Sensors and Materials, Vol. 31, No. 5 (2019) 1623–1634
MYU Tokyo

S & M 1885

Electrode Structure and Stimulation Frequency to Reduce the Estimation Error of Reagent Concentration Determined Using Measured Impedance

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(Received April 5, 2018; accepted March 20, 2019)

Keywords: interdigitated electrode, impedance, PCB substrate, saline, concentration estimation, lock-in amplifier

We present the structure of an electrode and the excitation frequency that reduces the estimation error when the concentration of a reagent is measured using impedance. To compare the performance characteristics of interdigitated electrodes with one to five fingers, five electrodes of the same type were fabricated independently on printed circuit board (PCB) substrates. A fluidic channel was created on each substrate using double-sided tape and a polycarbonate (PC) cover so that the solution was equally distributed over all electrodes. The impedance was measured with stimulation signals of various frequencies for various concentrations. The measured impedance was used to calculate a linear parameter between the concentration and the impedance, and this parameter was used to calculate the error between the estimated value obtained from the impedance and the original concentration. Using this estimation error as a measure, we investigated the electrode and stimulus frequency suitable for each application requiring either lot or individual calibration. Experimental results show that measuring impedance at high frequencies with a single-finger electrode is advantageous for applications requiring lot calibration, while multiple-finger electrodes are suitable for individual calibration applications at low frequencies.

1. Introduction

Measurements of DNA or protein using fluorescent labels require expensive and precise equipment, and may create an unexpected interference with detection systems.[1–6] In comparison, electrical sensors enable label-free detection by measuring electrical signals instead of fluorescence brightness.[5,7–11] This is a very promising biosensing method owing to the very

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https://doi.org/10.18494/SAM.2019.2283

ISSN 0914-4935

low detection limit and the relatively rapid completion time.[2,11,12] This method has recently been called electrochemical impedance spectroscopy (EIS) because it primarily measures the impedance change depending on the concentration of DNA or the amount of protein captured in the antibody.[6,7,13,14] Another electrical biosensing method, called electrical cell-substrate impedance spectroscopy (ECIS), is used to measure micro-motion and response to drugs or to assess the barrier function of cancer or stem cells.[15–22] ECIS can be regarded as a type of EIS that exploits the phenomenon of impedance increase between electrodes as the cell mobility increases.

All of these electrical sensors need electrodes. For example, to determine the concentration of a prostate-specific antigen associated with prostate cancer, three gold electrodes were prepared by photolithography on a silicon substrate.[13] In other cases, an electrode array made using CMOS technology was used for DNA detection.[6] Alternatively, in order to observe the motion of a cell, an electrode is formed on a glass or printed circuit board (PCB) substrate.[16,17,23–25] Various types of electrodes are used to measure biological quantities. Among these, interdigitated electrodes have advantages such as an easy-to-understand structure, a high reaction rate, and a high signal-to-noise ratio.[2,3,12,13,18,22,26,27] However, there has been scant research on the optimal number of fingers or the stimulus frequency.

In this paper, we describe our investigation into the effects of the number of fingers and the stimulation frequency of interdigitated electrodes when measuring the concentration of the reagent using impedance. Although the electrodes can be constructed in various ways, we used the manufacturing process of a PCB because of its stability. With manufacturing by other methods, fluctuations in the electrode characteristics may be caused by other factors arising from an unstable process. Since the PCB manufacturing process has become very stable with developments in information technology, fluctuations in the characteristics of an electrode manufactured by this process are expected to be relatively small compared with those observed by other processes.

