Transition metal oxide shells for encapsulation of (micro)organisms

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Introduction

Encapsulation of enzymes and proteins for preparation of biosensors has recently attracted attention of researchers (Luo 2005). Strong efforts were made on encapsulation of live single-cell microorganisms for bioremediation processes (Hammill 1997) and for prolonged enzymatic treatment, such as fermentation, in food technologies (Kosseva 2004). Developing capsules consisting of organic and especially biodegradable materials such as starch (Jankowski 1997), guar gum (Hammill 1997) or calcium alginate (Stormo 1992, Bienaime 2003) received major attention and have been proposed for treatment of disbacteriosis (Chandramouli 2004). A new and strongly growing direction is the search for systems able to host living microorganisms while hindering their proliferation (Premkumar 2002). Capsules of this kind should be highly resistant to biodegradation and the solution for their preparation is sought in inorganic sol-gel technology (Nassif 2002), and even proposed for creation of stable nanoshells for drug delivery (Caruso 1998).These efforts have, so far, concerned silica-based colloids and suffered of drawbacks such as too easy [SH1]release of whole-cell organisms by gel encapsulates (Nassif 2002) and inability to release the medically active compounds from the thick-wall nanospheres from colloid templation (Caruso 1998). We demonstrate here a possibility to solve these problems applying the Micelles Templated by Self-Assembly of Ligands (Kessler 2006) obtained by hydrolysis of metal (titanium and zirconium) alkoxides.

Material and methods

All operations with metal alkoxide precursors were carried out in dry nitrogen using a glove box with boiling nitrogen atmosphere. Metal alkoxides, 70% solution of $Zr(O^nPr)_4$ in ^{n}PrOH and pure $Ti(O^nPr)_4$, and the modifying agent, 2,2,6,6-tetramethylheptanedione (Hthd) were received from Aldrich and used without further purification. Isopropanol and n-propanol (Merck, p.a.) were purified by distillation over corresponding $Al(OPr)_3$, and hexane and toluene (Merck, p.a.) - by distillation over $LiAlH_4$. The inverted micelles are typically obtained when a small amount of polar solvent is introduced into a non-polar one and the droplets of the former are stabilized by a surfactant. The macro-droplets of emulsions were obtained by introduction of water solutions into hexane solutions of $Zr(O^nPr)_3$(thd) or $Ti(O^nPr)_3$(thd) (typically 0.2 g in 5 ml hexane) on vigorous shaking. The biological encapsulates within hydrated metal oxide spheres were studied using an Axiophot epifluorescence microscope (Zeiss, Oberkochen, Germany) with an excitation filter at 485 nm (band pass 20 nm) and emission wavelengths >520 nm. For photographs, images were obtained using a 20× or 100× oil immersion objective.

Results and discussion

The sol-gel process applying hydrolysis of silicon alkoxides is known to be in many cases a kinetically controlled reaction of inorganic polymerization, permitting to immobilize large biomolecules, organelles and whole organisms in the arising loose gel structure (Livage 2004). Biocompatibility of the resulting colloids is limited by the need to use quite high concentrations of alcohols to ensure miscibility of the silicon alkoxides with water. The hydrolysis of metal alkoxides is, in contrast to those of silicon, a kinetically unhindered process, resulting in formation of micellar

type particles with the organic ligands self-assembled on their surfaces. The ligands attached to hydrolyzed metal hydroxide units act as surfactants stabilizing the resulting micelles (see Fig. 1).

