Magnetic Sensors

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Separable Magnetic Sensors for the Optical Determination of Oxygen**

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Monitoring of oxygenation plays an important role in many areas, such as medical and biological research, bioreactor processes, and wastewater analysis.[1] Electrochemical and optical sensors are the two main types of sensors used for monitoring this parameter. Optical sensors have some significant advantages over electrochemical sensors: they do not require a reference element, and they can be miniaturized easily. For measurements in bioreactors with a total volume of over a liter, the Clark electrode is the most commonly used type of oxygen sensor. However, there is an increasing interest in the cultivation of cells and tissues in small volumes, for example, microtiter plates, which enable parallel multi-analysis. The commonly used bulk probes are not suitable for this purpose, because of drawbacks such as the sensor size, their high cost, and potential contamination.

An alternative method is to fix a sensor spot inside the sample vessel for measurements in microtiter plates or directly in the reactor.[2] Such sensors have many advantages: the sensor spot can be read out from the outside and isolated optically, the inhibition or toxicity of the dye towards sample components is minimized, and the sensor can be readily autoclaved. Nevertheless, each reaction vessel has to be loaded with the sensor spot individually, which increases costs and reduces the applicability. Moreover, sometimes fixing of the sensor spot inside the reactor can be cumbersome or even impossible. Alternatively, an oxygen indicator dye can be dissolved in the sample directly[3] or incorporated in dispersed micro- or nanobeads.[4]

Although such methods are very simple and readily adaptable, they suffer from significant drawbacks, such as interference with the sample, sensitivity to turbidity, potential toxicity, and the necessity for large amounts of dye when measuring in volumes above a few milliliters. The alternative strategy presented here uses sensor particles with magnetic properties. This allows the in situ formation of sensor spots by magnetic separation and, consequently, optical readout from the outside. Figure 1 shows the possible sensing configuration in a bioreactor. Magnetic optical particles combine the advantages of solid sensor spots with the ease of handling and universality of dissolved indicators. The handling of such magnetic sensors is simple; no centrifugation is needed for washing, and they can be merely added to the sample, collected with a bar magnet, and read out. If required, release and re-collection is also possible.

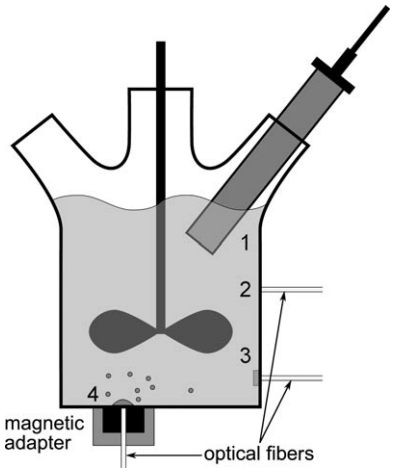

Figure 1. Schematic illustration of sensing concepts in bioreactors: electrochemical (Clark electrode) or fiber-optic sensor (1); external readout of a dissolved indicator (2), of a solid sensor spot (3), and of magnetic optical sensor particles collected with a magnet (4).

Herein, we present such magnetic sensor particles for the monitoring of oxygenation in bioreactors. Their preparation is based on the well-known silica sol–gel technology,[5] which yields a sol mixture with highly versatile composition. There are some reports on magnetic silica particles,[6] but the concept of combining optical sensor particles with magnetic properties is new.

Aspherical oxygen-sensitive particles were obtained from a sol–gel procedure that used tetramethoxysilane with dispersed Fe_3O_4 and TiO_2 nanobeads and the popular tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride ($[Ru(dpp)_3]Cl_2$) as encapsulated oxygen-sensitive indicator (Figure 2). $[Ru(dpp)_3]Cl_2$ was selected because of its bright luminescence and good resistance against leaching. The use of a phenyl-substituted Ormosil resulted in spherical sensor particles with superior selectivity as a result of the shielding of hydrophilic interferents by the hydrophobic phenyl groups. Furthermore, the matrix provided stable incorporation of the indicator.[7] The spectral properties of the indicator and the spherical sensor particles are reported in the Supporting Information.

