup>[14-16]</sup> However, particles made using a surfactant must then be  
34 extensively dialysed to remove the surfactant for use in applications such as drug delivery and photonics.<sup>[11,</sup>  
35 <sup>17, 18]</sup> There has therefore been a large amount of research into synthesising functional nanoparticles with  
36 diameters under 100 nm using “surfactant free” controlled radical polymerisation.<sup>[2, 19-25]</sup> One such technique  
37 is reversible addition-fragmentation chain transfer (RAFT) emulsion polymerization, in which a short  
38 amphiphilic di-block copolymer is prepared, then suspended in water to act as a surfactant for the emulsion  
39 polymerisation. The initial di-block subsequently becomes bound to the resulting latex particle via chain  
40 extension, conveniently imparting functionality from the hydrophilic section onto the resulting latex and  
41 avoiding the need for dialysis.<sup>[24, 26, 27]</sup> Such a synthetic approach can be time consuming and expensive when  
42 compared to standard emulsion polymerization,<sup>[28]</sup> although it allows access to a variety of functionalised  
43 particles with diameters below 100 nm.<sup>[29, 30]</sup> Nano-particles that are functionalised on their surface are also  
44 of particular interest in applications such as targeted drug delivery.<sup>[14, 17, 30-35]</sup> In particular, specific sugar  
45 moieties can be positioned at the surface of the particles, to target a class of protein receptors known as lectins.  
46 Previous research into using sugar-lectin targeting for drug delivery has shown that presenting the targeting  
47 sugar in a high concentration, such as that on the surface of a particle, increases the targeting efficiency of a  
48 drug delivery vector, taking advantage of the “glycocluster effect”.<sup>[36, 37]</sup> Recent developments in this area  
49 have applied the principle of targeting lectins with glycopolymers in more refined systems, using sequenced  
50 defined polymers alone and in combination with stimuli responsive polymers to elicit a more specific

biological targeting.<sup>[37-41]</sup> Amongst the many stimuli responsive systems reported, poly(*N*-isopropylacrylamide) is commonly used for temperature response, where heating above the cloud point of the system triggers a self-assembly or reveals a glycopolymer.<sup>[42, 43]</sup> Next to temperature, pH is arguably one of the most ubiquitously exploited stimuli, used to induce glycopolymer assembly and disassembly, or to release a covalently bound drug molecule *via* an acid cleavable linker; these amongst many other unique systems represent a growing and active field of research in drug delivery.<sup>[44-47]</sup>

Given their great potential, and current synthetic problems, it is therefore desirable to be able to synthesise functional polymer latex particles, under 100 nm in diameter with a low-cost and fast, one step synthesis that does not require further purification.

