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ORMOSIL oxygen sensors on polystyrene microplate for dissolved oxygen measurement

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Abstract

Oxygen sensors prepared from tetramethyl orthosilicate and dimethoxy dimethylsilane with tris(4,7-diphenyl-1,10-phenanthroline)ruthenium (II) as the sensing dye were coated onto the well bottom surface of a 96-well polystyrene microtiter plate to give a high-throughput system for dissolved oxygen measurement. The oxygen sensors give linear Stern–Volmer calibration plots, and produce reliable and reproducible results in the determination of IC_{50} values of drugs on a yeast model.
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1. Introduction

Oxygen consumption by microorganisms and aerobic cells is an important indicator on their metabolic activity and state of growth. Monitoring and measurement of dissolved oxygen concentration in cell cultures is thus of interest for drug screening, cell cultivation, toxicity tests and environmental analysis [1]. With the ever increasing number of samples and the limited availability of cells and expensive biochemicals, there is a need to develop high throughput systems for multi-sample analyses with small sample volumes. The rapid development of optical oxygen sensing by luminescence quenching of oxygen-sensitive dyes in the past decade [2,3] allows the monitoring of dissolved oxygen concentration in microtiter plates in a high throughput manner [4–8]. Although microtiter plates specially designed for dissolved oxygen measurement are now commercially available [9,10], these oxygen sensing platforms have a number of drawbacks that need further improvement. These microtiter plates were prepared by immobilization of oxygen-sensitive particles in a gas permeable polymer matrix on the bottom of the microwells. The oxygen-sensitive particles consist of an oxygen-sensitive luminescent dye, either tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) ($Ru(dpp)_3^{2+}$) or platinum porphyrin, coated on silica or polystyrene particles for better dispersion in the polymer matrix. The preparation of these particles requires special techniques that are not routinely practiced in most laboratories. It is also known that the performance of the oxygen sensors depends strongly on the properties of the immobilization matrix [11–19], and the Stern–Volmer plots for the dyes in the polymer matrix are usually nonlinear [20–22], which is a major drawback in sensor calibration. Moreover, microtiter plates are commonly made of polystyrene, the surface of which can be damaged by most organic solvents in which these polymers are soluble [23]. For this reason, water-based [24] or alcohol soluble polymers [5] are often used to coat the oxygen sensor films on microtiter plate, and this limits the choice of polymers in sensor fabrication. Recently, investigations on organically modified silicates (ORMOSILs) as supporting materials for $Ru(dpp)_3^{2+}$ indicated that certain ORMOSILs can yield linear Stern–Volmer calibration plots for the oxygen sensors [25–28]. This was attributed to the homo-

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geneous environment provided by the ORMOSIL matrix to the embedded dye molecules. Besides homogeneity, ORMOSILs are transparent, hydrophobic, thermally and photochemically stable and sufficiently porous to allow fast diffusion of oxygen. Moreover, condensation of ORMOSIL takes place in aqueous medium, which will not damage the surface of polystyrene microtiter plates. This paper describes a simple way to fabricate ORMOSIL oxygen sensors on polystyrene microtiter plates that can be easily carried out in most laboratories. The performance of the oxygen sensors was evaluated by determining the IC_{50} values of some antimalarial drugs on a yeast model and the results were compared to the conventional optical density method [29] by monitoring the absorbance of yeast cells at 595 nm.

2. Experimental section

2.1. Materials

$[Ru(dpp)_3]Cl_2$ (dpp = 4,7-diphenyl-1,10-phenanthroline) was synthesized and purified according to a literature method [30]. Tetramethyl orthosilicate (TMOS, 98%), and dimethoxy dimethylsilane (DiMe-DMOS, 95%) were purchased from Aldrich. Fine graphite powder (extra pure) was purchased from Merck. The yeast-peptone-dextrose (YPD) broth was purchased from USB Corp. Yeast nitrogen base and amino acid supplement mixture were purchased from Qbiogene. The antimalarial drugs, chloroquine (CQ) and quinacrine (QNC), were obtained from Sigma. Oxygen and nitrogen gases (99% purity) were purchased from Hong Kong Oxygen Company. Microtiter plates (no. 3603, 96 wells, flat clear bottom black polystyrene, TC-treated) were obtained from Corning. Aluminum microplate sealing foils were obtained from USA Scientific. All other chemicals and solvents were analytical-reagent grade and were used without further purification.

