Metal and chelator complexes- A newly discovered technology to purify immunoglobulins and without: Chromatography, polymers, membranes or specific-ligands.

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We introduce a new concept and a potentially general platform for purification of immunoglobulins that does not rely on any resins, chromatographic media, membranes or specific-ligands, rather, makes use of aromatic [metal:chelator] complexes. A hydrophobic chelator is combined with three different metal ions to establish our purification system. We experimented purification of IgG, IgM and IgA along with an artificial impurity background. Our impurity background contained 2600 of proteins along with 600 of membrane proteins. Our system captures the targets immunoglobulins quantitatively via [cation:pi] and [pi:pi] interactions and allow their recovery at high yields (80% to 88%, by densitometry) and purity (90% to 96%, by SDS-PAGE), while preserving their secondary structure (by circular dichroism, CD and native page gel electrophoresis) and monomeric state (by dynamic ligand scattering, DLS). The entire process is performed at pH 6-7 thereby avoiding complications that derive from exposure to harsh acidic conditions (e.g., aggregation, partial denaturation). The potential to upscale the technology was evaluated at laboratory scale. The leaching of the metal:chelator complex to the eluted antibodies was assessed and found to be less than 1%. The recycling of the chelator after the purification process generated a satisfactory yield of 95-97% .

Method- Purification strategy:

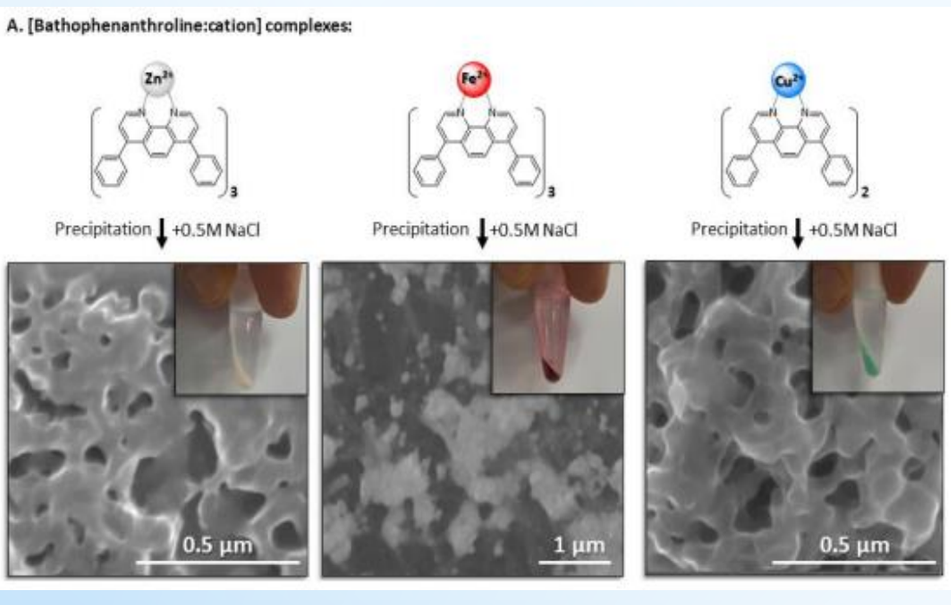

Figure 1: A. Chemical structure of the aromatic [metal:chelator] complexes and the morphology of their precipitates upon addition of 0.5M NaCl, as imaged by SEM. Insets show colored pellets of precipitated complexes

Results:

