

NOTIZEN

Nanoencapsulated * Fluorescence Indicator Molecules Measuring pH and pO₂ Down to Submicroscopical Regions on the Basis of the Optode-Principle **

D. W. Lübbers and N. Opitz

Max-Planck-Institut für Systemphysiologie, Dortmund

P. P. Speiser and H. J. Bisson

Pharmazeutisches Institut der ETH-Zürich, Zürich

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To measure pO₂ in gases or fluids and pH in solutions the fluorescence indicators pyrene butyric acid and β -methylumbelliferone, respectively, were nanoencapsulated to obtain nano-probes for measurement in physiological structures of nano-range. For pH the fluorescence changes of β -methylumbelliferone were monitored, for pO₂ the fluorescence quenching of pyrene butyric acid by oxygen were registered. Drawing $\log(S_1/S_2)$ versus pH, one obtains a straight line between the pH from 6 to 8. A linear increase in pO₂ between 0 and 400 torr yields a linear increase in the reciprocal fluorescence signal.

The optode-principle (Appendix 1) introduced by means of a macroscopical device, the so-called "optode"^{1–6}, has the advantage of being applicable down to submicroscopical regions if one succeeds in nanoencapsulating the molecules of the fluorescence indicators. Owing to the development of the nanoencapsulating technique (Appendix 2), the difficulties with the encapsulation of fluorescence indicators could be overcome. The capsules were prepared by H. J. Bisson (Galenische Abteilung [director: Prof. Dr. P. P. Speiser] des Pharmazeut. Inst. der ETH-Zürich).

As compared to the macroscopical membrane-faces manufactured usually, the nanoprobe (artificial organelles) have the advantage of enabling the measurement of physiological parameters in biological structures in the nano-range, *i. e.*, after injection of the capsules in blood vessels or tissues. Possible toxic influences of the indicator molecules on biogenic material are being negligibly reduced. Thanks to the special encapsulation technique the selectivity of the membranes can be controlled more effectively.

* Schweiz. Patentgesuch vom 4. 12. 1972 mit dem Titel: „Nanokapseln“.

** Deutsche Patentanmeldung vom 28. 2. 1975, DPA-Nr. P 2508637, Titel: „Vorrichtung zur Messung von Blutgasen“.

Requests for reprints should be sent to Prof. Dr. med. D. W. Lübbers, Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, D-4600 Dortmund.

When preparing the nanoparts the aqueous micells, generated by solubilisation and incorporating the dissolved indicator, are coated by a polyacryl-membrane. The formation of this "membrane" is obtained by polymerisation of the monomer wall material by means of γ -rays of a Co-60-source⁷. The mean diameter of the polyacryl-capsules was about 150 to 250 nm.

For the pH measurement we used polyacryl-capsules, containing saturated solutions of β -methylumbelliferone in 0.3 M NaHCO₃, in aqueous suspensions of 50 mg/ml. The transport of H⁺-ions through the polyacryl-membrane, which is impermeable for CO₂ molecules, was attained by 0.1% of *p*-nitrophenol. β -methylumbelliferone changes the spectral distribution of the corrected excitation spectra ($\lambda_{em} = 445$ nm) in dependence on the pH-value. The evaluation of the spectra in a calibration curve was done from a quotient, $F = S_1/S_2$, formed at two wavelengths, λ_1 and λ_2 , where the changes of the corresponding signals, S_1 and S_2 , were maximal.

For the measurement of oxygen partial pressure the nanoparts were prepared according to the "inverse process" of nanoencapsulation. The polyacryl-capsules contained saturated solutions of pyrene butyric acid and dimethylpolysiloxane in benzene, so that no CO₂ quenching effect occurred, which is observable in aqueous indicator solutions. The fluorescence of pyrene butyric acid was quenched by oxygen^{8,9}. The corrected excitation spectra ($\lambda_{em} = 375/395$ nm), shifted 2 nm towards longer wavelengths compared with the free indicator, were evaluated at a wavelength where the signal changes were again maximal. The corrected spectra were measured by a spectrofluorometer developed by Boldt and Lübbers¹⁰.

Results

Since the main bands of the corrected excitation spectra of encapsulated β -methylumbelliferone were not displaced as compared with the free indicator, the indicator can be assumed not to interact with the wall material of the capsule. In addition the intramolecular bondages of the indicator didn't seem to be affected by the γ -irradiation of the encapsulation process. A spectrofluorometrical analysis of the supernatant of a capsule suspension in dimethylformamid showed that all the indicator molecules were encapsulated.

By plotting semilogarithmically the formfactor F versus pH, one obtains a straight line of the calibration curve in the physiologically relevant pH-range from 6 to 8 (Fig. 1). Corresponding to the pCO₂-optode^{1–3}, the limit of the indicator sensitivity is



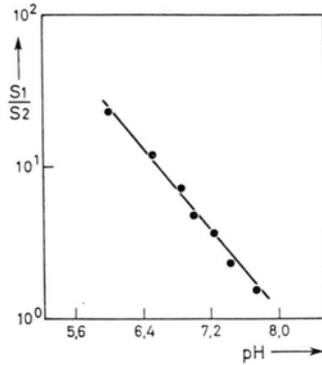


Fig. 1. pH Calibration curve of nanoencapsulated β -methylumbelliferon.

reached for pH-values lower than 6. In the alkaline range the fluorometrical pH measurement with nanoencapsulated β -methylumbelliferone is applicable up to pH 9. The response time t_{90} (90% of the final value) for the pH-nano-optodes was about 6 sec. Drawing the reciprocal fluorescence intensity ΔS_x versus pO_2 (with ΔS_0 = fluorescence intensity at $pO_2 = 0$ torr), the oxygen calibration curve shows linearity between 0 and 400 torr (Fig. 2), so that the fluorescence quenching of pyrene butyric acid by oxygen can be described in this range by the Stern-Volmer-equation¹¹: $\Delta S_0 = \Delta S_x \cdot (1 + K \cdot pO_2)$. The response time of the pO_2 -nano-optodes was $t_{90} = 3$ s.

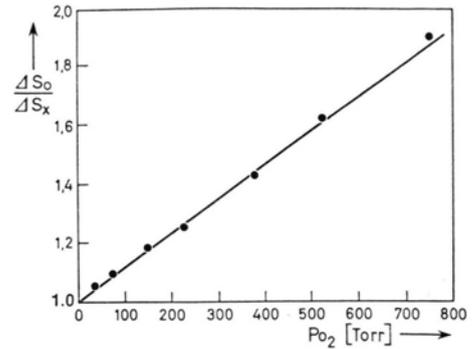


Fig. 2. pO_2 Calibration curve of nanoencapsulated pyrene butyric acid.

Appendix

1) Basic considerations of the optode-principle are: A fluorescence indicator solution is separated from the medium to be measured by a gas- or ion-permeable membrane. The gas molecules or ions to be determined diffuse into the indicator solution and interact chemically or physically with the fluorescence indicator molecules. The changes in indicator fluorescence are recorded by means of a fluorometer.

2) The nanoencapsulating technique allows the encapsulation of particles or droplets of submicroscopical size by means of a membrane.

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⁶ N. Opitz and D. W. Lübbers, Pflüg. Arch. **362**, Suppl. 1976, p. R 52, No. 208.

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