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Transmitted light pH optode for small sample volumes

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Abstract

An innovative low cost pH optode concept for small sample volumes is presented. The pH monitoring is based on the color changing effect of phenol red, which is a well known reagent in nutrition media for cell cultivation. The optode includes an USB spectrophotometer, a controllable LED, some reactor slides and a self-written LabVIEW software. Spectral behavior at 450 nm and 560 nm is indicative of the pH value of the investigated samples.

Keywords: pH optode, transmittance measurements, small sample volumes

Introduction

The new approach of fermentation-based bioreactors is the constant miniaturization of bioreactors in the framework of personalized medical care or high throughput. Such small-scale devices pose great challenges for measurement technology. Continuous monitoring of pH, pO_2 and pCO_2 are the most essential [1,2] for cell cultivation.

The measurement principles of optodes are well suited to overcome the challenges of monitoring small sample volumes. The main approach of pH optodes focuses on spectroscopic phenomena such as absorption, reflectance, luminescence and energy transfer based on the pH induced structure changes of indicators [3]. The objective of the present work is a pH optode based on transmitted light that can be easily used for micro-structured microfluidic arrangements.

In terms of validating optical behavior different measurement concepts were designed and manufactured [4]. Moreover, the possible application to small sample volumes enabling for high throughput screening as well as fast measurements without the need for fiber optics is particularly interesting. In this work, the pH optode is based on the transmittance change of phenol red in sample volumes less than 200 µl. Phenol red was chosen since it is a widely used pH indicator in nutrition media for cell cultivation.

Prototype

We used a path through setup for transmittance measurements of pH dependent color changing effects of phenol red. The experimental apparatus is shown in figure 1. A controllable LED (C) is used as light source. An USB spectrophotometer 2000+ from Ocean Optics (A) is used as the detection unit. For the spectral measurements the LED is underneath the sample, the light interacts with the sample and gives transmittance signals which are collected via the spectrophotometer. In this configuration there is no need of optical fibers and this allows to deal with a small portable and easy handling device. With the aid of rapid prototyping a small polycarbonate slide (E) with small holes was manufactured and is used as experimental mock-up for later micro bioreactors. The spectrophotometer is variably mounted (B) to overcome problems regarding individual thicknesses of different sample slides. Unique chamber geometrics of the shown polycarbonate slide (E) were used to define the physical detection limits for different possible approaches of micro bioreactors.

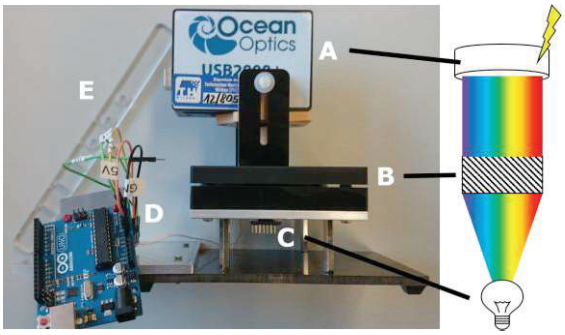

Fig. 1: pH optode; A) commercial available USB spectrophotometer; B) sample holder manufactured via rapid prototyping; C) LED; D) Arduino micro controller; E) Polycarbonate slide with bioreactor mock ups

Control and data acquisition

The pH optode prototype is working with two different hardware parts. On the one hand a LED connected to an Arduino microcontroller which is connected with an USB port to the laptop. On the other hand an Ocean Optics spectrophotometer USB2000+ which is similarly connected with an USB port to the laptop. A program based on LabVIEW was specifically developed for the controll and data acquisition of the gained spectral data. This program consists of the actual source code as well as the user interfaces, both shown in figure 2.

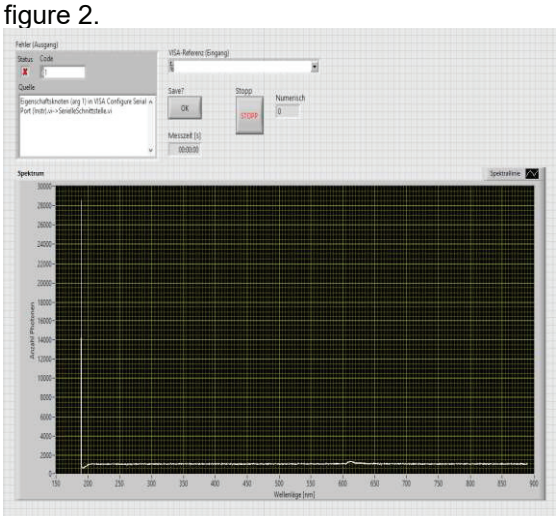

Fig. 2: self-written LabVIEW software; user interface with diagram for spectral data, a save button as well as the failure indicator (top left corner) are included

The software includes (i) a display of the spectral data collected with the spectrophotometer and (ii) the option to save single spectra as Excel files. The data analysis is made in a second stage exploiting Excel software.