IgA Purification

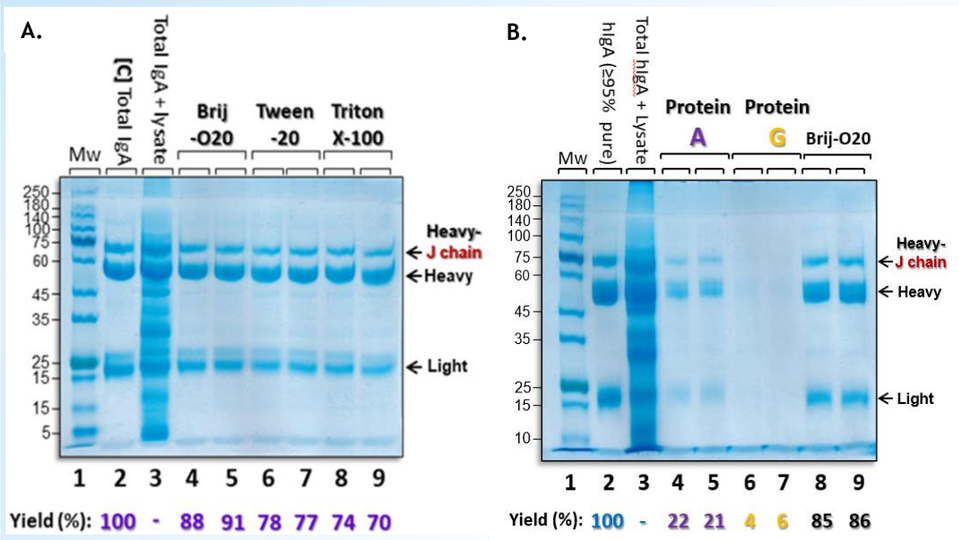

Figure 2. A. Comparison of efficiency of dIgA recovery (yield % dIgA) determined by three nonionic surfactant micellar platforms. Lane 1: molecular weight markers; Lanes 2-3: controls - commercial dIgA without (lane 2) or with (lane 3) E. coli lysate; Lanes 4-5, 6-7 and 8-9: dIgA, recovered from the conjugated surfactant mixed micellar aggregates Recovery yields are indicated below lane numbers and were quantitated using ImageJ (NIH). The gels are Coomassie blue stained.

B. Comparison of efficiency of dIgA recovery (yield % dIgA) determined by Protein A, Protein G chromatography and nonionic surfactant micellar platforms. Lane 1-3: as in 2.A; Lanes 4-5, 6-7 and 8-9: dIgA, recovered from the Protein A chromatography, Protein G chromatography and conjugated surfactant mixed micellar aggregates.

