Bioencapsulation

DOI: 10.1002/anie.200803307

Chemically Triggered Biodelivery Using Metal–Organic Sol–Gel Synthesis**

Vadim G. Kessler, Gulaim A. Seisenbaeva, Maria Unell, and Sebastian Håkansson*

The application of inorganic materials for drug delivery and bioencapsulation has recently attracted the attention of researchers. Silica-based mesoporous monoliths[1,2] and sol–gel-derived amorphous silica particles[3] have been actively studied for their slow release of adsorbed pharmaceutical agents, while soft silica gels have been demonstrated to act as highly biocompatible matrices for bacterial encapsulation.[4,5] Silica matrices have even been tested for biodelivery applications, specifically for medical treatments that exploit the delayed liberation of microorganisms able to attack target diseases. In particular, the liberation of viruses from silica gels has been studied for the development of viral gene therapy.[6,7] The disadvantages of silica gels for such applications are connected with the combination of high porosity and slow biodegradability, which means that the encapsulated viruses may cause an immune rejection before they are released in efficient amounts.

Many metal oxides, in particular titanium dioxide, are known to be highly biocompatible, at least when photochemical oxidation processes are prevented.[8] In addition, aluminum oxide gels, produced from inorganic salt precursors and washed to remove excess electrolyte, were demonstrated to be biocompatible hosts for microorganisms.[9] Titania nanotubes have even been investigated as a possible source in the context of slow drug release.[10] Development of titania-based sol–gel materials for bioencapsulation and controlled drug release has so far been hindered by the high reactivity of the metal–organic sol–gel precursors, titanium alkoxides, which require anhydrous media for the preparation of colloid solutions.[11]

We report herein the preparation of biocompatible aqueous titania sols and gels and their application in the encapsulation of microorganisms and pharmaceuticals with the possibility of controlled and chemically and biologically triggered release. Stabilization of metal alkoxide sols is usually achieved by modifying the precursors with chelating organic ligands. This chelation increases the reactivity of the alkoxides in hydrolysis/polycondensation, resulting in facile formation of self-assembled micellar aggregates. These structures, micelles templated by self-assembly of ligands (MTSALs), are covered by residual heteroligands.[12] 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 charged (protonated) by the addition of strong acids. The proposed stabilization mechanism for micelles is displayed in Figure 1. In our experiments, solutions of titanium alkoxides

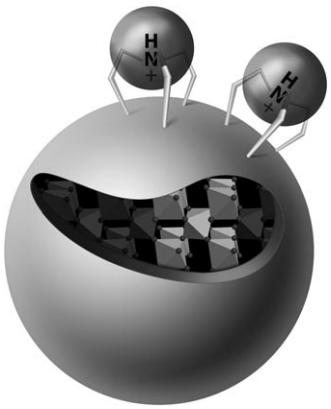

Figure 1. The structure of a metal alkoxide derived micelle templated by self-assembly of ligands (MTSAL). Positively charged ligands on the surface stabilize the produced colloids.

(20–40 vol %) in the parent alcohols were modified by 0.4–0.7 equiv of $N(C_2H_4OH)_3$ and hydrolyzed by 1.5–2 equiv of H_2O containing 0.1–0.15 equiv of H_3O^+. The resulting slightly viscous sols were immersed in deionized water or isotonic NaCl solution, which provided initially colorless and transparent aqueous sols. Depending on the amount of modifying ligand and the acid added, the gelation time for the aqueous sols could be controlled in the time span of minutes to several days. Drying the formed gels in ambient atmosphere resulted in completely amorphous xerogels, as deduced by powder X-ray diffraction. These xerogels are built up of hierarchically constructed closed mesoporous aggregated structures that are typical of xerogels formed through the coalescence of primary hydrolysis/polycondensation-derived particles[13,14] (Figure 2).