Before comparing the performance characteristics of different types of electrodes, it is recommended that instrumental errors be investigated. These errors may include electrode variations, electrical measurement errors, and experimental variations due to sample loading. To investigate interelectrode variation, we fabricated the same type of electrode repeatedly on one PCB board. To reduce the sample loading error, we fabricated a fluidic chip using double-sided tape and a polycarbonate (PC) cover on the substrate. Saline was used as a test reagent because its concentration has a very linear relationship with the measured impedance.[6,28]

In general, it is cost effective to achieve accuracy using calibration instead of making sensors infinitely precise. Calibration types can be categorized as individual calibration, in which parameters are applied to individual sensors, and lot calibration, in which the same parameters are applied to the entire production lot.[29–31] In individual calibration, the manufacturer or user applies the parameters obtained on the basis of the protocol determined for each sensor. When the calibration is performed by the manufacturer, additional storage is required for each sensor, whereas user calibration requires additional time and hassle. On the other hand, although some accuracy is lost, it is more cost effective to perform lot, rather than individual, calibration because lot calibration only estimates the average parameter for the entire or part of the lot. For

impedance sensing, it is unknown whether the structure or optimum frequency of the electrodes is similar to each other, regardless of which calibration method is used.

Since the saline concentration and the impedance form a strong linear relationship, the resultant error of the linear regression analysis between the impedances and the concentrations can be used as a measure of the electrode performance. Therefore, the linear parameters of all electrodes from one chip can be averaged to emulate the use of a lot calibration parameter, and each linear parameter can be used for the individual calibration.

Experimental results from this study showed that the different types of electrodes and the stimulus frequency should be selected on the basis of the calibration method. Better results were achieved by measurement at a high frequency using a single-finger electrode for lot calibration, whereas a multiple-finger electrode at a low frequency was the better choice for the individual calibration application.

2. Materials and Methods

2.1 Experimental setup

In this work, the most systematically accessible interdigitated type of electrode was employed to study the structure of electrodes. Specifically, our goal was to analyze the impedance measurement performance with respect to the number of fingers of interdigital electrodes. The number of fingers ranged from one to five. To investigate the variation between electrodes, five identical electrodes with the same number of fingers were arranged side by side on one PCB. The width and length of all fingers of the interdigitated electrodes were 100 μm and 1 mm, respectively, and the distance between all fingers was 100 μm. The electrode arrays with one to five fingers are shown in Figs. 1(a)–1(e). For reference, one of the 1-finger electrode arrays is magnified and shown in Fig. 1(f). To reduce the chemical reaction with the test solution, the electrode material was selected to be electroless nickel immersion gold.

In order to distribute the saline solution evenly on each electrode array, double-sided tape was used to fabricate a constant width channel. The size of the channel was 23 × 4 mm^2 so that the reagent spread evenly across all five electrodes [Fig. 2(b)]. Electrodes were labeled e1 to e5 starting from near the inlet hole. Both ends of the channel were rounded to ensure that the saline solution could be injected well into the channel. The fabricated channel is seen in Fig. 2(a). The channel was covered with PC and has an inlet and an outlet. The finished chip and the assembly diagram of the chip are shown in Figs. 2(a) and 2(c), respectively.

The saline solution was injected into the inlet hole of the fluidic chip, the inlet and outlet were sealed with tape, and then this chip was applied to the voltage divider. Care was taken to ensure that the injection did not result in air bubbles near the electrodes. A constant volume of saline solution, (35 μl) was required to fill the channel. The concentrations of saline solution used in the experiments were 0.625, 1.25, 2.5, 5.0, and 10.0 mg/ml. After the injection of the saline solution was completed, the inlet and outlet of the chip were sealed before measurements. Then, the fluidic chip was set on the measurement circuit.