The most desired characteristics of such magnetic sensor particles are good separability and high brightness of

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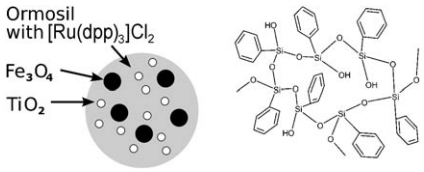

Figure 2. Schematic representation of a cross-section of the spherical sensor particles (Ormosil matrix) and the structure of the Ormosil material.

luminescence. Paramagnetic magnetite nanobeads allow the sensor particles to be magnetically separated and provide optical isolation from the sample after formation of the particle spot. Such isolation reduces interference with the sample. However, as a consequence of its black color, magnetite is responsible for the filter effect and self-absorption of the phosphorescence signal. Therefore, there is a need for a scattering additive, such as hydrophilic TiO_2, which makes the excitation of the indicator more efficient and, at the same time, increases the signal.

Two methods were evaluated to design magnetic microspheres. The first is based on condensation of the silica sol to form a monolith, which can then be ground in a mortar. These particles will be referred to as "aspherical" sensor particles. Alternatively, a method based on emulsion polymerization results in spherical sensor particles. Figure 3 shows microscopic images of both types of oxygen-sensitive particles. Aspherical ground particles (Figure 3b) form a toothed structure, which ensures higher stability against shear forces compared to spherical particles.

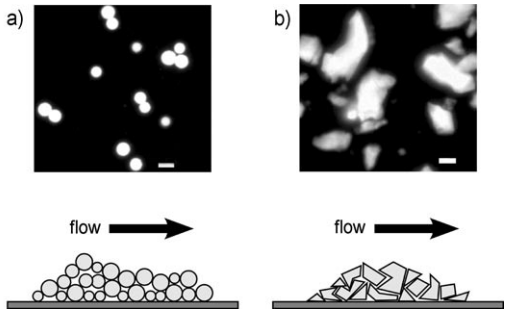

Figure 3. Microscopic phosphorescence images of sensor particles (400×): a) spherical particles, b) ground monolith. $[Ru(dpp)_3]Cl_2$ dye was excited using a 515–560-nm band-pass filter, and a 590-nm long-pass filter served as the emission filter. Scale bars 10 μm.

The particle size is also a relevant parameter for the applicability of magnetic spheres. When reducing the particle size, the magnetite content would have to increase to provide enough separability, but the self-absorption would increase at the same time, thus reducing the brightness of the signal. Hence, we focused on designing microspheres instead of nanospheres.

To investigate the influence of magnetite and titanium dioxide on particle separability, brightness, and quenchability, different aspherical, monolithic sensor particles containing identical amounts of indicator dye were prepared. The proportion of magnetite was varied (**A**–**E**) and afterwards the sensors were characterized within a capillary (see the Supporting Information). The results of this experiment are listed in Table 1. The more magnetite the sensor contained, the lower were the intensities obtained. The sensitivity of the sensor (τ_0/τ) was hardly affected but at the same time the phosphorescence lifetime decreased. After collection of the particles within the capillary at a flow rate of 2.03 cm s^{-1}, the pump velocity was raised. The maximal flow rate indicates the maximal pump velocity at which the particle spot remains in front of the optical fiber.

Table 1: Sensor characteristics of ground monoliths (aspherical sensor particles) with different contents of magnetite.

	A[a]	B	C	D	E
% Fe_3O_4 related to pure SiO_2	1	2.5	5	10	15
relative intensity (deaerated sample)		4400	3300	1900	900
τ_0 [μs]	–	4.06	3.96	3.85	3.68
τ_0/τ	–	1.61	1.62	1.63	1.66
maximal flow rate [cm s^{-1}]	–	2.40	4.04	8.10	9.71

[a] Not enough magnetite to separate the particles.