[*] Prof. V. G. Kessler, Dr. G. A. Seisenbaeva
Department of Chemistry, SLU
P.O. Box 7015, 75007 Uppsala (Sweden)
E-mail: vadim.kessler@kemi.slu.se
Dr. M. Unell, Dr. S. Håkansson
Department of Microbiology, SLU
P.O. Box 7025, 75007 Uppsala (Sweden)

[**] We thank Prof. S. Parola and Dr. F. Chassagneux at the University Claude Bernard Lyon-1 for help with TEM experiments and continuous fruitful discussions, and Prof. Y. Gunko at Trinity College Dublin for tests of the photochemical activity of titania colloids. Dr. K. Edsman, Dr. J. Näsström, and the Q-Med AB company in Uppsala are gratefully acknowledged for assistance with the studies of drug release from titanium dioxide gels. The Swedish Research Council (Vetenskapsrådet) is gratefully acknowledged for financial support of the project "Molecular Precursors and Molecular Models of Nanoporous Materials". The Swedish Foundation for Strategic Environmental Research (MISTRA) is acknowledged for funding of the research program "Domestication of Microorganisms (DOM)".

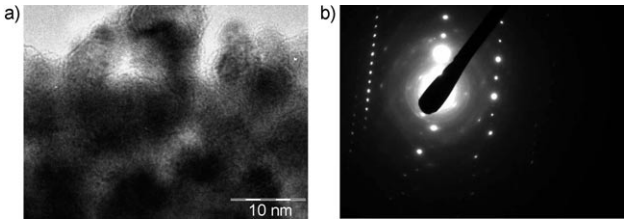

Figure 2. a) TEM image of a gel resulting from coalescence of MTSALs and b) electron diffraction image obtained from the crystalline core of a particle.

Examination of the fresh xerogels by transmission electron microscopy (TEM) 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 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 for soil bioremediation.[15,16] The yeast *Pichia anomala* J121 and the lactic acid bacteria *Lactobacillus plantarum* MiLab393 have both been shown to have antifungal activities of different modalities making them good biocontrol candidates for the prevention of mold growth in moist feed storage systems.[17–19]

According to observations by optical microscopy, the encapsulated microorganisms are 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 and lactate.[20] Treatment of gels containing encapsulated microorganisms with citrate buffer at pH 6 led to its complete dissolution within a few minutes and thus the release of the encapsulated microbes (see Figure 3), which could subsequently be analyzed for their survival. This complete and almost immediate liberation of the encapsulated material on action of a specific biocompatible chemical agent is a principally new feature previously not observed for any other inorganic or organic encapsulation matrix. It should be mentioned that the gels are otherwise highly chemically stable and do not dissolve on action of, for example, strong acids at $pH > 1.0$. The time required for dissolution of the gels in the presence of citrate ligands is clearly concentration dependent; this implies the possibility for chemically triggered and controlled release of encapsulated biological material.

The survival rates of encapsulated *P. anomala* and *Lb. plantarum* after dissolution of the hydrogels in citrate buffer was $95 \pm 10\,\%$ and $79 \pm 8\,\%$, respectively. The survival rate of encapsulated *A. chlorophenolicus* was significantly lower: $3.4 \pm 2.7\,\%$. The sensitivity to moderate levels of ethanol ($\leq 10\,\%$) did not differ between the different species (corresponding data are available on request from the authors).

Encapsulated *A. chlorophenolicus* displayed no detectable biological activity after repeated washing of the hydrogel with isotonic NaCl solution. However, encapsulated *P. anomala* and *Lb. plantarum* both displayed residual biological activity after several cycles of washing; we observed growth on culture plates, indicating that these microorganisms are capable of self-mediated release. The TEM study of heat-treated xerogels (air-dried hydrogels containing encapsulated *Lb. plantarum* heated at 400 °C in 30 min) provides proof of a continuous coating on the surface of the microorganisms through self-assembly of the primary particles. The surrounding gel transforms into hierarchical spherical aggregates outside the dense coating in the same manner as for gels alone (Figure 4). The entrapment of the microorganisms within the hydrogels is thus likely explained by the formation of a dense oxide coating on their surface. This structure is apparently responsible for one more peculiar feature of the obtained gel encapsulates: the produced shells prove to be rather dense and impenetrable even for relatively small organic molecules. For example, the samples of *A. chloro-*

