

Electrochemical electrode/SAW system for metal ions and glucose in solutions

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Abstract

An electrochemical electrode/surface acoustic wave (ESAW) system was developed to explore the possibility of the application of UHF waves, 300–3000 MHz for chemical analysis in solution. The ESAW system with a ST-cut SAW quartz transducer was prepared for detection of metal ions and glucose in aqueous solutions. The ESAW system has the advantages of very low cost, easy fabrication. A set of electrodes welded with long-distance wires and coaxial cables was used to contact to the metal shell of the 315 MHz SAW quartz resonator. The ESAW system was applied to detect various metal ions, e.g., alkaline metal, alkaline-earth metal and transition-metal ions. Without pre-concentration technique, the detection limit of Cu^{2+} ion with the ESAW detection system was estimated to be 1.2×10^{-4} mol/L (7.6 ppm), from an analytical sensitivity of 2.55×10^5 Hz/(mol/L) and the standard deviation of the blank signal of 10 Hz with a confidence level of 99.86%. The ESAW detection system was also applied as a biosensor for glucose to detect gluconic acid, a product of glucose oxidation by glucose oxidase (GOD) in aqueous solution. The glucose ESAW biosensor with glucose oxidase exhibited a linear frequency response to the log concentration of glucose with a slope of approximately 9.3×10^2 Hz decade⁻¹ ($\Delta\text{Hz}/\Delta\log M$). The ESAW detection system also showed a good selectivity and a good detection limit of $<10^{-3}$ M for glucose in aqueous solution. Furthermore, the ESAW detector showed much more sensitive than QCM crystal sensor for glucose. © 2007 Elsevier B.V. All rights reserved.

Keywords: Electrochemical surface acoustic wave (ESAW) sensor; Surface acoustic wave (SAW) sensor; Biosensor; Metal ions; Glucose; Glucose oxidase

1. Introduction

Starting from the 1980s, surface acoustic waves (SAW) devices expanded their uses to communication, signal processing, physical and chemical sensing [1,2]. The SAW-based devices provides the following advantages: simple structure, board ranging frequency, outstanding reproducibility and low-cost. A typical chemical SAW/quartz sensor applied in gas-detection design is to place the very thin coating materials on the surface of the SAW piezoelectric crystal. The sensing mechanism is that when the sensor is contacted with the gas chemicals, the interaction of the gas chemicals with the coating materials will lead to the changes of the electric properties and resonance frequency of the SAW device [3–15]. The SAW devices, which can be operated in the range of 0.1–1.0 GHz, also show greater sensitivity to the mass changes for gas adsorption than the tradi-

tional piezoelectric quartz crystal microbalance (QCM) sensors with an oscillating frequency of 8–30 MHz, as shown in our previous study [4,15].

The waves traveling on the surface of the ST-quartz substrate belong to the Rayleigh waves which have both a surface-normal component and a surface-parallel component with respect to the direction of propagation. When the chemical SAW/quartz resonator is contacted with a liquid phase, the quick decay of the surface-normal component of the Rayleigh wave leads to the large energy loss which limits the employments of the SAW/quartz devices in the solutions [16]. However, the Rayleigh-SAW/quartz still merits investigation. The Rayleigh-SAW/quartz has many advantages such as precision oscillator, temperature-stable resonator, narrow-band filter and lowest cost. The Rayleigh-SAW/quartz provides the frequencies ranging from several MHz to a few GHz, high radio frequency (HF) to ultra high radio frequency (UHF) waves. For the purposes to apply the Rayleigh-SAW/quartz in the liquid detectors, some kinds of the series-electrode detectors consisting with Rayleigh-SAW/quartz were even developed [17,18]. Although

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the series-electrode design offered the advantage of high sensitivity over classical conductivity in solution, it still had some disadvantages. One of the disadvantages of the series-electrode design is the SAW-device-frequency was still limited lower or just over the HF range, 3–30 MHz. Another disadvantage of the series-electrode design is the length of electrode-connection wires was requested as short as possible to prevent the quick energy-loss of waves. Thus, for the application of the SAW quartz transducers with the UHF waves, 300–3000 MHz, gas detections were developed in several studies but the liquid detections have not been found yet.

