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Self-Assembled Titania Micelles for Drug Delivery and Bio-Control Applications

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Introduction

Application of inorganic materials for drug delivery and bioencapsulation has recently attracted strong attention of researchers. Silica-based mesoporous monoliths (Vallet-Regi 2007) and sol-gel derived amorphous silica particles (Barbe 2004) have been actively studied as materials for slow release of adsorbed pharmaceutical agents, while soft silica gels have been demonstrated to act as highly biocompatible matrices for bacterial encapsulation (Nassif 2002, Avnir 2006) Many metal oxides, in particular titanium dioxide, TiO_2, are known to be highly biocompatible, at least, in the absence of photochemical oxidation processes(Crawford 2007). Titanium nanotubes have even been investigated as possible a matrix for slow drug release (Ayon 2006). Development of titanium-based sol-gel materials for bioencapsulation and controlled drug release has so far been hindered by high reactivity of the metal-organic sol-gel precursors, titanium alkoxides, which required application of anhydrous media for preparation of colloid solutions (Livage 2004).

Materials and methods

The biocompatibility of the hydrogels was tested by encapsulation trials using a number of model microorganisms. Initial precursor solution was obtained by dissolving either 4 ml $Ti(OEt)_4$ in 6 ml anhydrous EtOH and adding 1.0 ml of triethanolamine (for *A. chlorophenolicus*) or by dissolving 5 ml $Ti(OEt)_4$ in 5 ml anhydrous EtOH, adding 1.5 ml triethanolamine (for *P. anomala* or *Lb. plantarum* MiLab 393). 1 ml of hydrolyzing solution, prepared by mixing 0.5 ml 0.5 M HNO_3 with 2.0 ml EtOH, was added providing the organic sol and 1 ml of this sol was quickly added to 9 ml of suspension of the respective model microorganism in isotonic NaCl. Hydrogel encapsulates containing microorganisms (1 g) were added to 10 ml of citrate buffer solution (0.10 M, pH 6) and complete dissolution within 5 min of gentle agitation resulted in transparent suspensions of microorganisms. The obtained solutions were then subjected to 10-fold serial dilutions in isotonic NaCl and analysed for viable counts by spread on cultivation plates based on MRS (Oxoid Ltd., Basingstoke, England) for *Lb. plantarum*, Malt extract (Oxoid) for *P. anomala* and for *A. chlorophenolicus*, minimal medium (GM) as described previously (Westerberg 2000), supplemented with 0.1% yeast extract (Oxoid).

The size of initial particles in aqueous sols was measured by dynamic light scattering (ZetaSizer 3000 HSa, Malvern). FTIR spectra of sols and gels were recorded with Perkin-Elmer Spectrum 100 instrument without dilution in a cell supplied with CaF_2 glasses. The morphology of the xerogels was studied with a Hitachi TM-1000-μDeX 15 kV scanning electron microscope (SEM) and the agglomerate size and crystallinity were studied with a Topcon EM-002 B Ultrahigh resolution analytical electron microscope (TEM). UV-Vis spectra were recorded using Hitachi U-2001 spectrophotometer.

Results and discussion

In the present contribution we report the preparation of biocompatible aqueous titania sols and gels and their application in encapsulation of microorganisms and pharmaceuticals with the possibility of controlled and biologically triggered release. Stabilization of metal alkoxides sols is usually achieved by the modification of precursors by chelating organic ligands. Such reaction leads to increased reactivity of the alkoxides in hydrolysis-polycondensation resulting in facile formation of self-assembled micellar aggregates, Micelles Templated by Self-Assembly of Ligands – MTSALs, covered by residual heteroligands (Kessler 2006). The commonly applied ligands such as β-diketonates and carboxylates are hydrophobic which helps in the stabilization of the colloids in organic solvents. We have applied a hydrophilic ligand, triethanolamine, which is highly basic and easily chargeable via addition of strong acids. The proposed stabilization mechanism for micelles is displayed in Figure 1.

