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## ZIF-8 Incorporating Haemoglobin as Potential Red Blood Cell Substitutes

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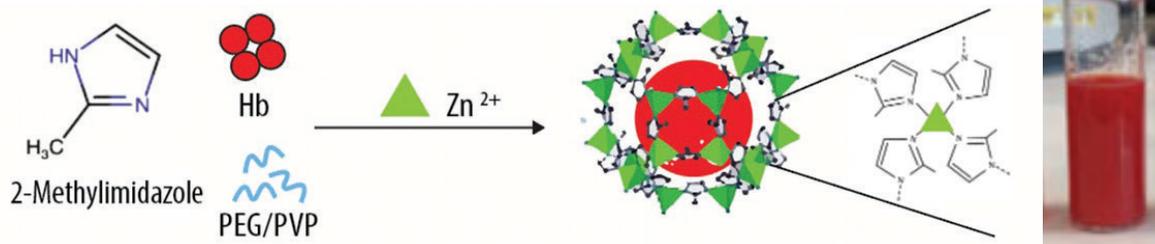
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Blood is crucial for our survival and the only way to substitute for blood is by using blood from donors. Even though blood transfusions are an essential procedure for saving lives, they present some important drawbacks. The short storage lifetime of blood-based products and its limited availability contribute to the current blood shortage, which will become even worse in the upcoming years. Thus, the creation of red blood cell substitutes is a pressing need in biomedicine. Despite decades of research, the fabrication of haemoglobin (Hb)-based oxygen carriers (HBOCs) with high Hb content remains as an important challenge.

Herein, we present a HBOC that makes use of the excellent oxygen carrying and delivering properties of Hb. The in-situ encapsulation of Hb in zeolitic imidazolate framework 8 (ZIF-8) is driven by a process known as biomineralization, where ZIF-8 grows around Hb, acting as a protective platform. This method gives rise to high entrapment efficiencies (~90%) and low leakage of biomolecules, thus reducing the free Hb toxicity problems reported in previous HBOCs. ZIF-8 nanoparticles incorporating Hb (Hb@ZIF-8) are synthesized in 10 minutes under mild conditions (**Figure 1**). Furthermore, this process is easy to scale-up, which is an essential requirement to meet the high oxygen demands of our body. Different screening processes were carried out to obtain a formulation with desirable size, zeta-potential, yield and high entrapment efficiency. The different Hb@ZIF-8 were also characterized by SEM, FTIR and PXRD. In addition, the effect of the incorporation of poly(ethylene glycol) (PEG) or poly(vinylpyrrolidone) in the resulting Hb@ZIF-8 was studied. A higher entrapment efficiency was observed when increasing concentrations of PEG. To improve the stability of the Hb@ZIF-8 in cell medium and PBS, a metal phenolic network (MPN) coating was successfully added. Finally, the functionality of Hb after its entrapment in Hb@ZIF-8, before and after the MPN coating, was evaluated using UV-vis spectroscopy and showed promising results. Future experiments will focus on cell studies and further evaluation of Hb's functionality after its incorporation in ZIF-8.



**Figure 1:** Schematic representation of the synthesis process and image of the Hb@ZIF-8.