Study on calibration method for the performance index of SPR sensors*

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Aiming at the problem that there isn't any standardized calibration method for the performance index of surface plasmon resonance (SPR) sensor, the calibration method for key performance indices of SPR sensor is summarized and proposed based on the comparison of relative methods and definition of each index. Experimental data of sucrose solutions with various concentrations are obtained by the self-building SPR instrument, and then the calibration method is used to determine the performance indices, such as noise, drift, sensitivity, resolution, linearity, dynamic range and reproducibility. Experimental results show that the definition of indices is reasonable, and the calibration method is correct, which has great significance for performance evaluation of SPR sensor.

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Surface plasmon resonance (SPR) has been researched and widely used since 1980. SPR biosensor has become a new research hot spot in the decade. Compared with enzyme labeled immunosorbent assay (ELISA), SPR biosensor has many advantages[1-5]. So far, a lot of researches have been made to improve the performance index of sensors by adopting different light sources, light paths and detectors[6-8]. The algorithms can also affect the performance index of sensors with angular and wavelength modulation[9,10]. However, ELISA has been made as national standard, while SPR is still not adopted as a standard. This is because there are so many kinds of SPR sensors, and there is no standard calibration method.

Noise, sensitivity, resolution and detection limit are often given to evaluate an SPR sensor. However, there is still no standard way to get the performance characteristics of SPR sensor[11-13]. Other performance characteristics, such as baseline drift, repeatability and dynamic range, are usually ignored in many reports. In this paper, we summarize and propose the definition and the method to obtain the main performance characteristics of SPR sensor. This paper has its significance for reference of evaluation of SPR sensor performance characteristics, which lays a foundation for the standardization of the SPR detection.

The main performance characteristics of SPR sensors include noise, drift, sensitivity, linearity, dynamic range, detection limit and repeatability[2].

Noise is stochastic in sensor output with blank sample running, and it's uncorrelated with the sample. Drift is a long-term shift of baseline due to the unstable light source and temperature. The noise is usually given in either peak-to-peak value or standard deviation. The drift is the change of baseline in hours. In American standard of testing materials published by American National Standards Institute, noise is the peak-to-peak value for 10 min to 60 min[14]. Among the papers about SPR, the standard deviation of deionized water for 10 min to 60 min at constant temperature is usually determined as noise[12,15,16]. For SPR sensor, it's common to give the standard deviation of baseline as noise level. In this paper, we give noise in both the standard deviation and the peak-to-peak value. The baseline drift is given as the shift per hour.

Sensitivity is the ratio of the sensor output change ΔY to the change ΔX of the measured sample. The sensitivity of an SPR sensor can be written as $S=\Delta Y/\Delta X$. In SPR sensor, the resolution usually means the refractive index resolution[2]. Sensitivity can be obtained from sensor fitting curve as shown in Fig.1, where X represents the input of sample, and Y represents the output of the sensor. For a sensor system with ideal linearity[17], the slope of the fitting curve is the sensitivity[12,15]. However, piecewise linear fitting[18] and exponential curve fitting[19] are common methods.

Resolution Δn refers to the smallest change in the refractive index, which produces a detectable change in the sensor output. Resolution Δn can be achieved by $\Delta n=\sigma/S$[1], where σ denotes the standard deviation of blank sample, and S denotes the sensitivity.

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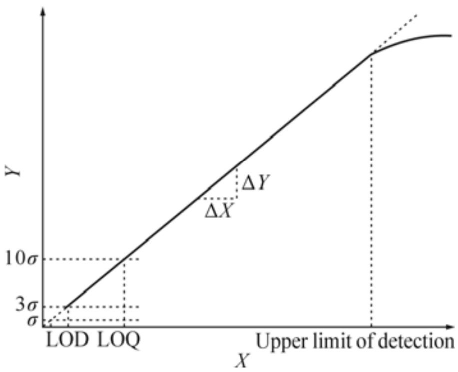

Fig.1 Sensitivity curve of the SPR sensor

Linearity represents the difference of output-input curve of sensor and the linear transfer function in dynamic range. For SPR sensor, the linearity is given as the linear correlation coefficient of fitting curve[20].