IgG Purification

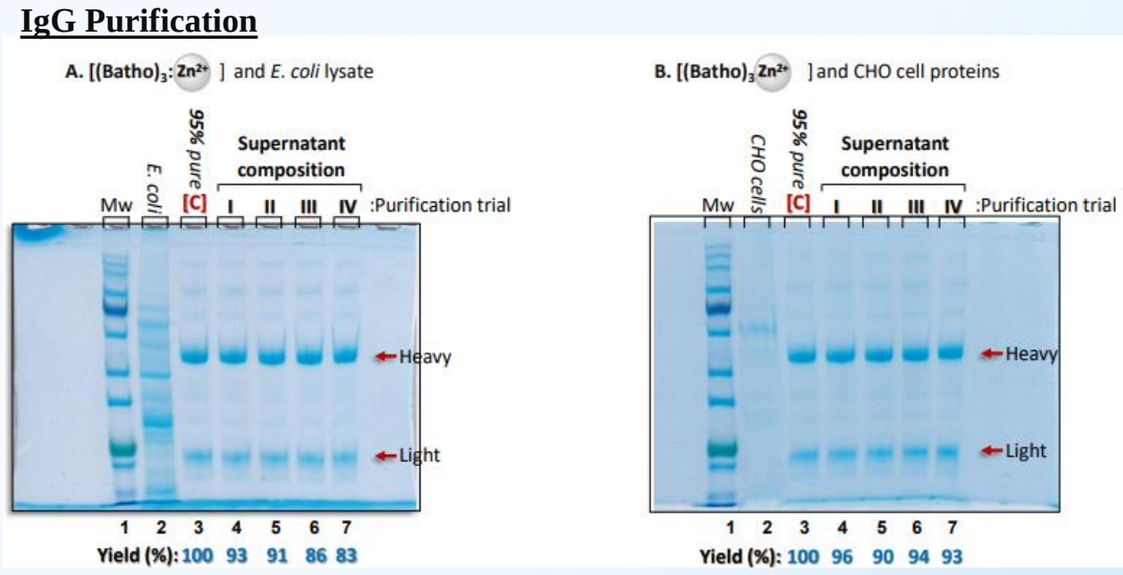

Figure 3: SDS polyacrylamide gels reveal hIgG purification process efficiency using zinc (A-B), divalent cations in the presence of the relevant impurity protein background. In all gels: Lane 1: molecular weight markers; Lane 2: total amount added of the artificial contamination background; Lane 3: total amount of commercial polyclonal hIgG (≥95% purity by HPLC) [C] added to each of the purification trials; Lanes 4-7: recovered hIgG after incubation for 30 minutes at 10°C in 50 mM NaPi (pH 7).

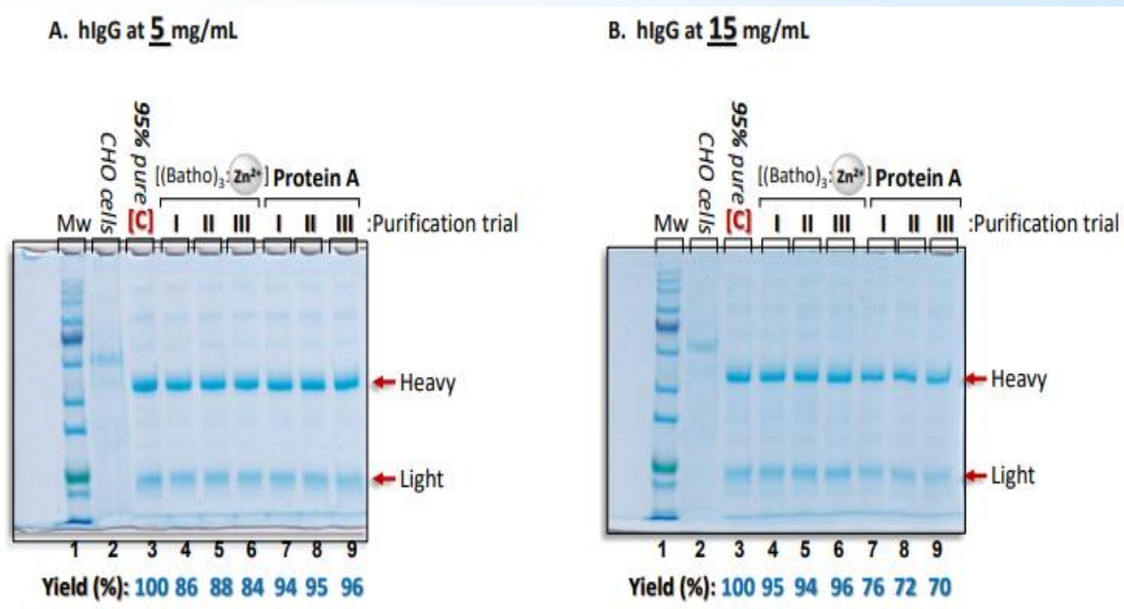

Figure 4: SDS polyacrylamide gels allow comparison of polyclonal hIgG purification efficiency via the [(batho)3:Zn2+] complex or with a Protein A column at antibody concentrations of either 5 mg/ml (panel A) or 15 mg/ml (panel B). A. Lane 1: Molecular weight markers; lane 2: total amount of CHO cell-extruded impurity proteins to be mixed with 5 mg/ml commercial polyclonal hIgG ([C]; lane 3) and added to each of the purification trials; lanes 4-6 and 7-9: Recovered hIgG via the [(batho)3:Zn2+] complex or eluted from a Protein A column (as described in the Experimental section). B. As in A, but concentration 15 mg/ml hIgG.

Conclusions:

1. **IgA and IgG can be purified without any chromatographic step or any ligand.**
2. **The gold standard ligand: Protein A, is not required.**
3. **IgA and IgG are recovered in good yields (81-91%) and high purity (>95%).**
4. **IgA and IgG preserve their secondary structure and are monodispersed.**