In order to be applied in liquid mediums, the SAW device with high dielectric constant (ϵ) material substrate, e.g., LiTaO₃ ($\epsilon = 47$) was developed to replace the device with the original common material quartz ($\epsilon = 4.6$ – 4.7). The high dielectric constant material substrate helps to confine enough electric field generated by the inter-digital transducers (IDTs) even in contact with water ($\epsilon = 75$) [19]. However, LiTaO₃ crystal membrane is quite more expensive and more difficult to be prepared than quartz crystal membrane. Furthermore, the oscillating frequency of the LiTaO₃ SAW transducer shows quite sensitive to the temperature [20]. Therefore, in this study, the electrode/SAW (ESAW) system was developed on the basis of the interference on the fringing electric field of the ST-cut quartz SAW transducer. The fringing electric field is generated by the source voltage applied on the IDTs of the ST-cut quartz SAW resonator. The spatially periodic fringing electric field proceed across the two sides of the IDTs, the piezoelectric substrate side and the vacuum-space side [1,9]. For the ESAW system, a set of electrode was designed and connected to the metal shell of the SAW resonator. The ESAW system was developed to explore the possibility of the application of the UHF waves, 300–3000 MHz for chemical analysis for metal ions and glucose in solutions and 315-MHz SAW quartz transducers were adopted in this study.

2. Experimental

2.1. Apparatus

Fig. 1 depicts the experimental set up of a ESAW system with a homemade computer interface. The parallel-plate electrodes connected with an SAW oscillator system was placed in a glass working cell. The volume of detection solution is

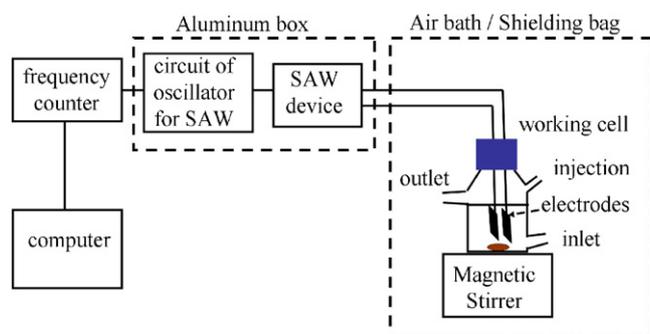


Fig. 1. Schematic diagram of the electrochemical SAW/electrode system (ESAW).

12 mL. The oscillation-circuit board, the SAW resonator and the frequency counter were obtained from Wenshing Electronics CD.LTD (Model: TWS-BS-6, Taiwan), Yoketan Co. (Model: TO-39 Type, one-port, Taiwan) and Lutron Inc. (Model: FC-2700, Korea), respectively. The oscillation-circuit device and the SAW device were firmly fixed in a 3 mm-thick aluminum box. The aluminum box, the home-made electrodes and the working cell were all covered in a home-made air bath/shielding bag which is made of plastic and aluminum films. Data processing and signals acquisition were automatically performed on a microcomputer (PC/AT) with a computer program written in BASIC.

The SAW one-port resonators with 315 MHz used in this study were made of 0.0294 cm² ST-cut SiO₂ quartz crystals with aluminum metallization and mounted on two pins. The resonator device was sealed in a 9 mm-diameter round copper box to prevent contact with the atmosphere. Fig. 2(A) shows the outward appearances of the whole SAW device. The ESAW was developed on the basis of the interference on the fringing electric field of the ST-cut SAW/quartz transducer. To establish the field effect on the frequency generated by the SAW device, the A-pin of the SAW device was cut off and a wire was welded on the metal shell of the SAW device in the following step. Another wire was welded on the ground position of the oscillation-circuit board. The ends of the two wires were welded with a coaxial wire to connect the two parallel electrodes (Fig. 2(B)). The two wires and a part of coaxial wire were fixed firmly in the aluminum box but another part of the coaxial wire outside the aluminum box is movable. The two parallel square-plate electrodes were home-made with copper foils.

2.2. Procedures

A typical procedure was performed as follows: pure water or solution (12 mL) was transferred into the working cell from the inlet, the solution was stirred by a magnetic stirrer. After the elec-

