Influence of Redox Attributes on the Self-Assembly and Functional Properties of Human Albumin Nanoparticles

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Human serum albumin (HSA) is widely used for nanoparticle-based drug delivery due to its biocompatibility, biodegradability, and intrinsic targeting properties, with clinically approved formulations such as albumin-bound paclitaxel. However, redox-driven self-assembly approaches—commonly employed for HSA nanoparticle (Nab) synthesis—rely on diverse reductants and reaction conditions, resulting in structurally heterogeneous systems and variable outcomes across studies. Here, we systematically investigate how redox conditions govern nanoparticle formation, thiol reactivity, and the resulting physicochemical and functional properties.

Using circular dichroism (CD), dynamic light scattering (DLS), and reverse-phase HPLC (RP-HPLC), we identified a three-stage redox-driven assembly process. Initially, HSA undergoes progressive disulfide reduction, followed by nanoparticle formation and a final ageing stage associated with partial re-oxidation and structural stabilization. Consistently, DTNB assays showed a progressive increase in free thiols during reduction and a partial decrease upon ageing, while MALDI-TOF revealed heterogeneous cysteine reactivity across assembly states.

Thiol capping using N-ethylmaleimide (NEM) enabled modulation of Nab thiols reactivity. Kinetic analysis of thiol reactivity using monobromobimane (mBBr), performed in the absence and presence of guanidinium chloride, showed that Nabs exhibit restricted thiol accessibility under native conditions, while partial denaturation increases both reactivity and total signal, revealing the coexistence of solvent-exposed and structurally buried thiols. Consistently, DTNB and mBBr assays confirmed that NEM efficiently blocks accessible thiols, abolishing their reactivity.

The resulting Nabs exhibited high reproducibility across batches. Structural and morphological characterization (DLS, SEM, TEM) confirmed the formation of stable, spherical nanoparticles with consistent size and dispersion, while zeta potential measurements supported their colloidal stability. Thiol capping using N-ethylmaleimide (NEM) enabled modulation of nanoparticle surface reactivity. Importantly, studies in A375 melanoma cells using Alexa Fluor labeling revealed that nanoparticle redox state and thiol availability significantly influence cellular uptake, with distinct behaviors observed between Nabs and Nab_NEM.