## Results and Discussion

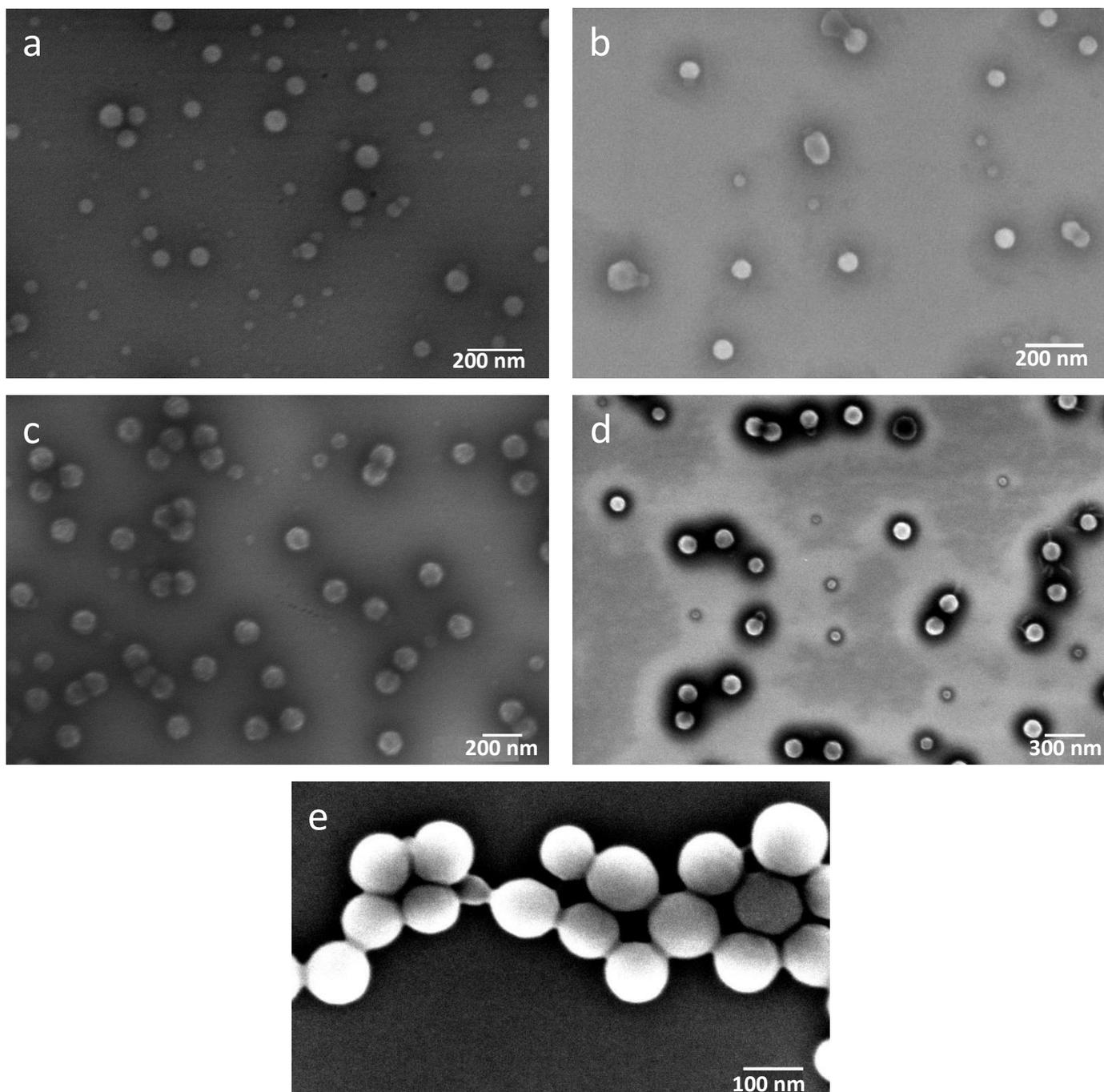
The full synthetic procedure for particle synthesis can be found in the supporting information. In brief: monomers and initiator were dissolved or suspended in water within a glass vial. This system was then, under an inert atmosphere, rigorously agitated with a magnetic follower at 800 RPM and heated to 70 °C for three hours. In all cases this resulted in the formation of well-defined nanoparticles ( $PDI \leq 0.1$ ), ranging in size from 50-350 nm (Table 1), which when broken up into constituent polymeric unimers, along with linear polymer in solution, showed dispersities as determined by GPC between 1.5-1.8 (SI: S2), and over 99% monomer conversion, determined by <sup>1</sup>H NMR (SI: S3).

	Composition of Free Radical Particle	Hydrophilic (μmol)	Hydrophobic (μmol)	ζ- Potential (mV)	Diameter by DLS (nm)	PDI <sup>a</sup>	Diameter by SEM (nm)
<b>A</b>	P(LactAm) <sub>1</sub> - <i>co</i> -(sty) <sub>5</sub>	4.711	23.55	-37.1	55	0.048	54
<b>B</b>	P(PEGA) <sub>1</sub> - <i>co</i> -(sty) <sub>5</sub>	4.711	23.55	-20.4	70	0.074	69
<b>C</b>	P(ManAm) <sub>1</sub> - <i>co</i> -(sty) <sub>5</sub>	4.711	23.55	-14.8	88	0.02	103
<b>D</b>	P(HEAm) <sub>1</sub> - <i>co</i> -(sty) <sub>5</sub>	4.711	23.55	-39.2	112	0.057	117
<b>E</b>	P(sty)	N/A	23.55	-37.3	108	0.063	106
<b>F</b>	P(LactAm) <sub>1</sub> - <i>co</i> -(BA) <sub>5</sub>	22.8	114	-31.3	85	0.1	-
<b>G</b>	P(LactAm) <sub>1</sub> - <i>co</i> -(BA) <sub>10</sub>	22.8	228	-38.1	198	0.058	-
<b>H</b>	P(LactAm) <sub>1</sub> - <i>co</i> -(BA) <sub>20</sub>	22.8	456	-43	260	0.051	-
<b>I</b>	P(LactAm) <sub>1</sub> - <i>co</i> -(BA) <sub>30</sub>	22.8	684	-37.3	256	0.06	-
<b>J</b>	P(LactAm) <sub>1</sub> - <i>co</i> -(BA) <sub>50</sub>	22.8	1140	-43.4	348	0.064	-

