The Sonochemical Synthesis of Proteinaceous Microspheres

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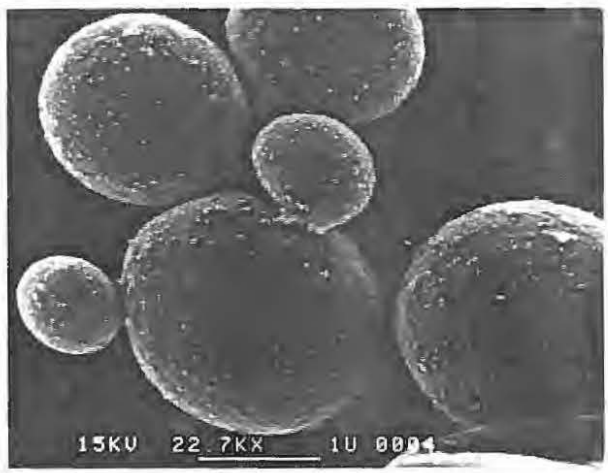
Microspheres have found diverse and important applications ranging from the microencapsulation of dyes, flavors and fragrances, to drug delivery systems, to the study of membrane structure, function and reactivity [1-3]. A sonochemical technique has been developed to synthesize microspheres composed entirely of albumin protein. Aqueous suspensions of proteinaceous microspheres filled with water-insoluble liquids can be synthesized (i.e., microcapsules) [4,5]. Scanning electron microscopy, optical microscopy, and particle counting characterization reveals spherical microcapsules with typical concentrations of 1.5×10^9 microcapsules/mL. The microcapsules synthesized have a narrow Gaussian size distribution (average diameter 2.5 μm ± 1.0 μm). Microcapsule formation is strongly inhibited by free radical traps, by superoxide dismutase (but not by catalase), by the absence of O_2, and by the lack of free cysteine residues in the protein. The microcapsules are held together by disulfide bonds between protein cysteine residues, and superoxide (sonochemically produced by acoustic cavitation) is the oxidizing agent which cross-links the proteins. These proteinaceous microcapsules have been developed and used for a variety of chemical and medicinal applications.



Present drug delivery methods primarily focus on water soluble drugs. Currently, in drug delivery research there are methods of introducing water soluble drugs into the interior of a microsphere and of attaching drugs to a solid microsphere [6-7]. We have discovered a new method that can be used to encapsulate water-insoluble or hydrophobic drugs in a microsphere which is composed entirely of albumin protein [8]. Aqueous solutions of spherical proteinaceous microcapsules filled with α-methyl-4-(2-propyl)benzene-acetic acid (i.e., ibuprofen) can be synthesized. These microcapsules have an average diameter of ≈2.5 μm with concentrations of $\approx 2.6 \times 10^8$ microcapsule/mL. Ibuprofen (trade names: Motrin IB™ & Advil™) was released over a 6-hour period at 38°C. Ibuprofen was encapsulated at a concentration of 14 mM.

The development of a non-invasive technique for *in vivo* temperature measurement has many potential clinical applications [9]. Microcapsules containing a nitroxide and a solid fatty acid have been synthesized. The fatty acid that the nitroxide is dissolved in undergoes a reversible phase change (solid to liquid) inside the microcapsule upon heating and cooling. The nitroxide free radical EPR signal is dramatically dependent on its environment, and consequently the temperature can be determined from a calibrated EPR linewidth. The current

microcapsules have a temperature range from 37 to 42°C with a temperature sensitivity of ≈0.5°C. These microcapsules are designed especially for optimizing the treatment of cancer *in vivo* with hypothermia.

Oxygen plays a critical role in physiological, pathophysiological and therapeutic processes. However, our present understanding of its role in living systems is limited due to the lack of techniques for the direct measurement of O_2 concentration. Low concentrations of O_2 are associated with a variety of normal as well as pathological processes and are difficult to measure. Electron paramagnetic spectroscopy (EPR) has previously been used for determination of O_2 even at low concentrations (<0.1 μM) [10,11]. An electron paramagnetic resonance spectroscopy technique for non-invasive *in vivo* oxygen measurement that uses both nitroxide free radicals and proteinaceous microcapsules has been developed [12]. Encapsulation of a nitroxide free radical dissolved in cyclohexane was accomplished. These microcapsules are easily penetrated by gases and the O_2 concentration can be quantitatively determined by experimentally measuring the observed EPR linewidth. The microcapsules do not inhibit the flow of oxygen or nitrogen as monitored by the change in nitroxide linewidth. An EPR spectrum from the lower back of a mouse was obtained. The signal was still strong after 45 minutes and no reduction in signal intensity was observed. Injection of Ketamine, a local anaesthetic, that reduces O_2 consumption in the mouse decreased the signal intensity. Thus, a reduction in physiological activity in the mouse was observed using this technique and this work is currently directed toward using these nitroxide-filled microcapsules as an *in vivo* O_2 determination.

In addition to producing microspheres containing a liquid or solid, microspheres that are air-filled (i.e., microbubbles) have been synthesized. These proteinaceous microbubbles are currently in clinical use as echo contrast agents for echocardiography [13,14]. Two-dimensional contrast echocardiography has become a valuable and routine procedure in diagnosing cardiac diseases. This diagnostic method is especially useful in monitoring myocardial perfusion and ventricular hemodynamics. To enhance image quality, a solution containing microbubbles may be injected intravenously to perfuse the cardiovascular system; these microbubbles change the acoustic impedance of the blood flow, resulting in dramatically improved echo contrast with the surrounding tissues. To permit unimpeded motion of the microbubbles through the circulatory system, the optimum diameter for such microbubbles is under ten microns. The sonochemically synthesized proteinaceous microbubbles are non-toxic, stable, micron size and have been used as contrast agents for echosonography [15-17].

Besides synthesizing biological materials using high intensity ultrasound, inorganic materials can also be formed. Under the conditions that maximize the cavitational heating (e.g., Ar, low vapor pressures, low ambient temperatures), carbon monoxide dissociates from iron pentacarbonyl ($Fe(CO)_5$) with the formation of iron powder. The powder synthesized during sonolysis was characterized with a variety of techniques including DSC, TEM, X-ray powder diffraction, BET, and SEM. The powder sonochemically synthesized is amorphous [18-20]. This amorphous iron powder is formed from the enormous heating and cooling rates (>5 x 10^6 K/sec) generated during acoustic cavitation. Recently we have observed the atomic emission lines during the ultrasonic irradiation of $Fe(CO)_5$ [21].

The amorphous iron is a soft ferromagnet. The coecivity at 5 K is ≈190 gauss and decreases to ≈10 gauss by room temperature. The saturation magnetization of the amorphous iron is ≈160 emu/g and is slightly less than crystalline iron (217 emu/g) [22]. The amorphous iron powder is also an active catalyst for the Fischer-Tropsch hydrogenation of CO and for hydrogenolysis and dehydrogenation of saturated hydrocarbons. The amorphous iron catalyst is ≈150 times more reactive than commercially available crystalline iron. This increase in catalytic activity is attributed to its larger surface area (≈120 m^2/g) compared to crystalline iron (0.8 m^2/g) [23].

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