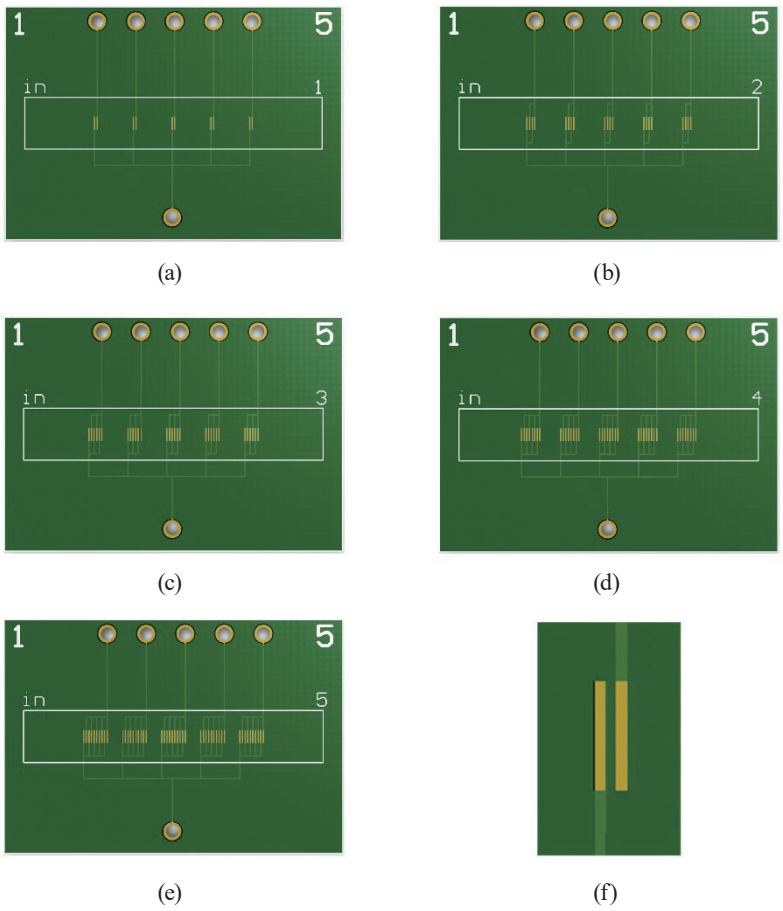

Fig. 1. (Color online) Five types of electrodes and a magnification of the 1-finger electrode. The gold-colored patterns are 100 µm × 1 mm and are separated by a distance of 100 µm. (a) 1-finger electrodes on PCB, (b) 2-finger electrodes on PCB, (c) 3-finger electrodes on PCB, (d) 4-finger electrodes on PCB, (e) 5-finger electrodes on PCB, and (f) a 1-finger electrode on PCB.

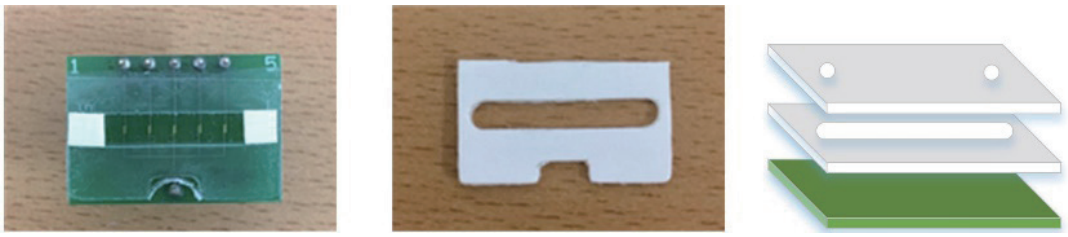

Fig. 2. (Color online) (a) Electrode fluidic chip. (b) The channel width and length are 4 and 23 mm, respectively. (c) The chip is constructed from a 100 µm PC, a 400 µm double-sided tape, and a PCB substrate from the top to the bottom.

Figure 3 shows the voltage divider for the impedance measurement, where the third electrode (e3) was ready to be measured. One end of each electrode was connected to the others and the point was probed with channel B of the oscilloscope. The other ends were probed with channel

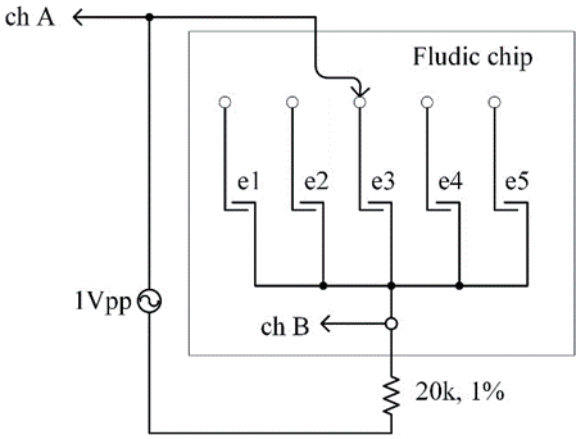

Fig. 3. Impedance measurement circuit diagram.

