

# Pilot Scale Production of Polymerized Human Hemoglobin

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## Abstract

Polymerized human hemoglobin (PolyhHb) is being studied as a possible red blood cell (RBC) substitute for use in scenarios where blood is not available. While the O<sub>2</sub> carrying capacity of PolyhHb makes it appealing as an O<sub>2</sub> therapeutic, the commercial PolyhHb PolyHeme® (Northfield Laboratories Inc., Evanston, IL) was never approved for clinical use due to the presence of large quantities of low molecular weight polymeric (LMW) Hb species (<500 kDa), which have been shown to elicit vasoconstriction, systemic hypertension, and oxidative tissue injury *in vivo*. Previous bench-top scale studies in our lab demonstrated the ability to synthesize and purify PolyhHb using a two-stage tangential flow filtration (TFF) purification process to remove almost all undesirable Hb species (>0.2 µm and <500 kDa) in the material to create a product that should be safer for transfusion. Therefore, in order to enable future large animal studies and eventual human clinical trials, PolyhHb synthesis and purification processes need to be scaled up to the pilot scale. Hence in this study, we describe pilot scale synthesis and purification of PolyhHb. Characterization of pilot scale PolyhHb showed that PolyhHb could be successfully produced to yield biophysical properties conducive for its use as an RBC substitute.

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