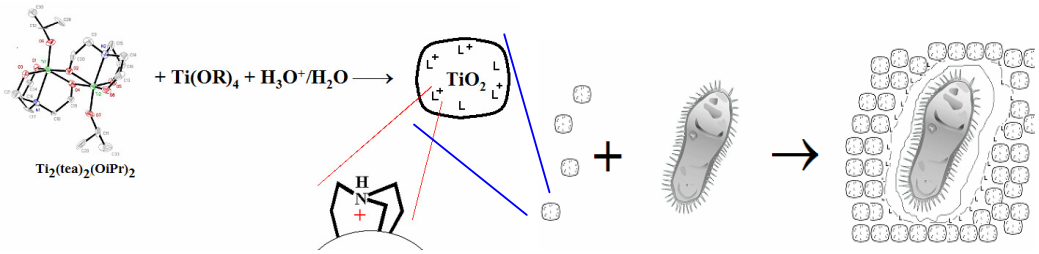

Figure 1 The structure of metal alkoxide derived micelle (MTSAL): positively charged ligands on the surface provide stabilization to the produced colloids

In our experiments, solutions of titanium alkoxides (20-40 vol %) in parent alcohols were modified by 0.4-0.7 eq. of $N(C_2H_4OH)_3$ and hydrolyzed by 1.5-2 eq. H_2O, containing 0.1 – 0.15 eq. H_3O^+. The resulting slightly viscous sols were immersed into de-ionized water or isotonic NaCl solution, which provided initially colourless and transparent aqueous sols. Depending on the amount of modifying ligand and the acid added the gelation time of the aqueous sols could be controlled in the time span of minutes to several days. Drying of the formed gels in ambient air offered completely amorphous xerogels, as deduced by powder X-ray diffraction, built-up of hierarchically constructed closed mesoporous aggregated structures that are typical of xerogels formed through the coalescence of primary hydrolysis-polycondensation derived particles (Werndrup 2004, Pązik 2008), MTSALs.

TEM examination of the fresh xerogels showed that the primary particles have a core-shell structure with a very small (2-3 nm) crystalline anatase core, clearly identifiable by electron diffraction analysis, and an outermost amorphous shell. The presence of a highly amorphous outer shell was a good indication that the particles would be able to precipitate from solutions onto heterogeneous surfaces thus forming continuous coatings.

Inclusion of microbial samples into the aqueous media permitted the preparation of gels encapsulating biologically active model microorganisms. The gram-positive bacterium *Arthrobacter chlorophenolicus* is capable of degrading phenolic compounds and is therefore of interest in soil bioremediation applications (Westerberg 2000, Unell 2007). The yeast *Pichia anomala* J121 and the lactic acid bacteria *Lactobacillus plantarum* MiLab393 have both been shown to have anti-fungal activities of different modalities making them good biocontrol candidates for the prevention of mould spoilage in moist feed storage systems (Melin 2007, Schnürer 2005, Passoth 2006).

According to observations by optical microscopy, encapsulated microorganisms were covered by a shell of hydrated oxide, and further incorporated into bits of gel measuring from tenths of micrometers to about 1 mm. Amorphous titania is soluble in the presence of chelating carboxylate ligands such as citrate or lactate. Treatment of gels containing encapsulated microorganisms with citrate buffer at pH = 6 leads to its complete dissolution within a few minutes and thus the in release of the encapsulated microbes (see Figure 2) which then could be analyzed for their survival. The survival rates of encapsulated *P. anomala* and *Lb. plantarum* after dissolution of the hydrogels in citrate buffer was 95% (± 10%) and 79% (± 8%), respectively. The survival rate of encapsulated *A. chlorophenolicus* was significantly lower: 3,4% (± 2,7%).

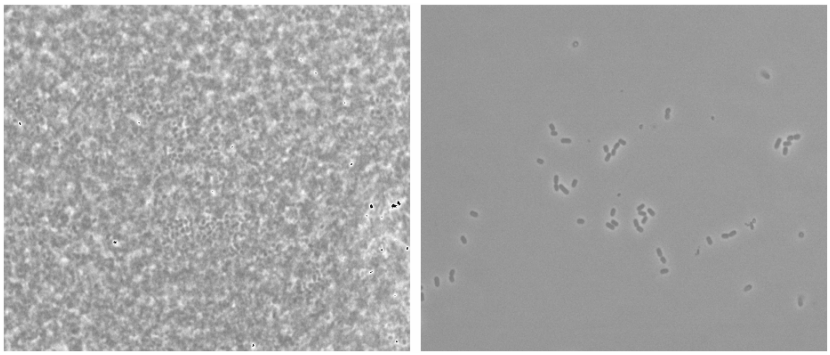

Figure (2) : Release of bacteria, encapsulated in a TiO_2 gel, by treatment with a citrate buffer: initial gel (left) and liberated bacteria (right).

We have no plausible explanation to why *A. chlorophenolicus* appears to be significantly more sensitive to this form of encapsulation as compared to the other model microorganisms used in this study. The sensitivity to moderate levels of ethanol (≤10%) did not differ between the different species.