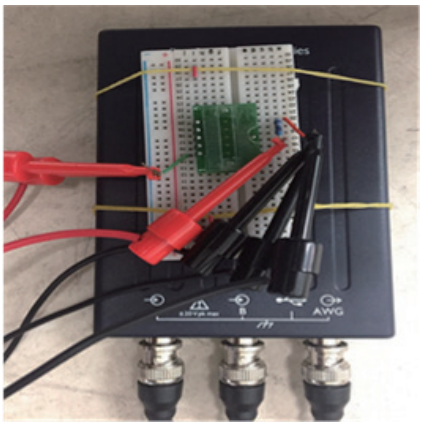

Fig. 4. (Color online) Experimental setup.

A in turn. A sinusoidal stimulation was applied on the voltage divider circuit with a known precision reference resistance (20 kΩ, 1%) using a USB oscilloscope with a function generator (Picoscope2204A, Pico Technology Ltd., England). The frequency of the stimulus signal was increased stepwise from 2 kHz to a final frequency of 100 kHz, which was the maximum frequency provided by the selected oscilloscope. The measurement frequency was set at 2, 4, 8, 16, 32, 64, and 100 kHz. Figure 4 shows the experimental setup.

The impedance ratio between each electrode pair and the reference resistor was estimated using the lock-in amplifier law. Assume that the impedance of the considered electrode is Z. Then, the complex gain between the voltages probed from channels A and B is

$$G = \frac{R}{R+Z}, \tag{1}$$

where G, Z, and R are the calculated complex gain, the impedance to be estimated at the electrode, and the resistance of the reference resistor (20 kΩ, 1%), respectively.

The complex gain G can be estimated using the following locked amplifier law:

$$real(G) = \int_0^T x(t)\cos\left(\frac{2\pi}{T}t\right)dt,\ imaginary(G) = \int_0^T x(t)\sin\left(\frac{2\pi}{T}t\right)dt, \tag{2}$$

where $x(t)$ and T are the voltage probed by channel B and the period of the stimulus sinusoidal function, respectively. The stimulus voltage is the cosine function in the integral of Eq. (2). The stimulus signal and the divided voltage $x(t)$ can be accurately acquired using the software development kit for MATLAB provided by the oscilloscope vender.

Before examining the performance of the electrodes, we examined variations in the electrical measurements and experimental deviations from the chip fabrication. To estimate the electrical measurement error, we measured the coefficient of variation (CV) by repeatedly measuring the chips after fabrication. In comparison, to estimate the experimental deviation, the chip

sealing system was disassembled and 100 μl of solution, which is about three times the channel capacity, was poured and the assembly was sealed again. After chip sealing, the impedance of all electrodes was measured at all frequencies. This experiment was repeated 5 times.

2.2 Electrode performance

To compare the performance of the electrode structures, we compared the performance of the concentration estimation using the measured impedance. The relationship between the saline concentration and the impedance measured at the electrode was highly linear on a log-log scale. Therefore, it was possible to obtain the linear parameter by using the impedances measured for various concentrations of saline solution and then to estimate the saline concentration from the measured impedance using the linear parameter.