Table 1: Characterisation for all polymer particles synthesised with surfactant free emulsion polymerisation. All polymerisations used an ACVA initiator concentration of  $3.13 \times 10^{-6}$ . <sup>a</sup> Determined using equation S1, see Supporting Information

73 Having optimised a one pot, single step method to produce nanoparticles, the effect of varying the  
74 hydrophilic monomer was investigated. It is proposed that in the early stages of this synthesis, hydrophilic  
75 homopolymer is predominantly formed (as it is fully soluble in the water phase with the initiator), which is  
76 then followed by the separate formation short copolymers with the small amount of hydrophobic monomer in  
77 solution. The copolymers formed then act as a surfactant to drive an emulsion mechanism, where nucleated  
78 particles are stabilised by the initiator and hydrophilic polymer, growing by both polymerisation and particle  
79 aggregation.<sup>[1, 48]</sup> As such, the choice of hydrophilic monomer used should have a large impact on the size of  
80 the resulting particle. To test this, the hydrophilic monomer was varied, whilst keeping all other reaction  
81 conditions the same. Lactose acrylamide (LactAm), mannose acrylamide (ManAM), hydroxyethyl acrylamide  
82 (HEAm) and poly(ethylene glycol) acrylate (PEGA) were employed, in order to assess the effect of monomer  
83 size and hydrophilicity. The same synthesis was also performed using no hydrophilic monomer, but with the  
84 negatively charged 4,4'-azobis(4-cyanovaleric acid) (ACVA) initiator as hydrophilic component. The  
85 polymerisation led to a range of particles which size varied from largest to smallest in the order: HEAm ≥  
86 ACVA > Mannose > PEGA > Lactose (Table 1, S4 and S5), confirming that the hydrophilic monomer used  
87 plays a large part in the stabilisation of this emulsion system and can be used to influence the size of any  
88 resulting particle.

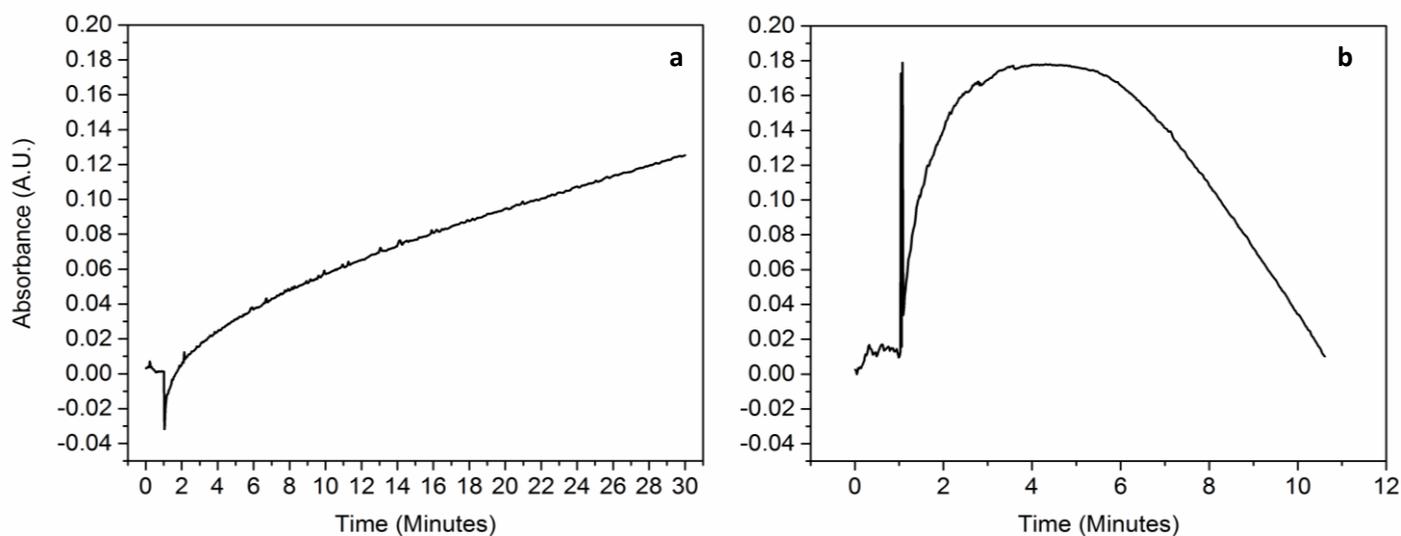
89 We hypothesise that monomers with greater steric hindrance, charge, and/or water solubility, give  
90 greater stabilisation, thus producing smaller particles, as they have a lower apparent number of aggregation.  
91 It is also noteworthy that emulsion polymerisation with only the ACVA initiator as the hydrophilic component  
92 produced well defined particles of 108 nm diameter (as established by DLS). We expect the stabilisation in  
93 this case is due to the charged carboxylic acid group of ACVA having been deprotonated by sodium hydroxide  
94 to ensure aqueous solubility, showing the importance of the initiator in stabilising the growing latex  
95 particles.<sup>[13, 49]</sup> In the case of the HEAm particles, the initiator potentially provided the majority of the particle  
96 stabilisation, as both the HEAm and ACVA-only particles were of an equivalent size and zeta potential.  
97 Lactose acrylamide produced the smallest particles 55 nm in diameter (determined by DLS and confirmed by  
98 SEM, giving an average size of 54 nm) (Figure 1). This suggested that lactose acrylamide provides superior  
99 stabilisation when compared to all other hydrophilic co-monomers tested, due to its 7 hydroxyl groups, which



