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Novel epoxy-silica nanoparticles to develop non-enzymatic colorimetric probe for analytical immuno/bioassays

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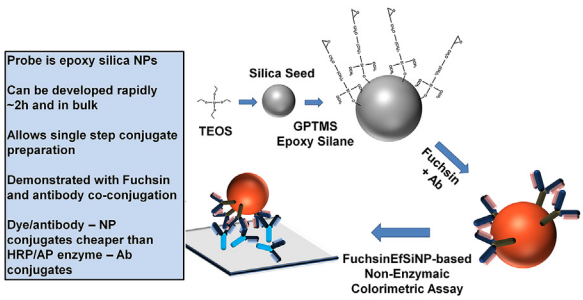
HIGHLIGHTS

- First report of very fast synthesis of epoxy functionalized silica nanoparticles in a scalable single pot approach.
- Epoxy functionalized silica nanoparticles can be used for single step immobilization of variety of dyes like fuchsin and biomolecules.
- Fuchsin conjugated nanoparticles can be employed as enzyme/chemical reaction-free colorimetric probe for faster assay to result turnaround time.

GRAPHICAL ABSTRACT



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ABSTRACT

We have developed a novel method to develop epoxy silica nanoparticles (EfSiNP) in a single pot. High surface coverage of epoxy functional groups between 150 and 57000 molecules per particles (~10^{13}–10^{16} molecules/mL of 200 nm EfSiNPs) was achieved for different preparation conditions. We then created a red colored probe by conjugating Fuchsin dye to the epoxy functionalities of EfSINPs. Anti-mouse IgG was co-immobilized with Fuchsin and their ratios were optimized for achieving optimum ratios by testing those in functional assays. Dye to antibody ratios were in good negative correlation with a coefficient of −1.00 measured at a confidence level of over 99%. We employed the developed non-enzymatic colorimetric immunonanoprobe for detecting mouse IgG in a direct immunoassay format. We achieved a sensitivity of 427 pg/mL with the assay.

1. Introduction

Physical properties, such as shape, size, charge, and functionalities of nanoparticles (NP) can easily be manipulated thus are widely used in analytical chemistry and biology [1–7]. This is

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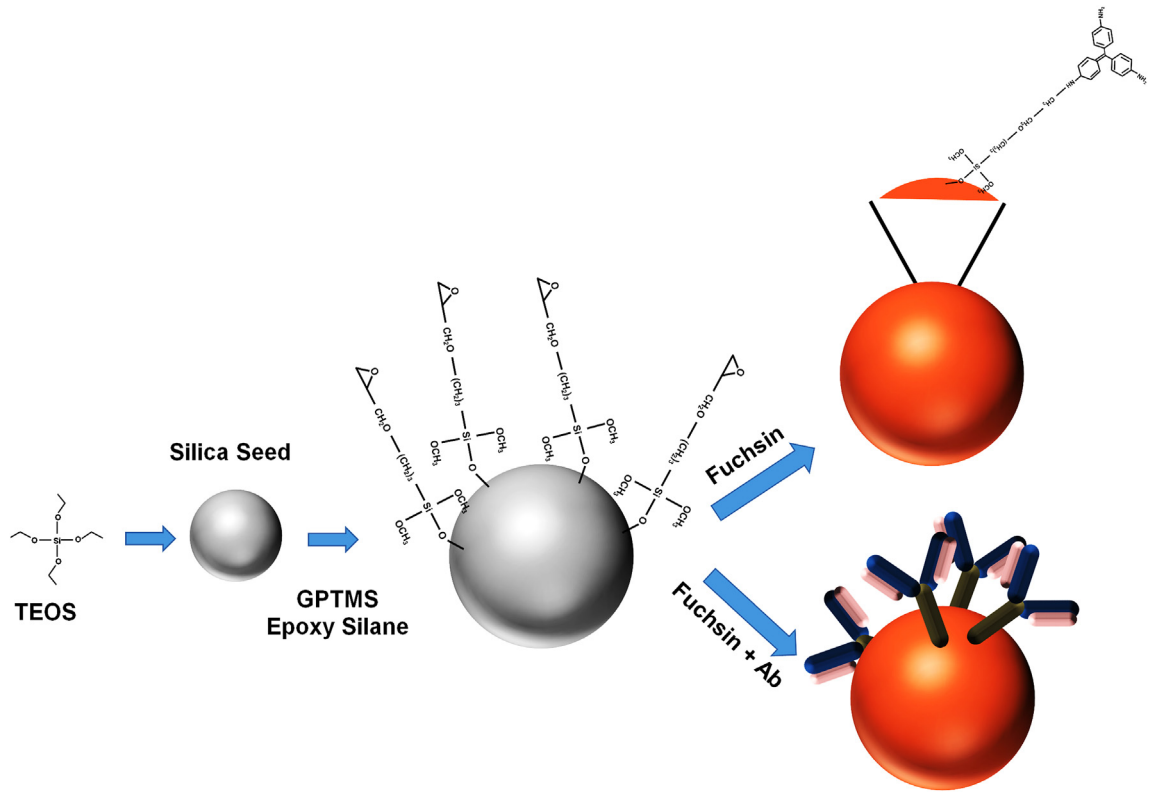
attributed to our better understanding of NP formation and surface functionalization [8–12]. However, silica nanoparticles (SiNP) are still mostly favored for applications associated with surface functionalization [10], due to the availability of self-grafting polymer precursors, such as silanes, that exist in constructs containing variety of functional groups [1,7,13].

