NOTIZEN

Nanoencapsulated * Fluorescence Indicator Molecules Measuring pH and pO_2 Down to Submicroscopical Regions on the Basis of the Optode-Principle **

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To measure pO_2 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 pO_2 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 pO_2 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 nanoprobes (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".

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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-source [7]. 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 $NaHCO_3$, in aqueous suspensions of 50 mg/ml. The transport of H^+-ions through the polyacryl-membrane, which is impermeable for CO_2 molecules, was attained by 0.1% of *p*-nitrophenol. β-methylumbelliferone changes the spectral distribution of the corrected excitation spectra (λ_{em} = 445 nm) in dependance 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 CO_2 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 (λ_{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 Lübbers [10].

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 pCO_2-optode [1–3], the limit of the indicator sensitivity is

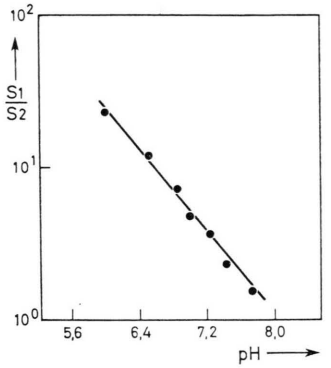

Fig. 1. pH Calibration curve of nanoencapsulated β-methyl umbelliferon.

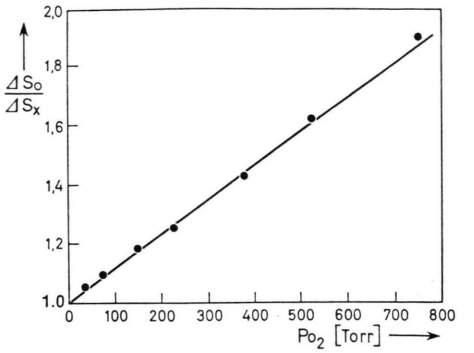

Fig. 2. pO_2 Calibration curve of nanoencapsulated pyrene butyric acid.

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-equation [11]: $\Delta S_0 = \Delta S_x \cdot (1 + K \cdot pO_2)$. The response time of the pO_2-nano-optodes was $t_{90} = 3$ s.

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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