Table 2 shows the sensor characteristics of particles containing different amounts of titanium dioxide (**F**–**I**). By addition of this high-scattering component, it was possible to increase the signal intensity. A surplus of titanium dioxide, on the other hand, resulted in dye quenching (**J**). Although the phosphorescence lifetime decreased gradually, the Stern–Volmer constant (K_{SV}) and sensitivity did not change considerably.

Table 2: Sensor characteristics of ground monoliths (aspherical sensor particles) with different contents of titanium dioxide.

	F	G	H	I	J
% Fe_3O_4 related to pure SiO_2	10	10	10	10	10
% TiO_2 related to pure SiO_2	0	10	25	50	100
relative intensity (deaerated sample)	1900	2500	2900	3600	2700
τ_0 [μs]	3.51	3.47	3.42	3.37	3.20
τ_0/τ	1.63	1.63	1.60	1.60	1.59

Pure sol–gel was not the matrix of choice for oxygen sensing in complex media because of a lack of selectivity. For this application, spherically shaped, phenyl-substituted Ormosil particles were employed. Typical nonlinear Stern–Volmer curves were measured for this type of sensor particle, which could be calibrated with the two-site model of Carraway et al.[8] (Figure 4b).

Figure 4a shows the response of a collected particle spot towards changes in oxygenation. The response is fully reversible. The delay in response reflects the delayed oxygenation of the sample solution. Individual, free-floating particles react within a few seconds, which ensures real-time oxygen monitoring.

Some examples of new potential applications of magnetic optical sensors are shown schematically in Figure 5. Such

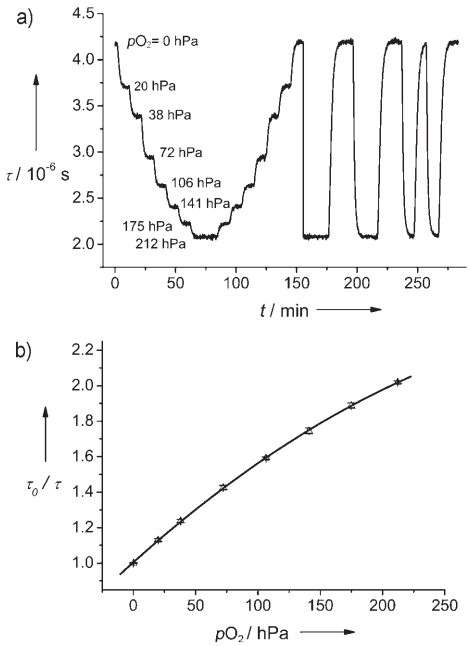

Figure 4. a) Response curve of collected spherical sensor particles at various concentrations of dissolved oxygen. b) Calibration curve of the oxygen-sensitive, spherical particles at various concentrations of dissolved oxygen. The calibration was validated with a commercially available oxygen sensor spot (PreSens, Germany).

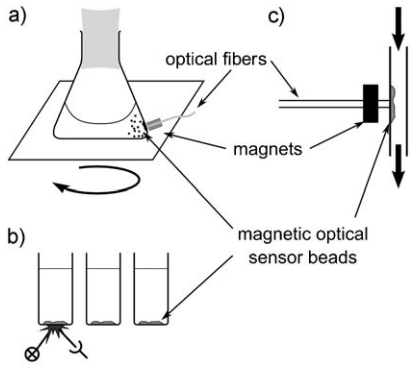

Figure 5. Schematic representation of the potential range of application of sensor beads in: a) a shaking flask, b) microtiter plate measurements, and c) flow-through systems.

magnetic sensors can be applied in shaking flasks to monitor cell cultivation. They can also be used in flow-through systems as well as in microtiter plates, where they form partially optically isolated spots that can be read out from the plate bottom. Most importantly, they can be manipulated inside the vessel.

The use of magnetically separable sensor particles instead of solid sensor spots offers a series of advantages, such as high flexibility in use. In particular, in vessels and fluidic systems with a complex geometry it is often difficult or even impossible to incorporate conventional chemical or electrochemical sensors. Here the new material offers an excellent alternative. The small amount of separable sensing material required to achieve a measurable signal is an especially attractive feature. Typically, particle concentrations around 10 mg L^{-1} were employed, but even concentrations below 1 mg L^{-1} resulted in significant signals.

