Engineering Multifunctional Nanoparticles with Dual Modality Imaging Capabilities

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Abstract:

Multifunctional nanoparticles with dual modality imaging capabilities offer the ability to deliver multiple optical imaging agents to targeted locations for use with various instruments. Here we describe the size distribution, NIR dye and MNP incorporation rate, and stability of nanoparticles formed with microemulsions using various concentrations of their respective components.

Introduction:

Multifunctional nanoparticles may be both synthesized and targeted to specific locations by a wide variety of processes [1, 2, 3] using a broad array of materials. Microemulsions, which consist of a thermodynamically stable emulsion of water and oil that forms a transparent dispersion in the presence of an amphiphilic surfactant [4, 5], have garnered particular interest as drug delivery systems due to their capacity to hold both hydrophilic and hydrophobic drugs, allowing simultaneous delivery of multiple drugs with little risk of causing an immune response [4, 6]. Typical imaging agents include near infra-red (NIR) dyes, whose emission and absorption spectra are not subject to background fluorescence overlap with tissues, and magnetic nanoparticles (MNP) used for imaging within magnetic fields [2, 3].

Within this study, we characterized several formulations used to engineer a dual modality nanoparticle carrier system encapsulating 10 nm MNP and the widely used NIR dye indocyanine green (ICG) [7]. The size of the nanoparticles and dye incorporation were primary areas of interest in examining each method.

Methods:

Nanoparticle Synthesis. Oil in water (O/W) microemulsions encapsulating ICG and MNP were prepared with varying concentrations of ICG, DOTAP, and purified MNP in 0.75 ml poly(sterene-*b*-arylic acid) (PSPAA) dissolved in 1 ml $CHCl_3$) with 0.25 ml surfactant (either polyvinyl alcohol -PVA, bovine albumin [BSA], or poly(maleic anhydride-alt-1-octadecane) [PMAO]). PVA was modified for targeting using a carbodiimide EDC crosslinking reaction. Emulsions were formed by sonication for two minutes and evaporated while stirring overnight. Excess ICG and MNP were purified from the nanoparticles via three rounds of centrifugation (dispersed in H_2O, 13K, 20 min).

Determining Maximum ICG Encapsulation. Purified particles were measured via bright field transmission electron microscopy (CM100 TEM). ICG encapsulation rates were determined using a Fluoromax4 fluorometer to compare ICG concentration in purified particles with the initial ICG in the preparation.

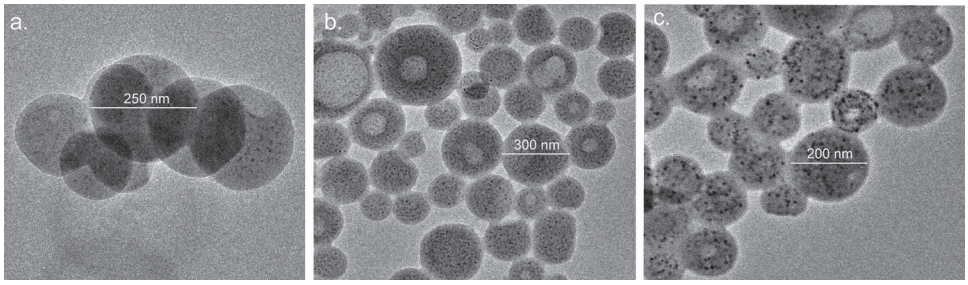

Figure 1: Nanoparticles formed with a) 1% PVA, b) 2% BSA, and c) 1% PMAO.

Results and Discussion:

We expected that ICG encapsulation rates would increase with greater concentrations of polymer (PS-PAA), lipid (DOTAP, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate), and ICG, and lower concentrations of surfactant (PVA, BSA, PMAO) and MNP. ICG has a dual hydrophobic and hydrophilic nature, causing it to preferentially partition into the aqueous phase, while the relatively hydrophobic MNPs might further push ICG out of the organic phase. Conversely, the addition of a lipid (DOTAP) might help to pull it into the organic phase. Likewise, it has previously been suggested that the fixed capacity of nanoparticles causes the concentration of polymer to play a role in determining dye loading [8].

In fact, only a small negative correlation between high MNP presence and ICG encapsulation was observed, although TEM images show that virtually all MNPs were taken into the organic phase (Figure 1). There was no certain relationship between surfactant concentration and ICG encapsulation rate. Adding increasing amounts of ICG increased the encapsulation rate up to 2 mg, at which point adding more ICG decreased fluorescence intensity (Figure 2). These results are consistent with earlier studies observing the self-quenching properties of ICG [7]. It was generally observed that higher percentage concentrations of PS-PAA resulted in greater ICG encapsulation efficiency, most likely since higher amounts of carrier polymer resulted in formation of more nanoparticles.

Although we expected that increasing the concentration of surfactant would result in small and more uniform particle size, we observed little relationship between surfactant concentration and particle size. Increased DOTAP concentrations resulted in higher dye loading, but combined with low surfactant concentrations, might have contributed to nanoparticle aggregation. Aggregation was also observed in particles formed using surface modified PVA, indicating that BSA, which formed relatively stable nanoparticles with a high ICG encapsulation rate and a 200-400 nm size distribution, might be better suited to future research with targeting molecules.

Future Work:

Future work could include the application of ICG / MNP encapsulated microemulsions to targeting studies in live cell lines.

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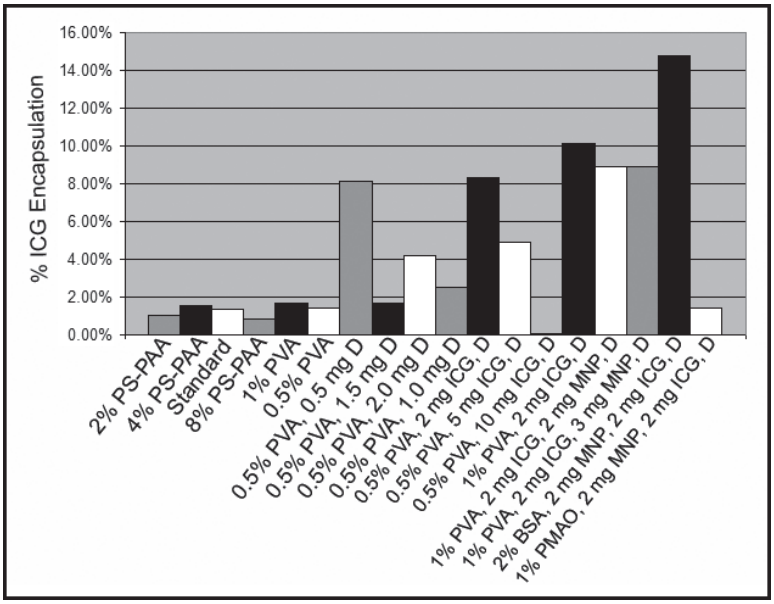

Figure 2: Standard was 1 mg MNP, 0.20 ml 6% PS-PAA, 0.70 ml 2% PVA, and 0.5 mg ICG. D = DOTAP (1 mg unless otherwise stated).