Bioconjugation onto nanoparticles serves an important role in various applications, such as immunoassays, drug delivery, and imaging [1,2,14–16]. SiNPs have been used for decades for these applications. Adding amine functionality on SiNP using 3-aminopropyltriethoxy silane [17] is by far the most common approach that allow for bioconjugation using several crosslinkers, such as amine to amine homobifunctional glutaraldehyde and carboxyl to amine heterobifunctional carbodiimide [1,18,19].

In this first ever report of creating epoxy-functionalized silica nanoparticles (EfSiNP) in a single pot proce**ss** (Scheme 1), we have synthesized EfSiNPs by customized modification of 'Bhakta method', which is our published novel approach [20] and employed it for rapid bioconjugation and assay applications. Previously, Ishimura's group synthesized one pot epoxy NPs by precipitating epoxy silane alone under different Stöber formulations [21]. They were able to synthesize NPs in only 0.5 mL batches with reaction times obscurely long extending between 1 and 3 days. There are several other reports grafting epoxy silanes on metal NPs, such as TiO_2 [22], Fe_3O_4 [23] etc. but only few reports were found pertaining to the epoxy functionalization of the pre-synthesized SiNPs [24–31]. The major disadvantage of grafting functionalities post synthesis is the poor surface coverage of the functionality-bearing chemical moieties. On the contrary several reports claim significant improvement of total number of functional groups and their distribution homogeneity on NP surface with co-condensation [6,32–34] with some reservations [35]. Therefore, we conceived the idea of functionalizing particles during their synthesis by adding epoxy silane into the backbone of the SiNP. This will also allow us to conjugate desired molecules on the surface of these particles, which we have demonstrated by conjugating fuchsin dye to the particle surface and created red colored silica nanoparticles.

In recent times several enzyme-free immunoassay approaches (Supplementary Table 1) have been developed. Enzyme-mimics, such as metal oxides [36,37], metal complexes/hemin [38], or Palladium-Iridium nanoparticles [39], can catalyze colorimetric substrate reactions similar to that of horseradish peroxidase constituting indirect colorimetric methods. Dye-doped colored particles have also been demonstrated as probes for enzyme-free immunoassays. Most of these particles are either colored dye-doped polymeric [40–44], *viz.* latex/polystyrene, or doped nanoparticles [2,29,45–47]. Inherently colored nanoparticles, such as gold nanostructures, are also routinely employed in immunosensing applications [48]. Several intuitive strategies, such as gold nanoparticle-catalyzed decolorization [49], have also been reported for enzyme-free colorimetric immune/bioassays. In the present manuscript we demonstrated the development of red colored EfSiNPs via surface conjugation of fuchsin dye and employed it as a signal probe in an immunoassay.

We present herein: (i) novel single pot synthesis of epoxy-functionalized silica nanoparticles, and (ii) an approach to develop Fuchsin-conjugated non-enzymatic color probe for performing immuno/bioassays. In order to achieve these goals, we developed a nanoprobe by single pot conjugation of Fuchsin dye and anti-mouse IgGs to the EfSiNPs and employed the conjugates for performing non-enzymatic colorimetric immunoassays.



Scheme 1. Schematic presentation of epoxy-silica nanoparticle synthesis. Step 1 represents the starting of condensation process of tetraethylorthosilica (TEOS) in water-ethanol medium with NaOH as base catalyst. Step 2 describes the formation of epoxy-silica nanoparticle after addition of (3-Glycidyloxypropyl)trimethoxysilane at different time. The time of epoxysilane addition controls the size and surface medication of these epoxy-silica nanoparticles. Step 3 demonstrates the application of these nanoparticles for easy and faster conjugation of amine-containing biomolecules; top is Fuchsin conjugation that imparted the red color while lower panel is Fuchsin and antibody co-conjugation.

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