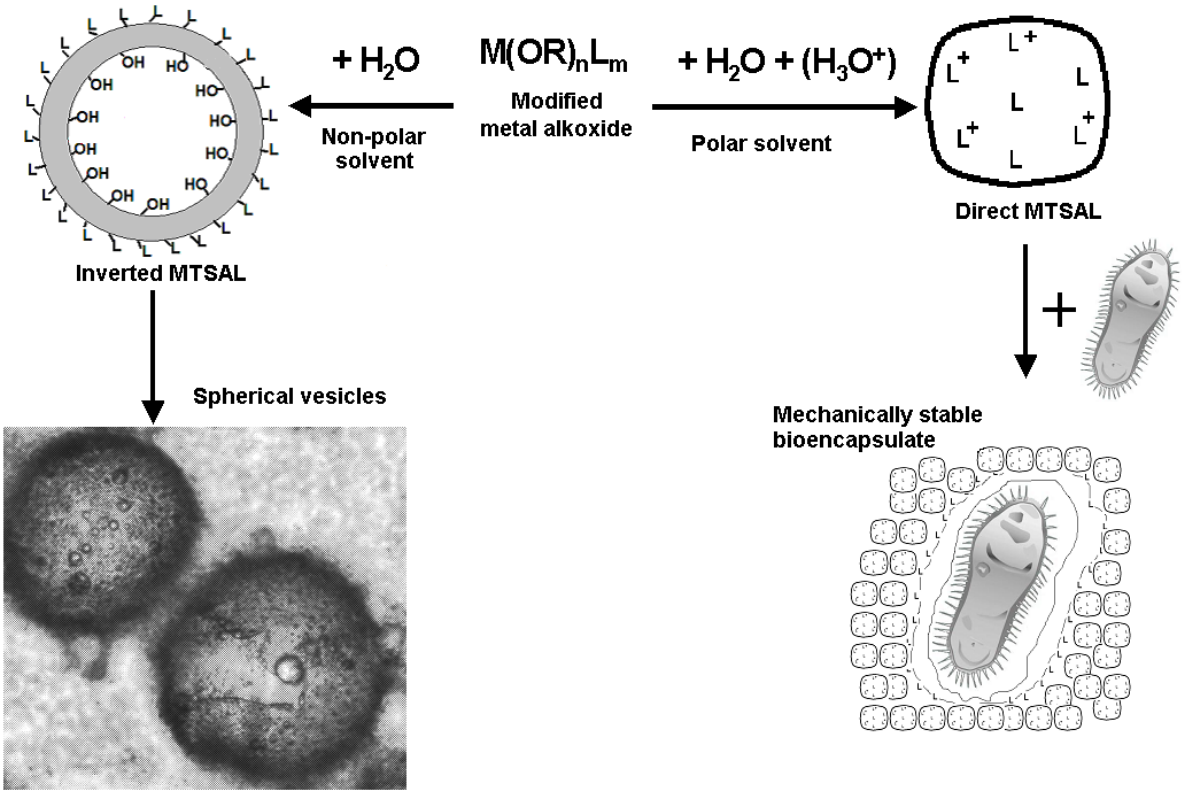

Fig.1 Schematic representation of the MTSAL mechanism. Formation of the direct and inverted micelles and encapsulation of living cells by direct micelles

In the present contribution we describe the preparation of inverted micelles. The inverted micellar systems are formed when a solution in a polar solvent, most often water, is introduced into a non-polar medium with controlled viscosity (solvent or oil) and stabilized by a surfactant. As the modified alkoxide produces units, acting as surfactant on hydrolysis, we investigated the formation of vesicles on introduction of water-based solutions of inorganic and organic dyes into hydrocarbon (hexane) solution of modified zirconium and titanium alkoxides on vigorous shaking. This procedure resulted in formation of spherical capsules with an approximate size of about 100 μm. The optical properties of the capsules indicated that they had dense walls: the color of the dyes was lighter in reflected and darker in transient light. That demonstrated the occurrence of the complete inner reflection phenomenon requiring the ideal mirror properties of the inner surface of the shell walls (see Fig. 2).

We wanted also to apply a sensitive marker to demonstrate that the hydrocarbon solvent does not diffuse efficiently inside the inverted micelles. As a marker, we have chosen the whole blood cells. The solution of erythrocytes was prepared by diluting 0.2 ml of fresh blood with 20 ml of isotonic NaCl solution. 0.5 ml of this bright red solution, containing undestroyed erythrocytes according to microscopic observation, were added by syringe on vigorous shaking to the of solution of $Zr(O^nPr)_3(thd)$ in 5 ml dry hexane. The water phase was immediately transformed into tiny light brick-red spheres hardly distinguishable by a bare eye. The solution above them remained completely clear for at least one hour, when light opalescence started to appear. The spheres were stable on shaking in the hexane solution but were destroyed by putting a cover glass over them. The

immediate observation of the water phase released then into paraffin oil (about 3h after encapsulation) showed a fraction of undestroyed erythrocytes (which are highly sensitive and destroyed rather quickly on contact with hexane).

