Sonochemically Produced Proteinaceous Microspheres

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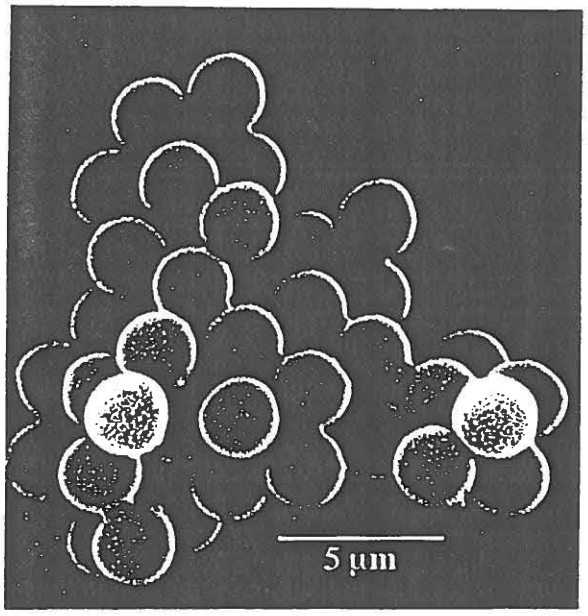
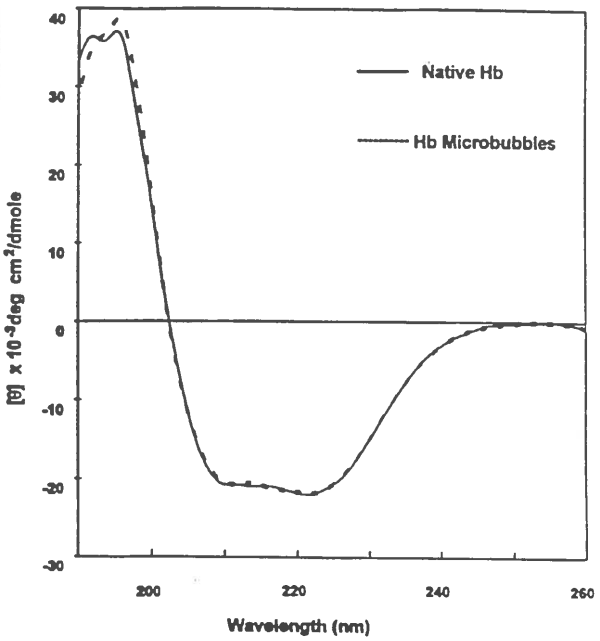
The field of microencapsulation has found increasing application since its industrial debut in carbonless copying paper some fifty years ago. Microencapsulated materials are found in a wide variety of technical, agricultural, food, and household products. There have been many methods developed for microencapsulation: from reverse-phase evaporation into liposomes to coacervation into polymeric microspheres. Previously, Suslick and coworkers have used high-intensity ultrasound to generate nonaqueous-filled serum albumin microspheres [1-3]. The mechanism involved the oxidation of cysteine residues by sonochemically generated superoxide to form disulfide bonds.

We have now extended this work to form a variety of other proteinaceous microspheres including air-filled hemoglobin (Hb), fluorocarbon-filled bovine serum albumin (BSA), and aqueous-filled lipase microspheres. The microspheres can be sonochemically generated in high yield (≈ 10^9 microspheres/ml) with a narrow size distribution (≈ 2-4 μm). The microspheres are stable over six months at 4 °C with minimal degradation (< 25%). The formation of these microspheres has been confirmed to involve superoxide (produced from sonolysis of water) and the action of this species on cysteine residues. From circular dichroism studies, the protein structure has not been significantly altered after sonication. More remarkably, protein func-tionality has been retained (and in some cases enhanced) upon microsphere formation.