Experimental Setup

Phenol red (0,04 g; P3532 Sigma-Aldrich) was dissolved in 1,13 ml NaOH (0,1 M) and filled with purified water up to 100 ml. Measurements were accomplished with sodium - / potassium phosphate buffers (SPPBs) in different concentrations, to get a stable array of pH-values from 5 to 8 in 0,1 pH-steps. The numbers reported in table 1 are some of the 30 collected data points. Phenol red and the specific SPPBs were mixed in a 1:10 dilution. All pH-values were checked with a pH electrode (Mettler Toledo InLab 490) in a 10 ml solution of each sample as well as in a common spectrophotometer (Thermo Spectronic Helios α) to verify the functionality of the prototype. 150 µl of each solution were then spread on the polycarbonate slide. The slide consists of 8 micro bioreactor mock-ups that can be analyzed separately because of the different spectral behavior.

Tab. 1: Overview of certain pH-values before and after the dilution of phenol red in different SPPBs

	pH	pH (1:10 dilution)	pH shift
Phenol red		-	-
SPPB_pH5	5,003	5,468	0,465
SPPB_pH5,5	5,555	5,777	0,222
SPPB_pH6	6,027	6,109	0,082
SPPB_pH6,5	6,511	6,572	0,061
SPPB_pH7	7,01	7,04	0,03
SPPB_pH7,5	7,533	7,573	0,04
SPPB_pH8	8,032	8,136	0,104

Results

Phenol red showed remarkable absorption bands at 430 nm and 560 nm for different pH values because of the ratio of protonated and deprotonated phenol red as shown in figure 3. The transmission spectra were collected using a commercial spectrophotometer in the range 190-890 nm every 150 ms. This short measurement time enables a quasi-instant pH value determination.

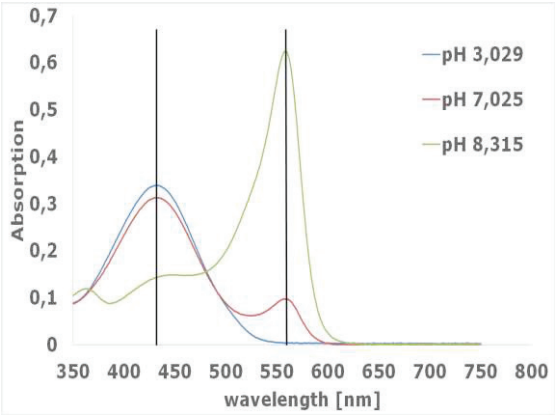

Fig. 3: Spectral data from the used commercial spectrophotometer Thermo Spectronic Helios α; absorbance measurement of phenol red in different SPPBs, best absorbance at 430 nm and 560 nm

The pH optode prototype, on the contrary to the absorbance measurements, did not use any type of monochromatic devices or a laser as light source but exploits directly the ligth from a LED. The LED spectrum shows two peaks at wavelengths of 450 nm and 560 nm and is therefore well suited for the pH monitoring with phenol red as reported in figure 4.

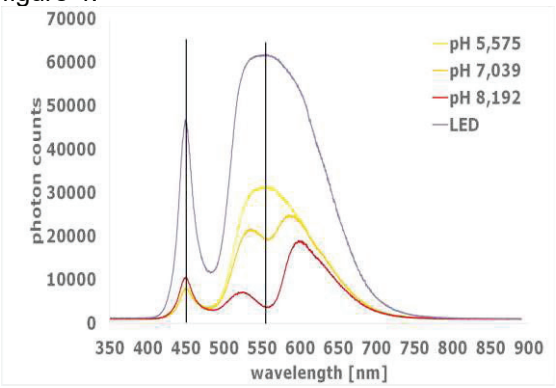

Fig. 4: Spectral data from the current pH optode prototype; spectral data of the LED and phenol red in different buffer solutions; two wavelengths were chosen for the pH dependent color changing effect of phenol red (450 nm and 560 nm)

It has to be underlined that the small difference between 430 nm (spectrophotometer) and 450 nm (pH optode) is not essential for the performance of the pH optode prototype. Over the range of 30 SPPBs with different pH-values a broad pH range was achieved for both 450 nm and 560 nm as shown in figure 5. The used pH range (5 to 8) reflects the vital pH range for common cell cultivation of human cells. Transmittance results of 560 nm showed a decreasing signal with higher pH values. Therefore, the absorbance of deprotonated phenol red at 560 nm rises, as shown also in the spectrophotometer measurements in figure 3. At the same time, transmittance signals (at 430 nm) increase with the pH value due to the decreasing effect on the absorbance of the protonated phenol red species. In all measurements of the pH optode no influences of scattered room light could be detected. Moreover, all signals were stable for more than 5 minutes.