2.2. Preparation of oxygen sensing films on microtitre plate

A solution of $[Ru(dpp)_3]Cl_2$ in ethanol (2.85 mg ml^{-1}) were prepared. The silica sol was prepared by mixing TMOS, DiMe-DMOS, 0.1 M HCl and deionized water in the volume ratio of 1.0:2.15:1.7:1.1. The sol mixture was stirred at 500 rpm for 3.5 h at 25 °C in a water bath. The sol was then centrifuged at 7500 rpm for 4 min. The thickened sol at the bottom of the centrifuge tube (1 ml) was pipetted out and thoroughly mixed with 250 μl of the ruthenium complex solution. The sol–ruthenium mixture (32 μl) was then transferred to each well of the microtiter plate and allowed to stand for 6 days at room temperature for gelation and drying. A layer of graphite-containing sol–gel coating was placed on the top of the oxygen sensing film to eliminate scattered light and background luminescence from sample solution. This light-insulating coating was prepared by pipetting 15 μl of a mixture of thickened sol (1 ml), graphite (0.1 g) and 0.1 M phosphate buffer (pH 7.0, 50 μl) onto the surface of each of the gelatinized oxygen sensing film and allowed to stand for another 6 days. After gelation, the microtiter plate was stored from direct light before use.

2.3. Instrumentation

All luminescence measurements were conducted on a BMG Labtech POLARstar microplate reader with a microcomputer. The wavelength of cutoff filters used for excitation and emission were set at 460 and 610 nm, respectively. The luminescence intensity was measured in the bottom plate reading configuration and at room temperature. Absorbance monitoring of yeast cell growth was performed on a Bio-Rad model 550 microplate reader.

2.4. Yeast strains and culture media

The yeast (*Saccharomyces cerevisiae*) strains YPH 499(pYX113), YHW 1052(pYX113) and YRP 3 used in this study were obtained from Larry M.C. Chow. This is a well-defined heterologous yeast model to address the problem of drug resistance [31]. The yeast strains YHW 1052 (*Dpdr5::TRP1, Dsnq2::hisG, Dpdr10::hisG*) and YRP 3 (*Dpdr5::TRP1, Dsnq2::hisG, Dste6::hisG, Derg6::LEU2*) were utilized for this study along with its isogenic parental strain YPH 499 (*MATa ade2-101oc, his3D200, leu2-D1, lys2-801am, trp1-D1, ura3-52*). Strain YPH 499 is wild type and refractory to drug substrates. Strain YHW 1052 is a mutant with deletions in the *PDR5*, *PDR10*, and *SNQ2* multidrug resistance genes and is thus more susceptible to drug substrates. Strain YRP 3 is another mutant with the *PDR5*, *SNQ2*, *STE6* and *ERG6* genes deleted. *ERG6* is a special gene responsible for synthesizing the plasma membrane lipid ergosterol on the yeast plasma membrane. Deleting this gene would result in a 'leakage' on the plasma membrane and therefore allowing drug substrates to enter the yeast cell. YRP 3 is therefore very sensitive to all kinds of drugs.

The yeast-peptone-dextrose (YPD) medium was prepared by dissolving YPD broth powder in deionized water followed by autoclaving at 121 °C for 20 min. The YPD medium used in all experiments was made up to a final concentration of peptone (20 g l^{-1}), yeast extract (10 g l^{-1}) and D-glucose (20 g l^{-1}).

As quinacrine (QNC) is insoluble in the presence of uracil, the synthetic complete medium minus uracil (SC-Ura) [32] was prepared for all experiments with QNC. The SC-Ura medium was made up to a final concentration of yeast nitrogen base (1.7 g l^{-1}), ammonium sulfate (5 g l^{-1}), D-glucose (20 g l^{-1}) and amino acid supplement mixture (0.8 g l^{-1}). It was sterilized by autoclaving at 121 °C for 20 min before use.

2.5. Measurement of dissolved oxygen concentration

Gas samples with different oxygen concentrations were prepared by mixing nitrogen and oxygen gases, the flow rate of each was controlled by a gas flowmeter, in a glass chamber. The outlet of the glass chamber was connected to a tubing for bubbling into various aqueous solutions. Solutions with different dissolved oxygen concentration were prepared from gas samples of different oxygen concentrations by bubbling the solutions for 30 min. The concentration of dissolved oxygen in the solutions were determined by a YSI 5000 dissolved oxygen meter (YSI Incorporated, Yellow Springs, Ohio, USA).

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