If the concentration of saline solution and the measured impedance satisfy a linear relationship, the following equation set is satisfied:

$$\log r_i = a \log \rho_i + b, \rho_i = \{0.625, 1.25, 2.5, 5.0, 10.0\}, \tag{3}$$

where ρ_i, r_i, a, and b are the i-th saline concentration, the corresponding impedance, and the slope and intercept of the relation, respectively. Note that the saline concentrations tested in this experiment were 0.625, 1.25, 2.5, 5.0, and 10.0 mg/ml. The above relationship is a function of the number of fingers, the electrode position, and the frequency. If n, i, and f are the number of fingers, the electrode number, and the frequency, respectively, the linear parameter vector can be expressed as the following vector:

$$\boldsymbol{p}(n,i,f) = \left(a(n,i,f), b(n,i,f)\right). \tag{4}$$

Calibration is required when the electrode is used to measure the reagent concentration. Two calibration methods were tested: one is lot calibration using the same parameters for all electrodes produced in one lot, and the other is individual calibration in which the parameter is calculated and used independently for each electrode. With the individual calibration, the concentration can be estimated by measuring the impedance using the parameters estimated from Eq. (3). Assuming the estimated parameter is $\hat{\boldsymbol{p}}(n,i,f) = \left(\hat{a}(n,i,f), \hat{b}(n,i,f)\right)$, the concentration can be estimated from the measured impedance using the following equation:

$$\hat{\rho} = \exp\left(\frac{1}{\hat{a}}\left(\log r_m - \hat{b}\right)\right), \tag{5}$$

where $\hat{\rho}$ and r_m are the estimated concentration and the measured impedance, respectively.

When applying lot calibration, all electrodes of one chip are considered to be in the same lot and the same parameters should be used for all electrodes of the chip. In this case, the concentration can be measured using the following parameter averaged over five electrodes:

$$\tilde{\boldsymbol{p}}(n,f)=\left(\tilde{a}(n,f),\tilde{b}(n,f)\right)=\frac{1}{5}\sum_{i}\hat{\boldsymbol{p}}(n,i,f)\,. \tag{6}$$

As a measure for comparing the performance of the electrode, the mean absolute error (MAE) between the concentrations estimated from Eq. (5) and the original concentrations was calculated as

$$MAE(n,f)=\frac{1}{5}\sum_{i}\left|\rho_i-\hat{\rho}_i\right|, \tag{7}$$

where n and f are the number of fingers and a member of the frequency set {2,4,8,16,32,64,100} in kHz, respectively. Note that the average is obtained over the five concentrations of the saline solution.

3. Results

To measure the electrical variations, 1.25 mg/ml saline was used to fill a 1-finger chip, which was then sealed with tape. The process of measuring the electrodes of the finished chip was, in turn, repeated five times. Assume that the measured value $r_m(i, f, n)$ is a function of the electrode number i, the frequency f, and the measurement number n. The CV over the repeated measurement was calculated as

$$CV(i,f)=\frac{\underset{n}{std}\left\{r(i,f,n)\right\}}{\underset{n}{mean}\left\{r(i,f,n)\right\}}, \tag{8}$$

where $CV(i, f)$, $\underset{n}{std}\{\cdot\}$, and $\underset{n}{mean}\{\cdot\}$ are the CV for the i-th electrode at the frequency f, the standard deviation, and the mean over the number of measurements, respectively.

Table 1 summarizes the CVs for each electrode number and the frequency. The electrical measurement was stable within 0.6% regardless of the position of the electrode or the stimulus frequency.

Table 1
CV for the electrical measurement (%).

Electrode ID	Frequency (kHz)						
	2	4	8	16	32	64	100
e1	0.1	0.1	0.3	0.2	0.1	0.3	0.2
e2	0.2	0.1	0.1	0.1	0.2	0.1	0.2
e3	0.6	0.3	0.1	0.2	0.2	0.2	0.3
e4	0.3	0.2	0.3	0.3	0.4	0.3	0.2
e5	0.4	0.3	0.2	0.2	0.1	0.2	0.1
Mean	0.3	0.2	0.2	0.2	0.2	0.2	0.2