100 Figure 1: SEM images showing particle size and morphology. Pictures a, b, c, d and e correspond to styrene particles  
 101 with shells of lactose acrylamide, PEGA, mannose acrylamide, hydroxyethyl acrylamide and no hydrophilic monomer  
 102 respectively. Average diameter for each in table 1  
 103 increase its number of hydration and water solubility, as well as good steric stabilisation from its bulky  
 104 disaccharide structure. These results are remarkable as surfactant-free polymerisation does not typically  
 105 provide access to particle diameters under 100 nm, due to insufficient stabilisation which causes particle  
 106 aggregation. Indeed, most examples to date use the initiator potassium persulfate (KPS) to provide sufficient  
 107 stabilisation, e.g. in the emulsion polymerisation of styrene and methyl methacrylate.<sup>[13, 50]</sup> In this work, we  
 108 show that styrene particles made with ACVA initiator and either lactose acrylamide, PEGA or mannose  
 109 acrylamide as a co-monomer provide access to particles of diameter 55, 70 and 88 nm respectively. Being

110 able to reliably produce particles under 100 nm in diameter without the use of a surfactant or initial polymer  
111 diblock shows the potential for using a charged initiator with sterically large or charged monomers,  
112 particularly glycomonomers, for their ability at stabilising a latex and to produce functional nanoparticles.

113 The surface functionality of the particles was assessed by a turbidimetric aggregation test between  
114 mannose coated particles (“particle C” Table 1), and the lectin concanavalin A (Con A), which is known to  
115 bind preferentially to mannose and glucose.<sup>[37, 51, 52]</sup> Latex was mixed in a UV-Vis spectrometer with a 1.3  $\mu\text{M}$   
116 solution of Con A in TRIS buffer at pH 7, and the absorbance tracked at 500 nm (Figure 2). This test was  
117 performed before and after removing any free sugar homopolymer in solution by precipitating the particles  
118 out of suspension using centrifugation for ten minutes at 13,500 rpm, and re-suspending the particle pellet in  
119 de-ionised water, a process repeated three times. This purification technique was preferred to dialysis, as it  
120 ensured that even the longest free radical polymer not anchored to the particle was removed, as there is no  
121 upper molecular weight cut off, which is necessary when purifying using a dialysis membrane.

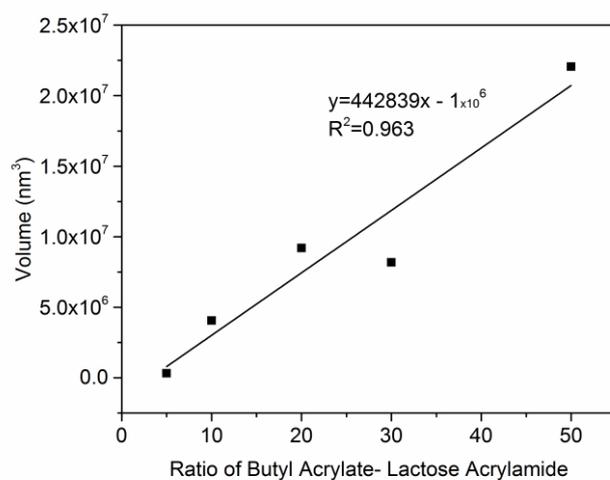


122

123 Figure 2: UV-Vis turbidimetric aggregation tests between mannose coated particles and concanavalin A. a) raw latex  
124 with free glycopolymer in solution b) purified latex with free glycopolymer removed from solution via centrifugation