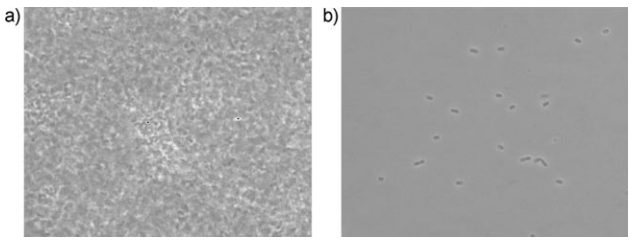

Figure 3. Release of bacteria encapsulated in a TiO_2 gel by treatment with a citrate buffer: images of the initial gel (a) and liberated bacteria (b). Optical microscopy images ×200.

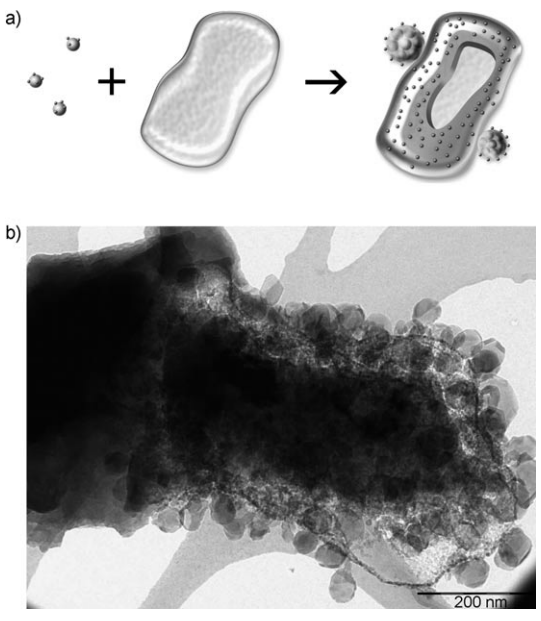

Figure 4. a) The mechanism of encapsulation of a cell in a metal oxide gel; the coalescence of MTSALs around a living cell with formation of a continuous oxide shell. b) TEM view of the formed oxide shell and MTSAL aggregates surrounding it.

phenolicus with the density of vital encapsulated bacteria as high as 2.18×10^7 colony-forming units (cfu; according to viable count after subsequent liberation via dissolution in a citrate buffer) did not display any detectable effect of chlorophenol degradation within several hours. This observation strongly indicates that this type of encapsulation can serve as chemical protection of microorganisms for, in particular, biocontrol and probiotic applications.

The residual activity and growth of *P. anomala* and *Lb. plantarum* after several cycles of washing ($78\pm17\%$ and $80\pm29\%$ 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 permits a significant fraction of the organisms to weaken the surrounding capsule by partial dissolution, leading to their release. The reason for the inability of viable *A. chlorophenolicus* to dissolve the hydrogel has not been clarified, but one possible explanation is that this strain is incapable of producing appropriate chelating ligands under these conditions.

These observations are important as they open prospects for the application of such encapsulates in tissue engineering. The inflammatory processes in the body are in fact associated with increased production of chelating carboxylate ligands. The 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 introduced these entities as components of the ligand systems in the MTSALs. For this purpose anhydrous 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 (Figure 5a). The molecules of the complexes are dimeric and pseudo-centrosymmetric with each the titanium atom attached to one ligand residue. The chiral structure of the ligands, however, provides chirality to both the complexes and their resulting packing. This is reflected by the fact that the crystals of both isopropoxo and *n*-propoxo complexes adopt the non-centrosymmetric monoclinic group *P*2(1).

