ISOFURE Methodology: A Novel Technique to Enhance the Loading and Stability of Nanoparticles

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Statement of Purpose: The unique chemical and physical properties of nanoparticles render them as effective carriers for various biomedical applications. For these applications, it is important that the surface chemistry of the particle is tailored precisely, and in most cases, it becomes difficult to prevent agglomeration during multistep functionalization processes due to changes in surface properties (e.g. charge neutralization, hydrogen bonding, interparticle crosslinking, etc). Herein, we introduce the ISOFURE (ISOlate-FUnctionalize-RElease) strategy using biodegradable poly(β-amino ester) (PBAE) hydrogels to synthesize functionalized nanoparticles with enhanced loading and stability.

Methods: A pre-synthesized macromer from isobutyl amine and poly(ethylene glycol)400 diacrylate was used to synthesize the PBAE hydrogel that can physically ISOlate the nanoparticles during functionalization. Both polymeric FUnctionalization via atom transfer radical polymerization (ATRP) and biomolecular loading of enzyme catalase was done inside the PBAE-NP composite. Degradation of the PBAE matrix via hydrolysis was finally carried out to RElease the functionalized stable nanoparticles (figure 1).

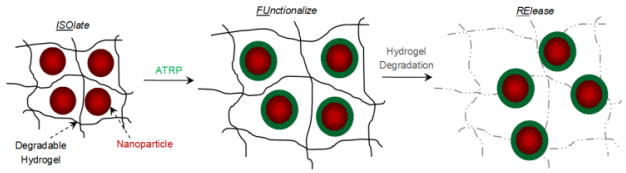

Figure 1. Scheme of ISOFURE methodology

Results/Discussion: UV-visible spectroscopy revealed the presence of similar sized GNPs from both added gold nanoparticles (GNPs) during polymerization as well as in-situ precipitated GNPs inside the pre-polymerized PBAE hydrogel matrix. Also, UV-visible spectroscopy confirmed the growth of a hydrogel shell over the nanoparticle, and dynamic light scattering analysis confirmed the presence of temperature responsive poly(ethylene glycol)n dimethacrylate crosslinked poly(N-isopropyl acrylamide) hydrogel shell. The PBAE matrix can be tailored by choosing the right macromer to tailor the size of the in-situ precipitated GNPs.

The aging/settling studies of the ISOFURE method synthesized ATRP GNPs showed higher stability than that of the solution based ATRP grown hydrogel coated GNPs (figure 2). The ISOFURE PBAE matrix physically entrapped the GNPs from aggregating with each other arising due to interparticle crosslinking during the shell growth. Further, no complex purification steps (centrifugation, rotary evaporation, etc) were involved in the synthesis of these polymer coated GNPs.

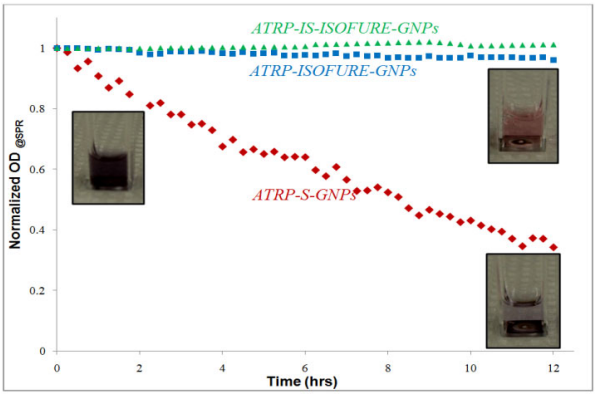

Figure 2. Settling kinetics of the ATRP grown crosslinked PNIPAAm hydrogel coated solution based GNPs and ISOFURE-GNPs clearly indicating that the hydrogel coated ISOFURE-GNPs exhibit enhanced stability than the S-GNPs.

The biotinylation of the GNPs was confirmed using UV-Vis spectroscopy. The dual action of NP release out of the degrading matrix and the diffusion of high molecular weight streptavidin resulted in the formation of streptavidin conjugated GNPs. An increased concentration of biotinylated catalase eventually resulted in the saturation of NP surface. Catalytic activity analysis using o-phenylene diamine assay showed enhanced loading of ISOFURE GNPs as compared to the 1:x charged thiol stabilized GNPs (table 1). This increased activity is due to the 100% availability of the NP surface area than the partially biotinylated 1:x GNPs.

Streptavidin GNPs + 8 µM Biotinylated Catalase	**Catalase Activity** (µU/GNP)
1:10 (no streptavidin; control)	~0.05
1:100	12
1:20	64
1:10	109
ISOFURE GNPs	327

Table 1. Increased activity of S-GNPs with increasing amounts of particle surface area occupied by biotin (decreasing amounts of charged stabilizer). The biotinylated ISOFURE GNPs devoid of stabilizer shows maximum catalase activity confirming enhanced biofunctionalization.

Conclusions: We have successfully demonstrated a route for forming stable functionalized nanoparticles using the ISOFURE methodology. Results show that the PBAE matrix can be used to provide increased particle stability. Also, this method eliminates the need for stabilizing agents, thereby utilizing the entire particle surface area for increased loading.

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