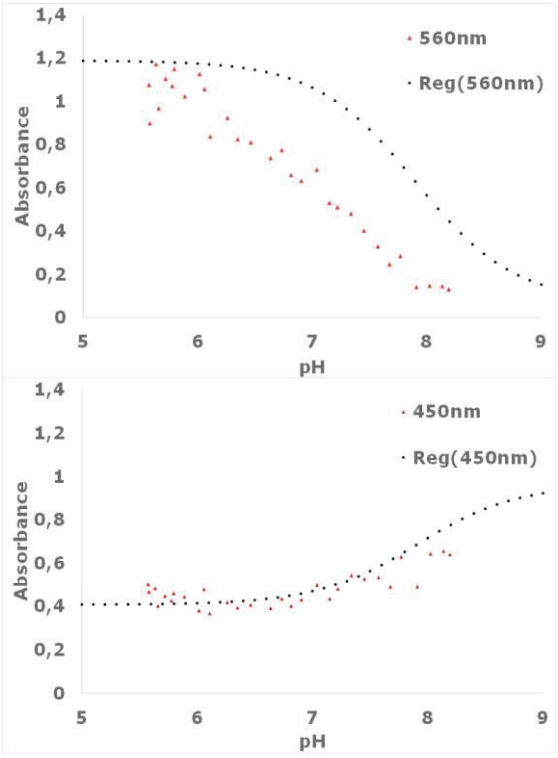

Fig. 5: Red triangles indicate transmittance of both 560 nm (top) and 450 nm (bottom) wavelength; black circles Reg(560nm) and Reg(450nm) are the correlating sigmoidal fit curves with a used pKa of 7.9

The pH dependent color changing effect of certain pH indicators is mostly described with a sigmoidal function [3]. As reported in figure 5, two different models (eq. (1) and eq. (2)) for the sigmoidal function were needed for the regression of both 560 nm and 450 nm. Equation (1) describes a decreasing signal (S) with higher pH values, whereas equation (2) describes an increasing signal.

$$S(560nm) = \frac{S_{max} - S_{min}}{1 + 10^{(pH - pK_a)}} + S_{min} \qquad (1)$$

$$S(450nm) = \frac{S_{max} * 10^{(pH - pK_a)} + S_{min}}{1 + 10^{(pH - pK_a)}} \qquad (2)$$

S(560 nm / 450 nm) is the predicted normalized transmittance signal for specific pH values. S_{max} and S_{min} are the maxima and minima values for 560 nm and 450 nm at pH values of 2.9 and 11.2, respectively. The acid dissociation constant pK_a for phenol red is 7.9. The actual regression for 560 nm shows a pH shift of about 1 to actual measurements whereas 450 nm fits perfectly. This phenomenon is directly associated with the used pK_a which is not a constant factor. The pK_a is dependent on the temperature and on the ionic strength of the solutions. Regarding these results, the pK_a for 560 nm and 450 nm should be determined individually in experiments using the specific operating conditions. Additionally to problems with pK_a taking blanks for each pH value is difficult. Each shown data point is taken from a whole spectrum. The spectra itself is not stable enough and thus gives failure when normalizing the spectral data.

Summary

The main benefits of the described pH optode are the contact free and automated pH measurement for cell cultivating systems as microfluidic batch and flow through devices. We demonstrated that there is no need for fiber optics which would increase the complexity of the system and therefore complicate the handling. Due to this new simple and effective setup more complex and expensive optical arrangements can be avoided. Optical transmission at 450 nm and 560 nm wavelengths were exploited to determine the pH value. By using two different sigmoidal fit models a calibration could be done for monitoring the pH dependent color changing effect of phenol red.

Outlook

Further steps include the determination of the lower detection limits for phenol red as well as a validation of the system with other pH indicators. While the device is already compact and easy to handle, the used spectrophotometer from Ocean Optics should be changed to even smaller and lighter detection units. Thus, future concepts will be based on small RGB-detection units instead of big and expensive spectrophotometers.

Acknowledgement

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