The time required for spot formation depends on various factors, such as sample velocity, reactor or vessel design, the magnetic adapter used for separation, and the sensor concentration. A concentration of 2 mg L^{-1} in a volume of 160 mL required up to 60 min for spot formation, whereas at higher concentrations (ca. 20 mg L^{-1}) stable signal intensities could be reached within a few minutes. Another challenge was the efficient collection of the particles in the optical field of view. A detailed study on the design of optimized magnetic adapters and the influence of the wall thickness, shear forces, and other parameters was recently published by Mistlberger et al.[9]

In the case of long-term use of a separated spot, as required in biotechnological or process monitoring, adhesion of cell material and biofouling can be an issue. The magnetic particles described herein allow release and re-collection of the sensor particles, which provides another key advantage.

To demonstrate and test the measuring properties of the calibrated particles, they were used to monitor oxygen concentration during bacterial growth. The spherical, oxygen-sensitive sensor particles were added to the culture medium in a baffled shake flask and separated from the suspension, to form a spot on the flask side. Oxygenation was controlled by simultaneous measurement with a commercially available solid sensor spot purchased from PreSens.

After approximately 2.5 h of growth, the culture became nearly deaerated and all oxygen diffusing from the atmosphere was consumed immediately for metabolism by the bacteria (Figure 6). The responses of our particle sensor spot

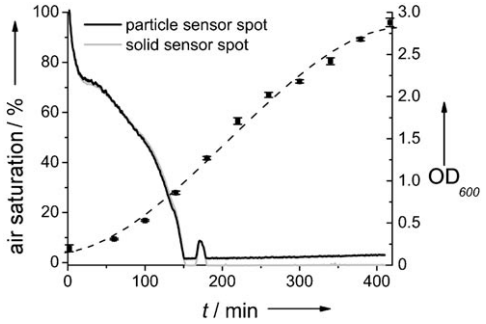

Figure 6. Oxygen monitoring in *Escherichia coli* culture (BL21 strain) in a baffled shake flask at 25 °C and 70 rpm. Black solid line: particle sensor spot; gray solid line: solid sensor spot. Bacterial growth was controlled by measurements of optical density (OD) at 600 nm (-----).

and the solid sensor spot from PreSens fit well. The slight difference at the baseline might be explained by background fluorescence of the cultivated bacteria. This experiment shows the principal usefulness of the presented sensors for oxygenation monitoring during fermentation, and also reveals the need for particles with improved properties, such as higher brightness and/or near-IR indicators.

In conclusion, we have designed separable optical sensor beads by equipping optical sensor particles with magnetic

properties. The use of these particles allows the reversible in situ formation of optical sensor spots suitable for the conventional, well-established readout techniques for solid sensor spots. These sensor particles have many advantages over solid spot sensors. Both the handling and the synthesis of such particles are simple. They can be collected at any desired position inside the sample vessel. Moreover, the sol–gel technology offers a flexible production method, so that the composition of the sensor materials can be controlled and varied in a wide range. Measurements in flow-through systems and in shake flasks confirmed the usefulness of the particles for oxygen monitoring. In an analogous manner to the method presented here, sensors for different analytes, such as pH, metal ions, and gases, can be developed.

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[1] a) F. G. Gao, J. M. Fay, G. Mathew, A. S. Jeevarajan, M. M. Anderson, *J. Biomed. Opt.* **2005**, *10*, 054005/1; b) P. Jamnik, P. Raspor, *J. Biochem. Mol. Toxicol.* **2005**, *19*, 195 – 203; c) R. A. Yotter, L. A. Lee, D. M. Wilson, *IEEE Sens. J.* **2004**, *4*, 395 – 411; d) M. C. Frost, M. E. Meyerhoff, *Curr. Opin. Chem. Biol.* **2002**, *6*, 633 – 641; e) W. Wang, P. Vadgama, *J. R. Soc. Interface* **2004**, *1*, 109 – 117; f) Y.-S. Yun, J. I. Park, J. M. Park, *Process Biochem.* **2005**, *40*, 1301 – 1306; g) M. Fitzgerald, D. B. Papkovsky, M. Smiddy, J. P. Kerry, C. K. O'Sullivan, D. J. Buckley, G. G. Guilbault, *J. Food Sci.* **2001**, *66*, 105.