In order to investigate the variation of the independent experiments, the sealing was released for the next experiment following the measurement, and the process of preparing the chip from the sample injection was repeated. We used only the 1-finger chip and 1.25 mg/ml saline, assuming that the other chips and concentrations showed similar trends. At reinjection, 100 µl (which was approximately three times the channel capacity of 35 µl) was poured into the solution to completely replace the existing solution. Through this process, each experiment was made as independent as possible. Figure 5 shows the frequency dependence of the CV in independent experiments at each electrode. It was found that the CV was 1.9% or less regardless of the frequency for all electrodes. A relatively low CV at a high frequency is shown. This tendency seems to be because the current flow between the electrodes at high frequencies does not extend far from the electrodes, hence the variation in solution height was not significantly influenced by the measurement.

In order to analyze the concentration estimation error from the impedance by the estimated parameters, we first investigated whether the relationship between the impedance and the concentration is reasonably linear. Figure 6 shows the relationship between the impedance and the concentration measured at electrode e1 of the 1-finger chip. The data points with the same

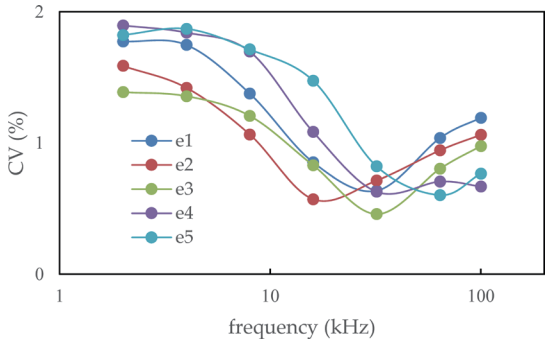

Fig. 5. (Color online) CV of the independent experiments (1-finger chip, 1.25 mg/ml).

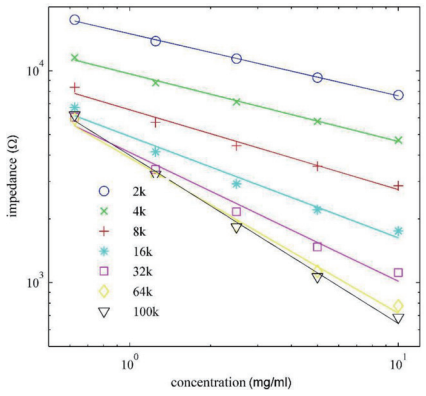

Fig. 6. (Color online) Linear relationship between saline concentration and the measured impedance (1.25 mg/ml, electrode e1, 1-finger chip).

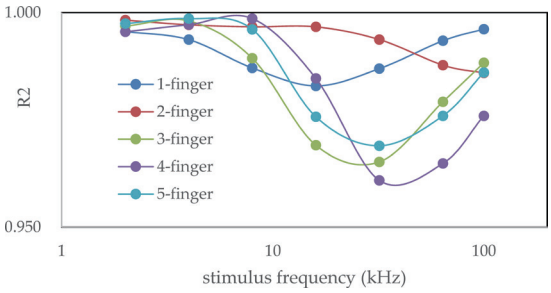

Fig. 7. (Color online) Coefficient of determination (R2) averaged over all the electrodes of each chip.

symbols are the impedances measured at the same frequency, and the solid lines with the same color as the data points represent the lines fitted to the data points. As shown in Fig. 7, the concentration and measured impedance were highly linear regardless of the frequency. Figure 7 shows the average of the coefficients of determination for the five electrodes of each chip. All of them are 0.961 or more, indicating good linearity regardless of chip, electrode, or stimulation frequency.

Since the concentration and measured impedance had a linear relationship, the linear coefficients were calculated and used to estimate the concentration from the measured impedance. The linear parameter averaged over the five electrodes was used to compare the electrode performance with lot calibration. In this case, we found that the MAE decreased at high frequencies, as shown in Fig. 8. In Fig. 8, the MAE of the 1-finger chip was 0.30 mg/ml at 100 kHz stimulation; this was the best performance. Interestingly, an odd number of fingers at high frequencies showed better performance than an even number.