Limit of detection (LOD) is the lowest quantity Y_{LOD} of a substance that can be distinguished from the substance (a blank value) within a stated confidence limit, which can be expressed as $Y_{LOD}=Y_{blank}+m\sigma_{blank}$, where Y_{blank} is the average value of blank sample, σ_{blank} is the standard deviation of blank sample, and m is a coefficient correlated with confidence level which is usually 2 or 3. LOD shown in Fig.1 can be obtained as $C_{LOD}=3\sigma_{blank}/S$. The detection limit is usually adopted to determine whether the analyte is contained in the blank sample. Meanwhile, the limit of quantitation (LOQ) is used to analyze the concentration. The limit of quantitation is expressed as $C_{LOQ}=10\sigma_{blank}/S$.

Dynamic range refers to the range of values which can be measured by a sensor. For SPR sensor, it refers to the range of refractive index or concentration of an analyte that can be measured. Dynamic range is often limited at one end of the range by LOD or LOQ, and we adopt LOQ in this paper[2]. The other end of the dynamic range is limited by linearity of the calibration curve as shown in Fig.1.

Repeatability is the variation of measurements taken by a single person or instrument on the same item and under the same conditions, given by the percent of full scale or the relative standard deviation of repeated measurements[17]. For SPR sensor, the relative standard deviation of the signal of the same sample in multiple experiments is reasonable to present the repeatability.

The lab-made SPR device as shown in Fig.2 is comprised of a Spreeta sensor (Texas Instruments Inc., USA), a microfludic chip (0.18 μL), an injection pump (MSP1-C1, Longer Inc., China), a high-performance liquid chromatography (HPLC) 10-position stream selector valve (Valco Instruments Inc., Switzerland), a sampling controller and PC workstation software[21].

Deionized water is adopted as buffer. Sucrose solutions (Guanghua Chemical Reagent Co. Ltd, AR) were prepared with concentrations of 0.025 g/L, 0.05 g/L, 0.1 g/L, 0.2 g/L, 0.4 g/L, 0.8 g/L, 1 g/L, 2 g/L, 4 g/L, 6 g/L, 8 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L, 60 g/L, 70 g/L, 80 g/L, 90 g/L, 100 g/L, 120 g/L, 140 g/L, 160 g/L, 180 g/L, 200 g/L, 250 g/L, 300 g/L, 350 g/L and 400 g/L. The refractive index was calibrated by using an Abbe refractometer (ST-1, Shanghai Precision & Scientific Instrument Co. LTD).

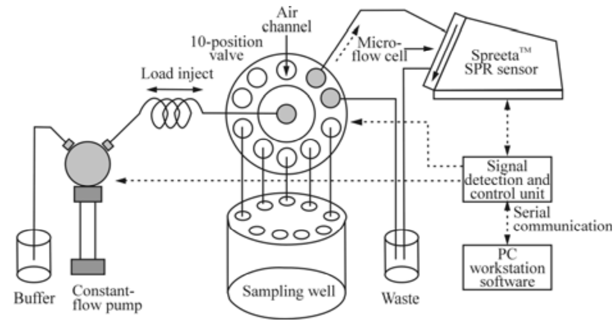

Fig.2 Schematic diagram of the SPR detector device

The sensor was placed in a temperature-controlled incubator at 25 °C. The buffer was injected at the rate of 50 μL /min for 2 h in baseline noise and drift test. 1 g/L sucrose solution was injected for 10 times in repeatability test. To obtain sensitivity, resolution, dynamic range and linearity, 0.025–400 g/L sucrose solutions were injected respectively. The buffer of 75 μL was injected, followed by sample of 85 μL and ended by buffer of 75 μL, which was defined as a cycle (75 μL water, 85 μL sample and 75 μL water). Each sample ran 3 cycles, and the average was calculated as the result.

The baseline monitoring curve of deionized water at constant temperature is shown in Fig.3. The horizontal axis P is resonance pixel, and the vertical axis t is time. Spreeta sensor is based on angular modulation. The SPR curve detected by CCD contains a resonance dip which is calculated and located by CCD pixel. The baseline drift is 0.06884 pixel for 2 h. We choose the data in the first 0.5 h to evaluate noise. The standard deviation of baseline is 0.00724, and the peak-to-peak value is 0.05009.

The relative standard deviation of the peak values of 1g/L sucrose solution for 10 times is 1.15%. The SPR sensor shows a good repeatability for sucrose solution.