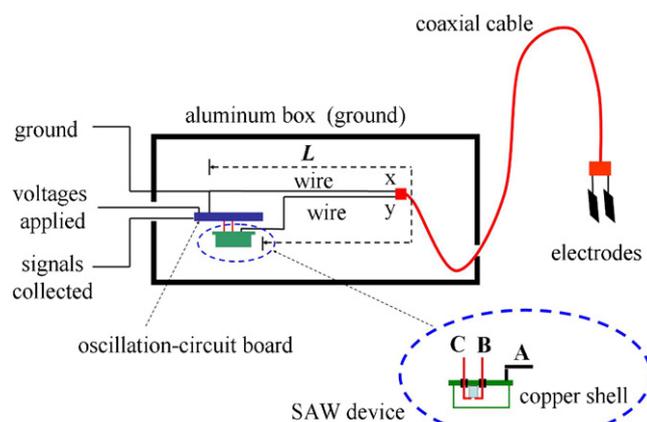


Fig. 2. (A) the side-view and the bottom-view of the 315-MHz SAW device. The A-pin is named as the pin connected directly on the bottom of the round copper box. The B and C pins are named as the two pins connected to the aluminum metallization and they are insulated from the round copper box. (B) The installation of the field effect on the wave generated by the SAW device and oscillation-circuit device.

trodes were put into the solution, the detection system was then turned on. In each experiment, different reagents were injected into the working cell within a volume of less than 100 μL each times.

2.3. Chemicals

Glucose-oxidase enzyme (GOD) used for the detection of glucose was bought from SIGMA (USA). All other chemicals were of analytical grade.

3. Results and discussion

3.1. Establishment of the FESAW system

For the electromagnetic wave propagating in the oscillation-circuit device, the wavelength (λ) can be calculated by the velocity of the wave (v) and the wave frequency (f):

$$\lambda = \frac{v}{f} = F_v \frac{c}{f} \quad (1)$$

The F_v is the velocity factor which is corresponding to the media of the propagating electromagnetic wave. The c is the velocities of the waves propagating in vacuum and it is equal to $3 \times 10^8 \text{ m s}^{-1}$. The velocities of the waves propagating in the atmosphere and in the oscillation-circuit device are different. The velocity factor is nearly equal to 1.0 in atmosphere and 0.5–0.9 in the electric transmission lines. Since the velocity of the wave propagating in the oscillation-circuit device is near $1.5 \times 10^8 \text{ m s}^{-1}$, i.e. $F_v = 0.5$, and the wave frequency induced by the SAW device is 315 MHz, the wavelength of the wave propagating in the oscillation-circuit is near 48 cm, or the half-wavelength is near 24 cm. Considering the property of stand wave, an important parameter to be controlled in this study is the length, L , between the A-pin position on SAW device and the ground-port position on oscillation-circuit device. A rapid way to find out the best condition of the length L in this study was developed as below. The base-line frequency, F_1 , and the frequency, F_2 were measured when the end of the two wires, x and y , were on open-circuit and touched together on short-circuit, respectively, changing the length of the two wires leads to the different frequency response. The frequency shifts were calculated from F_2 minus F_1 , $\Delta F = (F_2 - F_1)$. Fig. 3 shows that changing the length of the wires yielded the influences on the frequency shifts and there is a signal inversion when the length L is near 23 cm which is nearly consistent with the propagating half-wavelength (24 cm) of the SAW resonator used in this study. When the length L is near 23 cm, the frequency responses of open-circuit is equal to the ones of short-circuit. Similar results were obtained even when the wires outside the aluminum box were arranged in straight or circular form. It was confirmed that the propagation of the electromagnetic wave in this system follows the properties of the stand wave. This phenomenon obtained is similar to the properties of half-wave dipole, Hertz antenna. For the electromagnetic wave, the stand wave of electric voltage or current is provided by Hertz antenna which consists of a set of two-wire transmission lines with the open-circuit

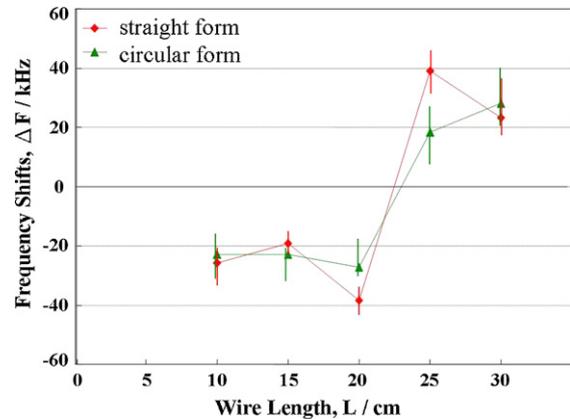


Fig. 3. The frequency shifts, ΔF , for the different length, L , of the wire between the A-pin position on SAW device and the ground-port position on oscillation-circuit device. The frequency shifts were calculated from F_2 minus F_1 , $\Delta F = (F_2 - F_1)$.

since the total length of the wire lines is equal to the multiples of the half-wave length. If the wire-length of the parallel transmission lines is equal to the odd-times of the half-wave length, the stand wave of the open-circuit has similar properties as of short-circuit.