125 The results showed a very slow increase in absorbance over 30 minutes when the raw latex was mixed  
126 with a Con A solution. However, when the same test was performed on the purified latex, a much faster  
127 increase in absorbance, and subsequent reduction (due to large particulate aggregates sedimenting out of  
128 solution), was observed over 11 minutes. We hypothesise that the reduced response from the raw latex is due  
129 to binding competition from the free sugar homopolymer in solution. This result confirmed that the particles

130 have a covalently bound glycopolymer shell. A modified turbidimetric test was also performed with the  
131 addition of free mannose post-aggregation in excess to compete for ConA binding sites and induce aggregate  
132 break up, confirming that aggregation was due to sugar-lectin binding (SI: S6). Gravimetry was also used to  
133 quantify the amount of hydrophilic glycopolymer bound to the surface of the particles. The mass of 1 mL of  
134 raw latex was compared to that of 1 mL the purified latex, after drying both solution in a vacuum oven at 40  
135 °C for 16 hours. The average difference in mass after three repeats represented the mass of polymer in solution,  
136 and was used differentially to determine the amount of polymer bound to the particles, 41% of the polymer.



137 Figure 3: Particle size calibration with varying molar ratios of hydrophilic lactose acrylamide monomer to  
138 hydrophobic butyl acrylate monomer plotted against the resulting particle volume

139 To determine if this emulsion process can reliably control particle size over a larger range, and be used  
140 with a different hydrophobic monomer, five latexes with varying ratios of lactose acrylamide to butyl acrylate  
141 were synthesised and the monomer ratios plotted against the resulting particle volume. The results (Table 1  
142 and Figure 3) show that by varying the ratio of lactose acrylamide: butyl acrylate from 1:5 to 1:50, the diameter  
143 of the resulting particle could be reliably adjusted to between 85 and 348 nm (321,555 – 22,066,629 nm<sup>3</sup>),  
144 with a linear relationship between hydrophobic component and resulting volume. Particle diameters under 100  
145 nm were also produced with butyl acrylate, showing the ability of this method to produce functionalised  
146 nanoparticles with monomers other than styrene. To further explore the limits of our system, we  
147 experimentally determined the maximum particle concentration possible using this synthetic method at 15-20  
148 wt% of monomer. This was determined by varying the total solid content of the emulsion whilst maintaining  
149 a molar ratio of 1:10 lactose acrylamide to styrene. Well-defined particles were produced up to 15 wt% of

150 monomer (SI: S7). At 20 wt% and above, defined particles were not obtained and formed aggregated to such  
151 an extent that a reliable size was not obtainable by DLS.

## 152 Conclusion

153 We have shown that a surfactant free emulsion can reliably produce nanoparticles between 50-350nm in  
154 diameter in a one-step synthesis, with little or no purification. The initiator ACVA and glycomonomers,  
155 particularly disaccharide monomers with a large number of hydration such as lactose acrylamide, are  
156 extremely good at stabilising emulsion polymerisations and provide a useful tool for synthesising low  
157 diameter, well defined carbohydrate coated nanoparticles suitable for biological use.

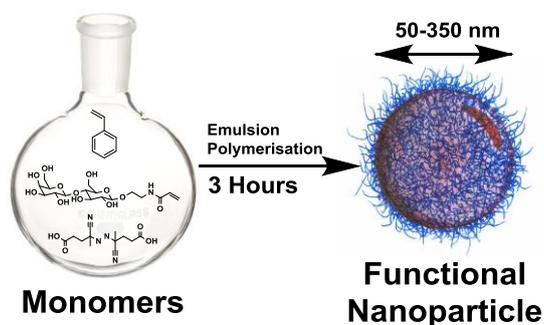
## 159 Acknowledgments

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161 S.P.), are acknowledged for financial support.

162

## 163 Table of Contents Summary Text

164 **A rapid one-step approach to synthesising sub-100 nm polymeric glyco-nanoparticles** without the use of  
165 a surfactant is presented. The free radical method takes advantage of the exceptional ability of  
166 glycomonomers, particularly lactose acrylamide to stabilise a growing latex particle, with the use of the  
167 charged initiator ACVA to go from monomer to final latex in under three hours.



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