In contrast to lot calibration, the MAE performance for individual calibration was better at low frequencies, as shown in Fig. 9. The smallest MAE was measured using a 5-finger electrode at 4 kHz and was 0.1 mg/ml. At low frequencies, the experimental variation tended to be large, as shown in Fig. 5. Therefore, the fluidic channel should be carefully designed to prevent any fluctuation in liquid height. The 2-finger electrode showed the best performance when using 16 or 32 kHz, as the experimental variation was relatively small. Since the linearity was relatively small in this frequency range (Fig. 7), nonlinear parameter methods were also worth considering. Experimental results, however, suggest that fluid channels designed to have small experimental variations should use multiple-finger electrodes, and a stimulus signal of less than 8 kHz is recommended.

In summary, it is advantageous to use different measurement methods in accordance with the calibration method. In the case of lot calibration, measurement at a high frequency using a single-finger electrode is better, whereas a multiple-finger electrode at a low frequency promises better performance for an individual calibration application.

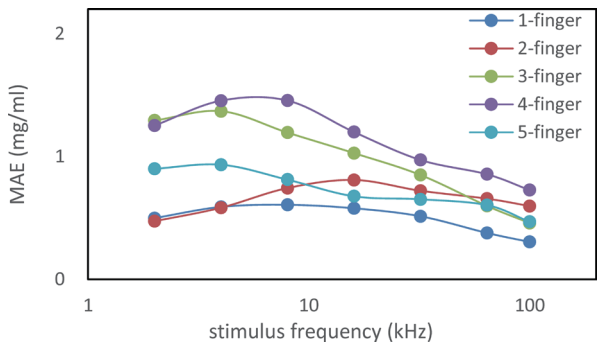

Fig. 8. (Color online) MAE when the concentration was estimated from the measured impedance with the linear parameter averaged over the electrodes (lot calibration example).

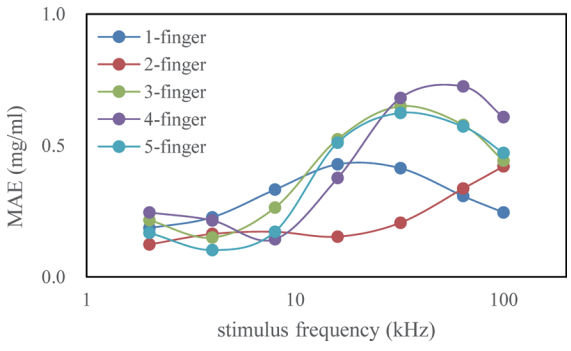

Fig. 9. (Color online) MAE when the concentration is estimated from the measured impedance with the linear parameter of the individual electrode (individual calibration example).

4. Conclusions

Fluidic chips were fabricated with electrodes on a PCB substrate, and experiments were conducted to compare the reagent concentration estimation performance characteristics with respect to the electrode structure. Five electrode structures were investigated: 1-finger to 5-finger interdigitated types. Experimental results show that the electrode structure and stimulus frequency should vary depending on the calibration method. The best performance was obtained at 100 kHz with a 1-finger electrode for lot calibration, whereas a multiple-finger electrode at 4 kHz performed the best for individual calibration.

We investigated the experimental variation in a limited manner. Once the application and its structure are determined, it must be carefully examined. This is because, with individual calibration, the linearity and estimation error perform very well when using the multiple-finger electrode at a low frequency, whereas the experimental variation tends to be poor. This reasoning contrasts with the relatively simple strategy of measurement at high frequencies with a single finger when using lot calibration.

We presented the experimental results for electrodes on a PCB substrate. A similar methodology can be used for glass or silicon substrates.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT, & Future Planning (NRF-2017R1A2B4002679), and by the Leading Human Resource Training Program of Regional Neo Industry through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT, & Future Planning (NRF-2016H1D5A1909654).

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