It is essential to control the condition of the voltage applied on the oscillation-circuit device. This study shows the 5.3-V voltage provided the best sensitivity of the frequency shifts in an 8.2 mM KNO_3 solution between 3 and 6 V (Fig. 4) Considering the better stability of the signals, a 5.2-V voltage was used in the following study. It is not recommended that higher voltage be applied on the ESAW system because more dissipative heat was produced in the oscillation-circuit device.

3.2. Properties of the electrodes

The effects of area and the distance of the two parallel square-plate electrodes were investigated. This study in the electrode-area effects on the frequency shifts for the KNO_3 solutions revealed that among these three electrodes, C#1, C#2, and C#3 with 4, 10 and 17 mm widths, respectively, the electrode with 10-mm width revealed the best sensitivity of the frequency

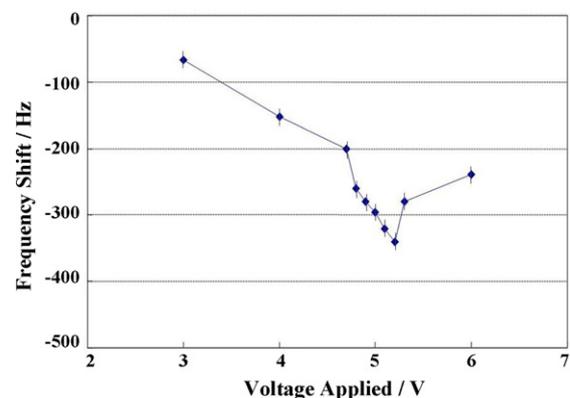


Fig. 4. The effects of the voltage applied on the oscillation-circuit device. The frequency shifts were measured in a 8.2 mM KNO_3 solution and the frequency respond of pure water was defined as the base-line frequency.

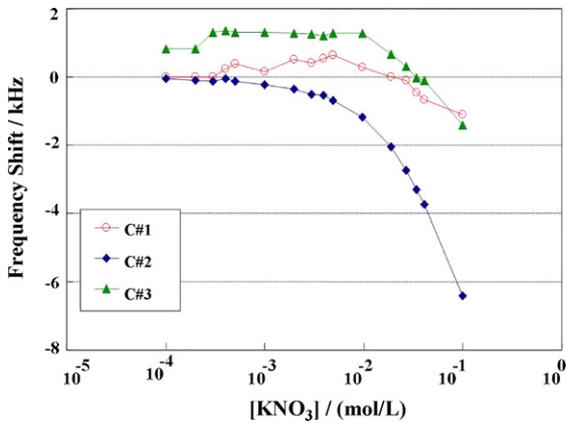


Fig. 5. The electrode-area effects on the frequency shifts for the KNO_3 solutions. C#1, C#2, and C#3 were the names of the C-type electrodes with different widths in 4 mm, 10 mm, and 17 mm.

response (Fig. 5). The standard deviation of the each data in Fig. 5 is less than 30 Hz. The distance effects of the two electrodes on the frequency shifts for the $\text{Cu}(\text{NO}_3)_2$ solutions were studied. C#4, C#5, and C#6 were the names of three same-size electrodes. All the copper foils were cut into square size with 10-mm width and 10-mm height. Those electrodes were designed with different distances between the two copper foils. Fig. 6 depicts that the frequency shifts appeared the fluctuation properties of the electric-magnetic wave. The standard deviation of the each data in Fig. 6 is less than 30 Hz. In a 4.1 mmol/L of $\text{Cu}(\text{NO}_3)_2$ solution, whatever which electrode, only certain distances of the electrodes leads to the acquisition of higher sensitivity. In the later chemical-analysis study, the distance of the electrodes was always adjusted before each experiment to make sure that high sensitivity was guaranteed. The best condition for the sensitivity of the ESAW installation was checked by detecting 2.0 mM $\text{Cu}(\text{NO}_3)_2$ solution to get a frequency response between 500 and 520 Hz.

