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Fluorescent organosilica micro- and nanoparticles with controllable size

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Abstract

This paper reports on the synthesis of uniformly dye-doped organosilica particles with narrow size distribution. The particle size can be controlled from tenths of nanometers up to several micrometers, whilst still maintaining monodispersity. Microparticles were observed to swell in various solvents up to ∼2.5 times their original volume, suggesting the presence of a gel-like internal structure. As shown by confocal microscopy, this morphological control of particle swelling has important implications for the encoding of the nano/micro particles with organic dyes, such as rhodamine B isothiocyanate. Swelling allows the dye to penetrate the organosilica matrix and produce uniformly dye-doped nano- and microparticles. Finally, we suggest a coagulation model for the particle formation which significantly differs from conventional Stöber synthesis.

Keywords: Colloids; Ormosil; Organosilica; Nanoparticles; Microspheres; Dyes; Biomolecules

1. Introduction

Optically-encoded silica particles can be utilised in bioanalytical applications such as screening of biomolecules [1], intracellular sensing [2], colloidal encoding [3,4], or signal amplification [5–7]. The usefulness of silica-based materials stems from the variety of surface modification and immobilisation procedures available for the coupling of biomolecules [6]. Key criteria that particles should satisfy in order to be suitable for these applications are monodispersity, control over particle size, uniform surface loading and uniform distribution of internal encoding elements such as organic dyes or quantum dots.

Stöber et al. achieved the controlled growth of monodisperse nonporous silica particles with diameters ranging from 20 nm up to approximately 2 μm [8]. This technique utilised silicon alkoxide precursors dissolved in short chain alcohols in the presence of ammonia. Other approaches to synthesising silica microspheres are based on aerospraying [9], micron-sized injection nozzles [10], and ultrasonic oscillation [11]. However, these methods result in a high degree of polydispersity in size distribution. In comparison, Barbe et al. produced spherical silica particles with narrow size distribution over an extensive size range of 10 nm–50 μm, combining sol–gel synthesis with surfactant-stabilised water-in-oil emulsion polymerisation chemistry [12].

Thiol- and amine-based organosilanes can be incorporated into the silica network by a seeded growth technique [13,14], to allow for the covalent attachment of thiol- and amine-reactive organic dyes and the conjugation of biomolecules. We recently developed an alternative surfactant-free two-step approach to synthesise thiol-functionalised organosilica microparticles, based on acid-catalysed hydrolysis and condensation followed by base-catalysed condensation [1,15]. By carefully controlling the base-catalysis, these organosilica microparticles proved to be monodisperse with mean particle diameters ranging from 2 to 5 μm.

Recent approaches in diagnostic bead-based assays have required the implementation of optical encoding strategies, whereby the uniform incorporation of encoding elements into

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nano- and microparticles is of major importance [7,16]. Traditional silica microsphere production has utilised the well-known Stöber synthesis method, as mentioned above. However, the structure of the internal network of these silica particles does not allow the access of large organic molecules such as fluorescent dyes [8,17]. In order to internalise encoding elements such as fluorescent dyes, several possibilities are available. These include the generation of porosity within the silica network using surfactant template strategies [18–21], template-free strategies [1] and the incorporation of encoding elements during synthesis of the silica material [13,22–24].