These complexes were added to 8–10-fold excess of unmodified titanium alkoxides and treated with triethanolamine and acidic aqueous solution to produce organic sols, which were converted further into aqueous sols and gels by immersion into aqueous media. The aqueous gels were then placed in isotonic NaCl solution 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 with the same amount of Ibuprofen-free gel. The increase in the concentration of the released drug (Figure 5b) can be described rather accurately using a logarithmic approximation within the first 300 min (released fraction $=0.1656\ln(t)-0.2043$, $R^2=0.9705$) with complete release in over 500 min; these characteristics are at least as good as those for reported earlier mesoporous silica matrices.[1,2] The total fraction released between 500 and 1400 min

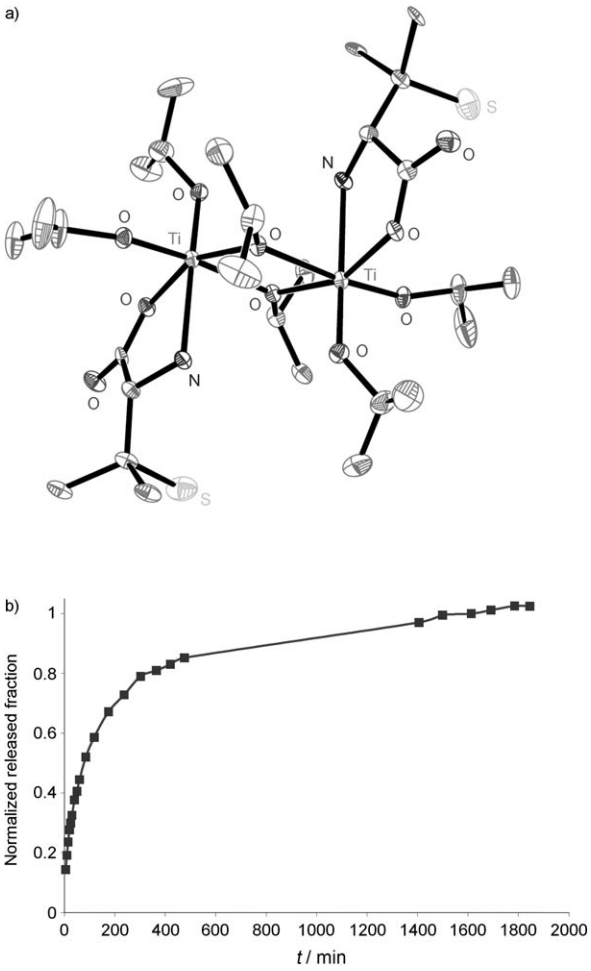

Figure 5. a) Molecular structure of the titanium alkoxide complex with the antirheumatic and immunodepressive drug D-penicillamine. b) Kinetics of release of a model drug (Ibuprofen) from the TiO_2 gel.

was less than 10%, justifying the interruption of measurements overnight. The residual 5% of the loaded drug was released in the following 600 min. It should be mentioned that despite their UV-absorbing properties, the prepared sols and gels do not display any noticeable photochemical activity according to the standard rhodamine test.[21] This particular feature can be explained by the core–shell structure of the primary particles making up these colloids: the crystalline cores absorb UV light at rather short wavelengths, resulting in the formation of an electron–hole pair. The mobility of the holes is, however, strongly decreased in the amorphous shell leading to recombination of the photochemically generated pairs without further transformation.[22]

In conclusion, the proposed modification approach permits the preparation of highly biocompatible and photochemically inactive titania sols and gels with principally new encapsulation characteristics. The biocomposite materials produced from these new colloids contain microorganisms within dense shells impenetrable even by relatively small organic molecules; this opens new possibilities for the protection of biomaterials for biocontrol and probiotic applications. The encapsulated material can, however, be liberated completely and rapidly; the release mechanism can

be controlled chemically and, possibly, even biochemically by addition of chelating carboxylate ligands in solutions buffered at neutral pH.