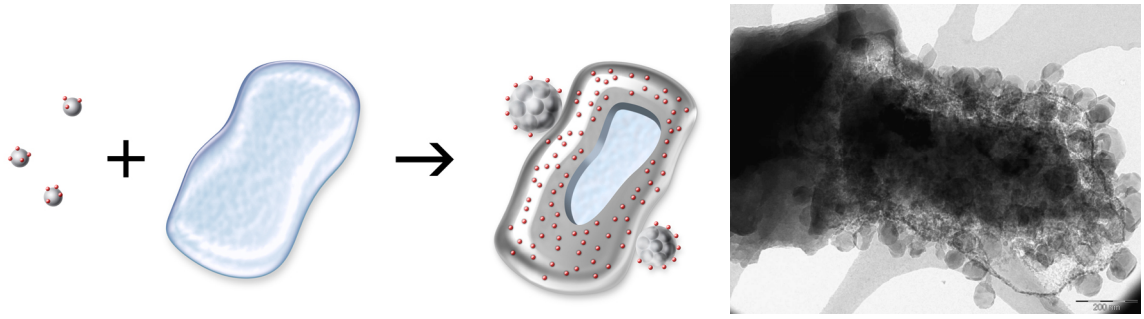

Figure (3) : The mechanism of encapsulation in a metal oxide gel: the coalescence of MTSALs around a living cell with formation of a continuous oxide shell (left) and the TEM view of the shell and surrounding it MTSAL aggeragates (right).

Encapsulated *A. chlorophenolicus* displayed no detectable biological activity after repeated washing of the hydrogels with isotonic NaCl solution. However, encapsulated *P. anomala* and *Lb. plantarum* both displayed residual biological activity after several cycles of washing in the form of growth on culture plates, indicating that these microorganisms where capable of self-mediated release. The TEM study of heat-treated xerogels (air-dried hydrogels containing encapsulated *Lb. plantarum* subjected to thermal treatment at 400°C in 30 min) provided proof of a continuous coating on the surface of the microorganisms through self-assembly of the primary particles. The surrounding gel transformed into hierarchical spherical aggregates outside the dense coating in the same manner as for gels alone (see Figure 3). The entrapment of the microorganisms within the hydrogels was thus likely due to the formation of a dense oxide coating on their surface.

The residual activity and growth of *P. anomala* and *Lb. plantarum* after several cycles of washing (78±17% and 80±29% of the living cells released, respectively) most probably originated from the ability of these microorganisms to produce chelating lactate, citrate or other carboxylating ligands. This permitted a significant fraction of the organisms to weaken the capsule around them by partial dissolution leading to their release.

These observations are principally important as they open up prospects for application of such encapsulates in tissue engineering. The inflammatory processes in the body are in fact associated with increased production of chelating carboxylate ligands. Immobilization of biological material on the surface of implants may open ways for site-directed pharmaceutical treatments. We therefore tested the possibility of encapsulating anti-inflammatory drugs in the prepared gels and the conditions of their subsequent release. We have chosen to introduce these entities as components of the ligand systems in MTSALs. For this purpose the anhydrous compounds ibuprofen (2-[4-(2-methylpropyl)phenyl]propanoic acid) and D-penicillamine ((2*S*)-2-amino-3-methyl-3-sulfanyl-butanoic acid) were used as modifying ligands for titanium alkoxides, producing individual heteroligand complexes. These complexes were added to 8-10-fold excess of unmodified titanium alkoxides and treated with triethanolamine and acidic water solutions to produce organic sols, which were converted further into aqueous sols and gels by immersion into aqueous media. The aqueous gels were then placed into excess isotonic NaCl and the concentration of released ibuprofen was monitored by UV-Vis measurements. The intensity of the peak at 224 nm was corrected for the absorption of TiO_2-particles themselves (broad maximum at 320 mn) using a reference test with the same amount of ibuprofen-free gel. The increase in the free drug concentration, was essentially linear within the first 300 min with complete release in over 500 min – these characteristics were at least as good as those for mesoporous silica matrices [1,2].

Conclusions

The modification approach proposed in this work permits preparation of highly biocompatible and photochemically inactive titania sols and gels, capable of encapsulating living microorganisms and pharmaceuticals with the release mechanism controllable in a chemical and possibly even biochemical way via addition of chelating carboxylate ligands in solutions buffered at neutral pH.

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