Incorporation of encoding dyes into common polymeric particles is easily achieved by controlled swelling in suitable solvents. While Stöber silica does possess branched, microporous internal structure, incorporation of dyes is negligible as any swelling is of a very small scale [25]. In comparison, some organically-modified silicate (ORMOSIL) bulk materials possess enough structural flexibility to allow solvation, resulting in material swelling not unlike a polymer gel [26]. Research by Rao et al. investigated the swelling properties of ORMOSIL gels in aqueous solutions and their applications for microdevices [27,28]. These bulk materials were touted as environmental sensors and shown to swell in response to changes of pH, temperature, and an applied field. However, these ORMOSIL gels were not adopted for particle synthesis. In comparison, Goller et al. investigated the swelling of organosilica microparticles in the nonpolar solvent *n*-heptane [29]. These microparticles were prepared using a combination of bi- and trifunctional silanes and resulted in relatively small particles with diameters below 1.5 μm. Overall, the organic components of these ORMOSIL materials have consisted primarily of alkyl chains. In contrast to these materials, Walcarius et al. produced mercapto-functionalised organosilica particles using the co-condensation of tetraethoxysilane (TEOS) and 3-mercaptopropyltrimethoxysilane (MPTMS) [30–32]. The resulting particles were highly functionalised and successfully removed large amounts of mercury from aqueous solutions. However, the high polydispersity of these particles renders them unsuitable for most bioanalytical applications.

It is evident from the research to date that there has not been a successful synthetic route developed which brings together all four desirable elements required in a particle designed for biomolecular synthesis and screening namely: monodispersity, selective functionality, controlled size and robust dye incorporation.

In this report we describe a method which achieves all of these objectives. The modified synthetic route demonstrates the size control of novel organosilica micro- and nanoparticles and an aggregation based model for the formation of emulsion droplets which condense to form solid particles is proposed. Control of particle size (50 nm–3 μm) is achieved by varying the total monomer concentration. In addition, dye molecules are shown to be covalently bound throughout the interior of both organosilica nano- and microparticles, enabled by solvent assisted swelling of the organosilica matrix.

2. Materials and methods

2.1. Materials

3-Mercaptopropyltrimethoxysilane (MPTMS, 95%) was obtained from Lancaster and used as supplied. Triethylamine (TEA, 99%), hydrochloric acid (37%), dimethylformamide (DMF), and rhodamine B isothiocyanate (RBITC) were obtained from Sigma–Aldrich and used as received. Commercial silica particles with a diameter of 5.17 μm (standard deviation unspecified) were purchased from Bangs labs.

2.1.1. Synthesis

Organosilicate particles were produced using a two step process. The initial acid catalysis step is described in detail by Miller et al. [15]. The resulting emulsion was centrifuged and the supernatant was separated from the oil. The supernatant phase was then diluted with pH 3.5 water, to obtain various concentrations (0.1, 1, 5, 10, 50, and 100%) of the supernatant phase. These supernatant solutions are referred to as "0.1%, . . . , 100% solutions." Finally in the base-catalysed step, 70–100 μl of TEA was rapidly added to 100 ml of the supernatant–water mixtures under continuous stirring.

Approximately 30 min after the addition of TEA, the formed particles were separated from solution by centrifugation and then washed in ethanol. This procedure was applied for samples with supernatant concentrations >3%. Due to a slower particle formation process at lower concentrations the samples with supernatant concentrations of 0.1 and 1% were left under basic conditions for 24 h before being centrifuged and washed.

2.1.2. Dye incorporation

For investigations into dye localisation, the organosilica particles were covalently labeled with rhodamine B isothiocyanate (RBITC) in DMF. Once dye incorporation was complete, bead pellets were washed from DMF into ethanol for confocal microscopy analysis.

2.2. Methods

2.2.1. Electron microscopy

Scanning electron microscopy (SEM) images of platinum coated samples were collected on a JEOL JSM 6400F, using an accelerating voltage of 5–10 kV. Images were analysed with Image-Pro software to determine size distributions, using a minimum of 200 particles.

Transmission electron microscopy (TEM) studies on organosilica particles were carried out on a JEOL 2010 microscope at an operating voltage of 200 kV.

2.2.2. Optical transmission microscopy

The swelling of organosilica and silica microparticles (Bangs Labs) in different solvents (water, ethanol, DMF) was investigated by suspending dried microparticles in approximately 1 ml of solution and left for at least 48 h. A Nikon Eclipse TE2000-E with a Photometrics CoolSnap HQ camera attachment was used to image microparticles at 100× magnification