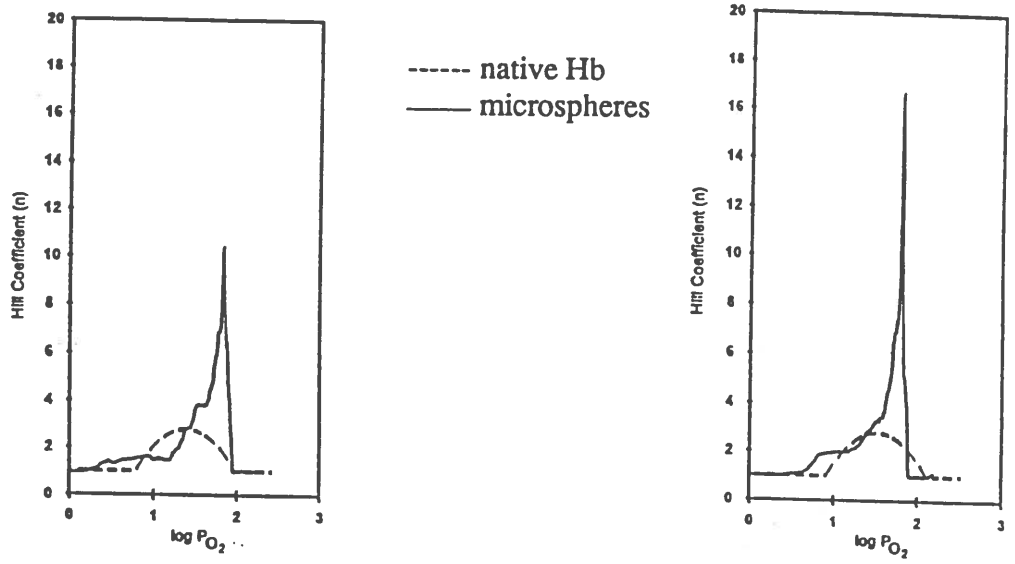


Hb Microspheres



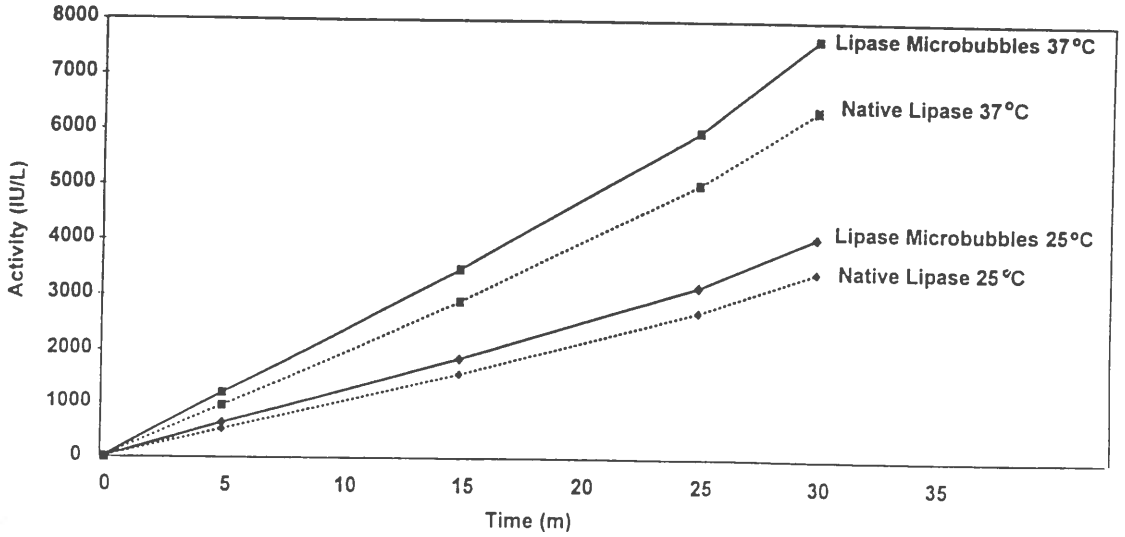
The Hb microspheres possess many of the desired characteristics of the ideal blood substitute [4-6]. The microspheres can still bind oxygen reversibly over many cycles. Oxygen binding studies indicate the microspheres possess a similar $P_{1/2}$ (in the absence or presence of phosphates) as native Hb. The maximum degree of cooperativity (represented by the Hill coefficient) has increased by roughly six-fold over that of native Hb (in the presence of phosphate). The Hill coefficient represents the number of interacting subunits. Tetrameric native Hb has a maximum Hill coefficient of about 2.8. In the Hb microspheres, maximum Hill coefficients of 10 and 18 are detected in 0 and 1.7 mM inositol hexaphosphate (IHP),

respectively. This indicates that there is an interaction between the crosslinked Hb tetramers within the microsphere shell upon oxgenation. Calculation shows that there are approximately 4 x 10^6 Hb molecules in the 35-60 nm shell (determined from transmission electron microscopy). The Hb microspheres have twice the oxygen carrying capacity as whole blood. Preliminary *in vivo* transfusion studies are very promising.



Perfluorononane (n-C_9F_{20})-filled BSA microspheres have also been generated as a potentially new class of ^{19}F magnetic resonance imaging (MRI) agents [7]. ^{19}F MRI shows the accumulation of the n-C_9F_{20} microspheres within the reticuloendothelial system (RES, liver and spleen). Furthermore, the surface of the BSA microspheres can be chemically modified to prolong circulation time.

So far, only air- and nonaqueous-filled protein microspheres have been sonochemically generated. We have recently developed the sonochemical synthesis of aqueous-filled lipase microspheres. Studies not only show the lipase structure to be unaffected during sonication, but also that the enzymatic activity of the protein has been retained in the microspheres. The biodistribution of the lipase microspheres has been studied by the encapsulation



of the FDA-approved MRI agent gadopentetate dimeglumine (Gd-DTPA). *In vivo* studies show that Gd-DTPA solution and liposomes accumulate in the liver and are excreted through the kidneys, as expected. However, Gd-DTPA lipase microspheres are seen in the small intestine. Native lipase is produced in the pancreas and is secreted into the gastrointestinal tract during digestion. The same trend has been seen with fluorescently-labeled BSA microspheres in the liver and fluorocarbon-filled pepsin microspheres in the stomach. More studies are underway to investigate this extraordinary targeting mechanism.

References

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