Experimental Section

The biocompatibility of the hydrogels was tested by encapsulation trials using a number of model microorganisms. The initial precursor solution was obtained either by dissolving $Ti(OEt)_4$ (4 mL) in anhydrous EtOH (6 mL) and adding 1.0 mL of triethanolamine (for *A. chlorophenolicus*), or by dissolving $Ti(OEt)_4$ (5 mL) in anhydrous EtOH (5 mL) and adding 1.5 mL of triethanolamine (for *P. anomala* or *Lb. plantarum* MiLab 393). Hydrolyzing solution (1 mL), prepared by mixing 0.5 M HNO_3 (0.5 mL) with EtOH (2.0 mL), was added to provide the organic sol, and 1 mL of this sol was quickly added to 9 mL of a suspension of the respective model microorganism in isotonic NaCl solution. Hydrogel encapsulates containing microorganisms (1 mL) were added to 0.10 M citrate buffer solution (9 mL, pH 6). Complete dissolution within 10 min of gentle agitation resulted in transparent suspensions of the microorganisms. The obtained solutions were then subjected to tenfold serial dilutions in isotonic NaCl and analyzed for viable counts by spreading on cultivation plates based on MRS (Oxoid Ltd., Basingstoke, England) for *Lb. plantarum*, malt extract (Oxoid) for *P. anomala*, and minimal medium (GM) as described previously[15] supplemented with 0.1% yeast extract (Oxoid) for *A. chlorophenolicus*.

The size of the initial particles in the aqueous sols was measured by dynamic light scattering (ZetaSizer 3000 HSa, Malvern). FTIR spectra of sols and gels were recorded with a Perkin–Elmer Spectrum 100 instrument without dilution in a cell fitted with CaF_2 windows. 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 a Hitachi U-2001 spectrophotometer.

The release of pharmaceuticals was studied by putting 10 mL of the gel into 300 mL of the designated medium (isotonic NaCl solution or solutions modeling the compositions of body fluids by addition of relevant buffers and lactate and citrate salts) and stirring the mixture (70 rotations per minute) at constant room temperature (25 °C). Aliquots (3 mL) were removed at the prescribed times. The removed solution was replaced immediately by the same volume of fresh medium. The Ibuprofen content in the removed samples was determined spectrophotometrically. All the experiments were conducted two or three times to check the reproducibility.

Kinetic studies of the effect of the concentration of the release medium on the process of complete delivery were carried out by shaking a standard portion of the gel (0.2 mL) with a chosen volume of citrate buffer at pH 6.0, containing the amount of citrate sufficient for complete dissolution of titania (5 mL for 0.100 M and 0.020 M, 10 mL for 0.010 M and 20 mL for 0.005 M citrate buffer). The observed times of total dissolution of the gel were 59 s for 0.100 M, 305 s for 0.020 M, 864 s for 0.010 M, and about 2500 s for the 0.005 M solution.

Crystallography: L-penicillamine derivative of titanium isopropoxide (see Figure 5a). The data collection was carried out at room temperature (23 ± 2 °C) using a SMART CCD 1k diffractometer with $Mo_{K\alpha}$ radiation ($\lambda = 0.7083$ Å) in the 2θ range: 1.7–28.5°. $C_{28}H_{62}N_2O_{10}S_2Ti_2$, $M_w = 746.72$ Da, monoclinic, space group $P2(1)$, $a = 10.631(7)$, $b = 16.674(12)$, $c = 12.156(8)$ Å, $\beta = 109.280(14)°$, $V = 2034(2)$ Å^3, $\rho_{calcd} = 1.219$ g cm^{-3}. Subsequently, the data were reduced after integration to the resolution of 1.0 Å to remove the domains with too low fraction of observed data (4345 symmetrically independent reflections with $R_{int} = 0.0296$). The structure was solved by direct methods. The coordinates of all non-hydrogen atoms were obtained from the initial solution and refined in isotropic and then anisotropic approximation. The hydrogen atoms were added by geometrical calculation and refined isotropically using a riding model. Final discrepancy values were $R1 = 0.0678$, $wR2 = 0.1730$ for 3082 reflections with $I > 2\sigma(I)$).