[2] a) M. A. Hanson, X. Ge, Y. Kostov, K. A. Brorson, A. R. Moreira, G. Rao, *Biotechnol. Bioeng.* **2007**, *97*, 833 – 841; b) F. Kensy, G. T. John, B. Hofmann, J. Buechs, *Bioprocess Biosyst. Eng.* **2005**, *28*, 75 – 81; c) S. Arain, S. Weiss, E. Heinzle, G. T. John, C. Krause, I. Klimant, *Biotechnol. Bioeng.* **2005**, *90*, 271 – 280; d) G. T. John, D. Goelling, I. Klimant, H. Schneider, E. Heinzle, *J. Dairy Res.* **2003**, *70*, 327 – 333; e) D. Weuster-Botz, R. Puskeiler, A. Kusterer, K. Kaufmann, G. T. John, M. Arnold, *Bioprocess Biosyst. Eng.* **2005**, *28*, 109 – 119; f) C. Wittmann, H. M. Kim, G. John, E. Heinzle, *Biotechnol. Lett.* **2003**, *25*, 377 – 380; g) L. Tolosa, Y. Kostov, P. Harms, G. Rao, *Biotechnol. Bioeng.* **2002**, *80*, 594 – 597; h) P. Harms, Y. Kostov, G. Rao, *Curr. Opin. Biotechnol.* **2002**, *13*, 124 – 127; i) B. H. Junker, D. I. C. Wang, T. A. Hatton, *Biotechnol. Bioeng.* **1988**, *32*, 55 – 63.

[3] F. C. O'Mahony, C. O'Donovan, J. Hynes, T. Moore, J. Davenport, D. B. Papkovsky, *Environ. Sci. Technol.* **2005**, *39*, 5010.

[4] a) S. M. Buck, Y. L. Koo, E. Park, H. Xu, M. A. Philbert, M. A. Brasuel, R. Kopelman, *Curr. Opin. Chem. Biol.* **2004**, *8*, 540; b) E. Schmälzlin, B. Walz, I. Klimant, B. Schewe, H.-G. Löhmannsröben, *Sens. Actuators B* **2006**, *119*, 251 – 254; c) E. Schmälzlin, J. T. van Dongen, I. Klimant, B. Marmodée, M. Steup, J. Fisahn, P. Geigenberger, H.-G. Löhmannsröben, *Biophys. J.* **2005**, *89*, 1339 – 1345.

[5] a) A. Van Blaaderen, A. Vrij, *Langmuir* **1992**, *8*, 2921 – 2931; b) J. Lin, C. W. Brown, *TrAC Trends Anal. Chem.* **1997**, *16*, 200 – 211; c) M. Kato, K. Sakai-Kato, T. Toyo'oka, *J. Sep. Sci.* **2005**, *28*, 1893 – 1908.

[6] a) D. Mueller-Schulte, T. Schmitz-Rode, P. Borm, *J. Magn. Magn. Mater.* **2005**, *293*, 135 – 143; b) Y. Deng, C. Deng, D. Yang, C. Wang, S. Fu, X. Zhang, *Chem. Commun.* **2005**, *44*, 5548 – 5550; c) V. Salgueirino-Maceira, M. Spasova, M. Farle, *Adv. Funct. Mater.* **2005**, *15*, 1036 – 1040.

[7] I. Klimant, F. Ruckruh, G. Liebsch, A. Stangelmayer, O. S. Wolfbeis, *Mikrochim. Acta* **1999**, 131, 35 – 46.

[8] E. R. Carraway, J. N. Demas, B. A. DeGraff, J. R. Bacon, *Anal. Chem.* **1991**, *63*, 337 – 342.

[9] G. Mistlberger, P. Chojnacki, I. Klimant, unpublished results.

