141d Engineering High Oxygen Affinity Oxygen Carriers

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Oxygen carriers based on glutaraldehyde polymerized hemoglobin (PolyHb) are currently being developed for use in human subjects needing blood transfusions. Though there has been a great deal of research on the glutaraldehyde (G) hemoglobin (Hb) reaction, a systematic study of the effect of varying the G-Hb molar ratio on the resulting PolyHb physical properties (molecular weight distribution and oxygen binding parameters) has not been conducted to date. The results of this study show that increasing the G-Hb molar ratio elicits a general decrease in the P50 and cooperativity, and a simultaneous increase in the weight averaged molecular weight of the PolyHb dispersion and methemoglobin (MetHb) level. Three PolyHb dispersions (20:1, 30:1, and 40:1 G-Hb molar ratios) exhibited potential as oxygen carriers. The 20:1 PolyHb dispersion resulted in the presence of more intramolecularly crosslinked and non-crosslinked tetramers versus crosslinked species that were larger than a tetramer (~75% tetrameric and ~25% higher order species), lower MetHb level (8%), and P50 (20.1 mmHg) similar in magnitude to that of non-crosslinked Hb. The 30:1 PolyHb dispersion consisted of more higher order species (~76%), higher MetHb level (28%), and lower P50 (13.3 mmHg). The 40:1 PolyHb dispersion resulted in a similar P50 of 13.0 mmHg, similar MetHb level (30%), however this PolyHb dispersion only consisted of species larger than a tetramer. Taken together, the results of this study should aid in engineering appropriate molecular weight distributions and oxygen binding properties of potential oxygen carriers.