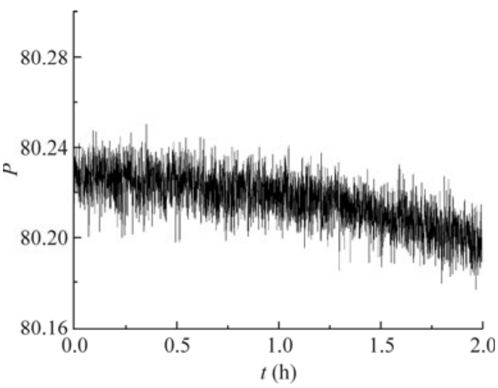

Fig.3 Noise and drift of deionized water at constant temperature

The refractive indices of deionized water and sucrose solutions with different concentrations from 10 g/L to 400 g/L were measured by the Abbe refractometer. The concentration and the refractive index show a linear relationship as shown in Fig.4. The formula of linear fitting is R=0.000147786C+1.3318, where R refers to refractive index, and C refers to concentration. With this formula, we can calculate the refractive index for sucrose solution with different concentrations.

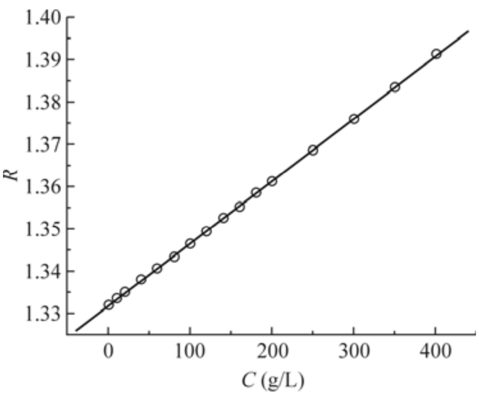

Fig.4 Refractive index of sucrose solution versus concentration

Sucrose solutions with different concentrations are injected and measured by SPR sensor to obtain the resonance pixels. The result is shown in Fig.5. The resonance pixels at 300 g/L, 350 g/L and 400 g/L are obviously out of the dynamic range with ideal linearity. We use quadratic curve to fit with the remaining data, and the formula is P=–13498.65R^2+34769.62R–22286.04. The sensitivity S=dP/dR correlates with R. The sensitivity S is –2216.7 pixel/RIU at 1.37. We can obtain the resolution Δn which is 3.27×10^{-6} RIU. As shown in Fig.5, the dynamic range is from 1.33 RIU to 1.37 RIU. The data of sucrose solutions with different concentrations from 0 g/L to 250 g/L show a dynamic range with a correlation coefficient of 0.9894. We can determine the limit of detection and dynamic range for sucrose solution by the concentration and the refractive index formula. The performance indices of the SPR sensor are listed in Tab.1.

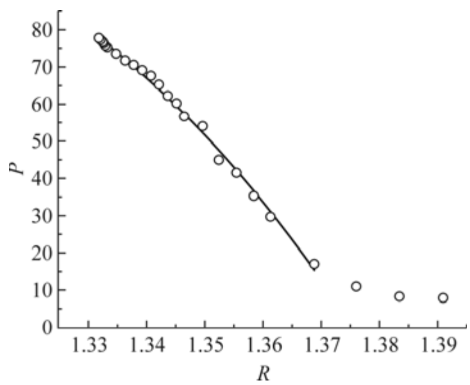

Fig.5 Calibration curve of the SPR sensor

Tab.1 Performance indices of the SPR sensor

Noise (pixel)	Drift (pixel/h)	Linearity	Sensitivity (pixel/RIU)	Resolution (RIU)	Dynamic range (RIU)	Repeatability (%)
0.00724	0.03442	0.9894	2216.7	3.27×10^{-6}	1.33−1.37	1.15%

We summarize and propose the definition and a reasonable calibration method for key performance indices of SPR sensor. Experiments of sucrose solutions with different concentrations are performed, and the calibration method is used to determine performance indices, such as noise, drift, sensitivity, resolution, dynamic range and repeatability. The definition of indices is reasonable, and the calibration method is correct, which has great significance for performance evaluation of SPR sensor and lays a foundation for the standardization of the SPR detection.

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