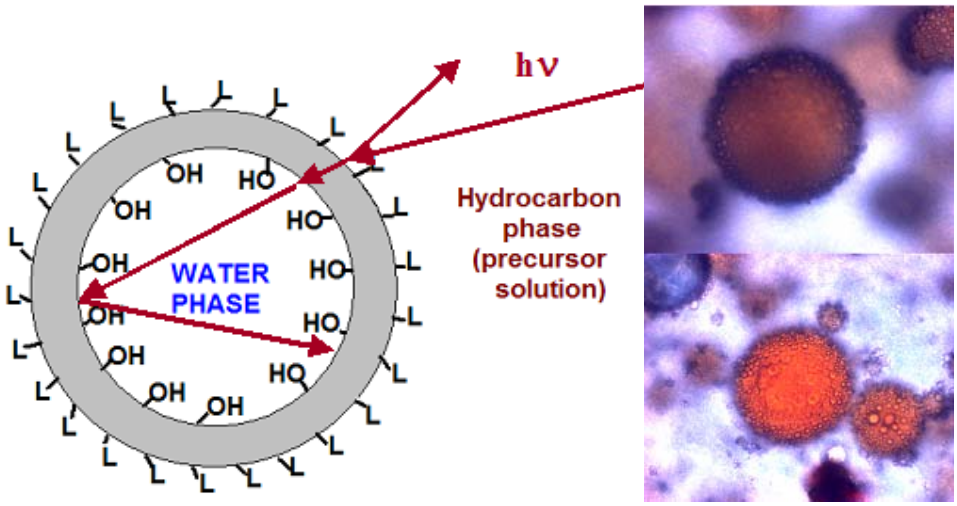

Fig. 2. Optical manifestation of the dense structure of the vesicle walls through complete inner reflection phenomenon: emulsion supported by metal oxide core (upper right) and an unsupported mechanically produced emulsion (lower right).

We sought also a possibility to demonstrate and exploit the amphiphylic nature of the walls of the prepared vesicles. This has been achieved by encapsulation of a water suspension of green fluorescing 2.2 μm polystyrene microspheres (Duke Scientific Corp., Palo Alto, CA, USA). These suspensions are commonly used in the microbiological studies and are stable in water. Polystyrene, however, is a weakly hydrophobic material, which meant it should have been extracted into the amphiphylic shell in the process of encapsulation. The microscopic observations (see Fig. 3) were completely in accordance with our expectations – the microspheres have not escaped from the droplets into hexane solution as the formation of the shells is a quick process, but turned to be located inside the shells and not in the volume of the droplets.

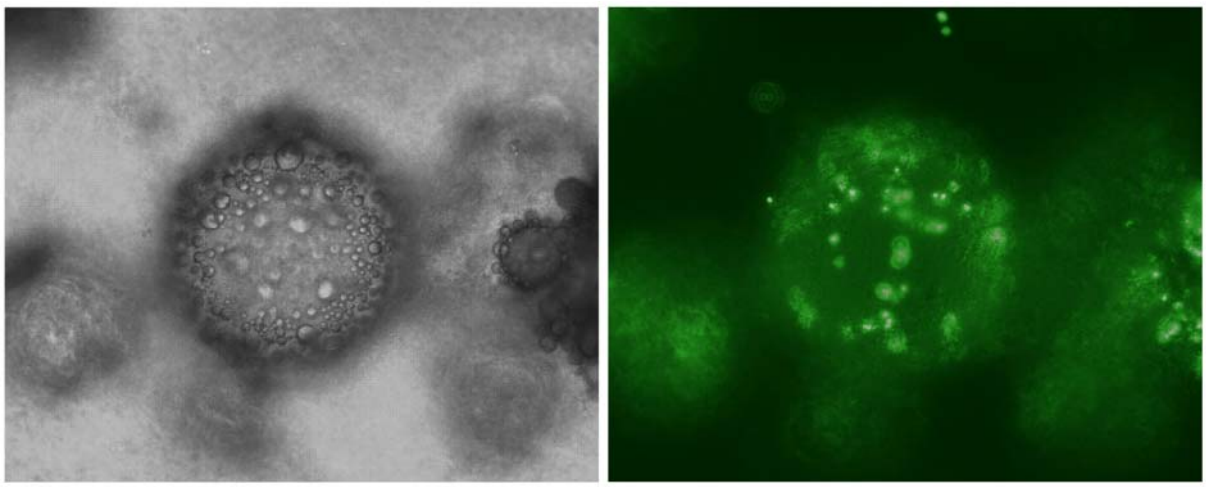

Fig. 3. Luminescent microspheres localized within the shells formed on the interface of a water suspension in hexane solution of thd-modified zirconium propoxide

A series of experiments on encapsulation of microorganisms and of plant seeds applying direct micelles prepared from titanium and zirconium alkoxides has also been carried out, demonstrating

biocompatibility of the produced encapsulates and also their high mechanical stability. The details of this work will be reported separately in a near future.

Conclusions

Highly biocompatible encapsulates with dense walls transparent for diffusion of only small molecules can be successfully prepared through hydrolysis of modified metal alkoxides producing Micelles Templated by Self-Assembly of Ligands. The inverted micelles provide spherical vesicles with dense smooth and uniform inner walls. They are stable in suspension, but have rather poor mechanical stability. Highly mechanically stable encapsulates can be produced from direct micelles.

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