Received: July 8, 2008
Published online: October 1, 2008

Keywords: biodelivery · drug delivery · metal alkoxides · sol–gel processes

[1] M. Vallet-Regi, F. Balas, M. Colilla, M. Manzano, *Solid State Sci.* **2007**, *9*, 768.
[2] M. Vallet-Regi, F. Balas, D. Arcos, *Angew. Chem.* **2007**, *119*, 7692; *Angew. Chem. Int. Ed.* **2007**, *46*, 7548.
[3] C. Barbé, J. Bartlett, L. G. Kong, K. Finnie, H. Q. Lin, M. Larkin, S. Calleja, A. Bush, G. Calleja, *Adv. Mater.* **2004**, *16*, 1959.
[4] N. Nassif, O. Bouvet, M. N. Rager, C. Roux, T. Coradin, J. Livage, *Nat. Mater.* **2002**, *1*, 42.
[5] D. Avnir, T. Coradin, O. Lev, J. Livage, *J. Mater. Chem.* **2006**, *16*, 1013.
[6] M. Koskinen, M. Toriseva, M. Jokinen, H. Jalonen, J. Salonen, V.-M. Kähäri, *Mol. Ther.* **2005**, *11*, 422.
[7] R. Viitala, M. Jokinen, S. L. Maunu, H. Jalonen and J. B. Rosenholm, *J. Non-Cryst. Solids* **2005**, *351*, 3225.
[8] G. A. Crawford, N. Chawla, K. Das, S. Bose, A. Bandyopadhyay, *Acta Mater.* **2007**, *3*, 359.
[9] M. Amoura, N. Nassif, C. Roux, J. Livage, C. Coradin, *Chem. Commun.* **2007**, 4015.
[10] A. A. Ayon, M. Cantu, K. Chava, C. M. Agrawal, M. D. Feldman, D. Johnson, D. Patel, D. Marton, E. Shi, *Biomed. Mater.* **2006**, *1*, L11.
[11] J. Livage, T. Coradin, *Handbook of Sol-Gel Science and Technology* (Ed.: S. Sakka, H. Kozuka), Kluwer, Dordrecht, **2004**, p. 485.
[12] V. G. Kessler, G. I. Spijksma, G. A. Seisenbaeva, S. Håkansson, D. H. A. Blank, H. J. M. Bouwmeester, *J. Sol-Gel Sci. Technol.* **2006**, *40*, 163.
[13] P. Werndrup, M. Verdenelli, F. Chassagneux, S. Parola, V. G. Kessler, *J. Mater. Chem.* **2004**, *14*, 344.
[14] R. Pązik, D. Hreniak, W. Stręk, V. G. Kessler, G. A. Seisenbaeva, *J. Alloys Compd.* **2008**, *451*, 157.
[15] K. Westerberg, A. M. Elvang, E. Stackebrandt, J. K. Jansson, *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 2083.
[16] M. Unell, N. Kabelitz, J. K. Jansson, H. J. Heipieper, *FEMS Microbiol. Lett.* **2007**, *266*, 138.
[17] P. Melin, I. Sundh, S. Håkansson, J. Schnürer, *Biotechnol. Lett.* **2007**, *29*, 1147.
[18] J. Schnürer, J. Magnusson, *Trends Food Sci. Technol.* **2005**, *16*, 70.
[19] V. Passoth, E. Fredlund, U. Druvefors, J. Schnürer, *FEMS Yeast Res.* **2006**, *6*, 3.
[20] I. Truijen, I. Haeldermans, M. K. Van Bael, H. Van den Rul, J. D'Haen, J. Mullens, H. Terryn, V. Goossens, *J. Eur. Ceram. Soc.* **2007**, *27*, 4537.
[21] J.-H. Wang, J. D. Bartlett, A. C. Dunn, S. Small, S. L. Willis, M. J. Driver, A. L. Lewis, *J. Microsc.* **2005**, *217*, 216.
[22] A. G. Agrios, P. Pichat, *J. Photochem. Photobiol. A* **2006**, *180*, 130.