The other kinds of electrodes for the ESAW system were investigated as shown in Fig. 7. The C-type electrodes were made of two copper square-plate foils. The outside surfaces of the two copper foils were protected with polyethylene polymers. The Q-type electrodes, round-shape silver-coating quartz

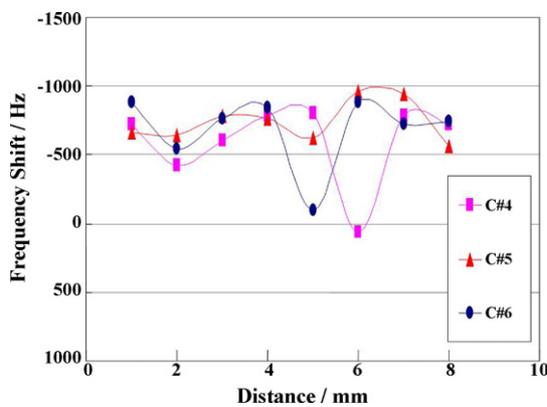


Fig. 6. Effect of distance between two electrodes on the frequency shifts for the $\text{Cu}(\text{NO}_3)_2$ solutions. C#4, C#5, and C#6 were the names of three same-size electrodes.

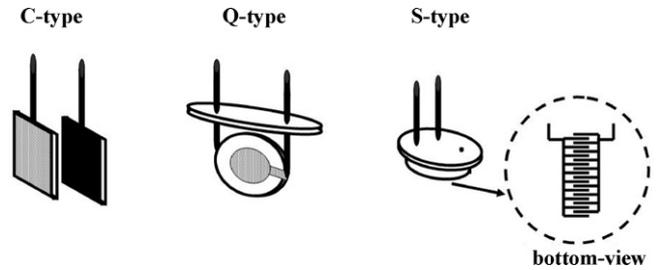


Fig. 7. Schematic diagram of the three types of the electrodes.

electrodes, were cut down from the QCM devices. The S-type electrodes, aluminum-coating one-port inter-digital transducer (IDTs) electrodes, were cut down from the SAW/quartz devices. The plate and the inter-digital shapes of the of electrodes induce the different penetration modes of the UHF electric-magnetic wave into the solution [1]. The C-type and the Q-type electrodes owned better sensitivities than S-type in the $\text{Cu}(\text{NO}_3)_2$ solutions as shown in Fig. 8. When the inside surfaces of C-type electrodes were coated with Ag glue, named as the C/Ag1-type electrodes, the sensitivity of the electrodes did not change in dilute $\text{Cu}(\text{NO}_3)_2$ solutions as shown in Fig. 8. This phenomenon supposed that the properties of the electromagnetic waves propagating in the aqueous solutions were affected by changing the shapes of the metallic electrodes, but were not affected by the kinds of the metallic materials.

Furthermore, the measurement of temperature effect on the ESAW system was also made. The temperature coefficient of the ESAW is less than $-0.065 \text{ ppm}/^\circ\text{C}$, i.e. $-20 \text{ Hz}/^\circ\text{C}$ for 315 MHz. The effect of the dielectric property of the liquid was also detected by filling various alcohols into the cell of ESAW system, respectively. Several-kHz frequency drops, from small to large, were observed for methanol ($\epsilon = 32.6$), ethanol ($\epsilon = 24.3$) and isopropyl alcohol ($\epsilon = 18.3$).

3.3. Analysis of glucose with the ESAW system

The ESAW detection system was also applied as a biosensor for glucose in this study. The ESAW system was employed to

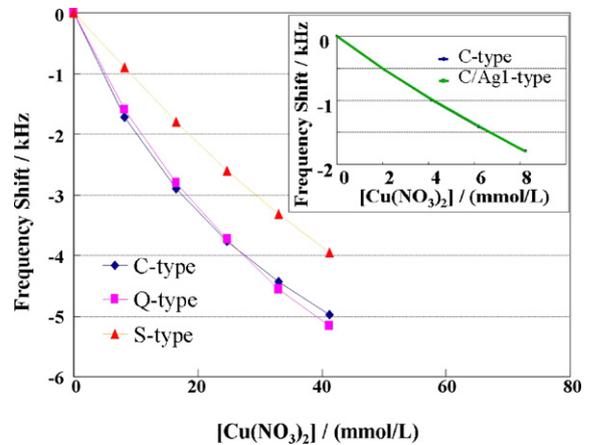


Fig. 8. The frequency responses for the three kinds of the electrodes in $\text{Cu}(\text{NO}_3)_2$ solutions. The inside surfaces of C-type electrodes coated with Ag glue were named as the C/Ag1-type electrodes.

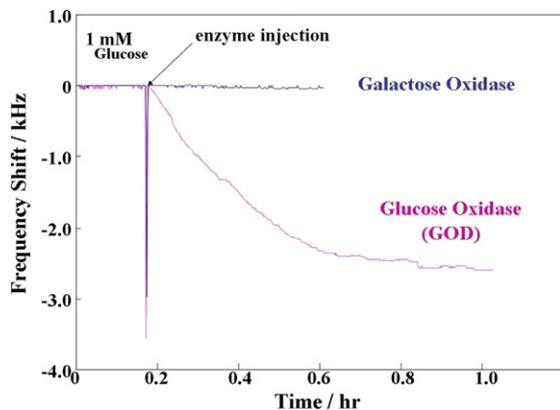


Fig. 9. Frequency responses of ESAW for glucose with glucose oxidase (GOD) or galactose oxidase (GALAC oxidase).

detect gluconic acid, a product of glucose oxidation by glucose oxidase (GOD) in aqueous solutions. The catalytic oxidation of glucose by glucose oxidase results in the consumption of oxygen was as follows:



As shown in Fig. 9, the ESAW detection system exhibited sensitive frequency responses for glucose with GOD enzyme, in contrast, no any frequency responses were found for glucose with other enzymes, e.g., galactose oxidase (GALAC oxidase) and without GOD enzyme. With GOD, glucose can be oxidized to produce gluconic acid which can be dissociated to ions, while no gluconic acid and ions are produced for glucose without GOD. These results seem to indicate that the frequency response of ESAW detection system for glucose with GOD is attributed to the variation of ionic strength or conductivity around electrodes in aqueous solution. Similar behavior for quartz crystal microbalance (QCM) resonant frequency in metal electrolyte solutions was also reported [21]. In addition, The ESAW glucose biosensor with the GOD enzyme seems to respond sensitively to glucose and responds almost not at all to other bio-species such as galactose as shown in Fig. 10. This result seems to indicate that the ESAW glucose biosensor with glucose oxidase has demonstrated quite good selectivity to glucose.

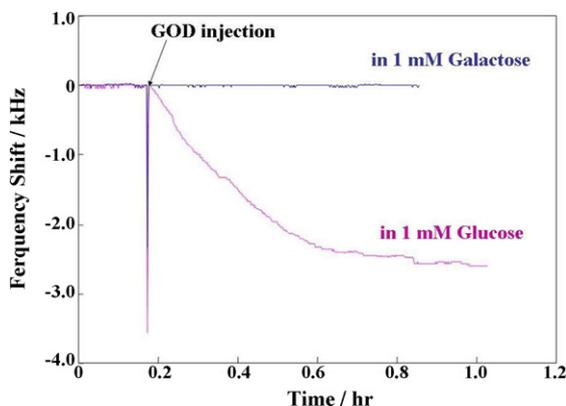


Fig. 10. Frequency responses of ESAW system for glucose and galactose with GOD in solutions.

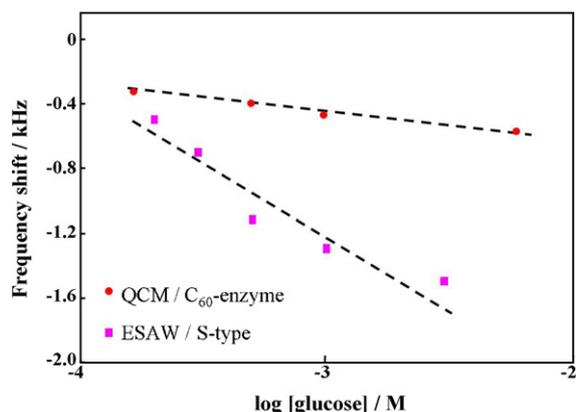


Fig. 11. Effect of concentration of glucose on the frequency responses of ESAW system with GOD enzyme.

As shown in Figs. 9 and 10, a sharp peak of the frequency drop appears when the sample was injected into the solution by using the syringe. The longer the metal needle of the syringe stays in the cell of the ESAW, the larger the frequency drop appears. The field of the UHF electric-magnetic wave, penetrating in the sample solution, was very easily interfered by immersing the metals into the solution.

The effect of glucose concentration ($\log M$) on the frequency response of the ESAW bio-sensor was also investigated, as shown in Fig. 11. The glucose ESAW bio-sensor with glucose oxidase exhibited a linear frequency response to the log concentration of glucose with a slope of approximately $9.3 \times 10^2 \text{ Hz decade}^{-1}$ ($\Delta\text{Hz}/\Delta\log M$). According to the Michaelis–Menten equation [22], the rate of the enzyme-catalyzed reaction is nearly proportional to log concentration of the substrate ($\log M$). Thus, the linear response of the ESAW glucose bio-sensor to the log concentration of glucose can be understood. Furthermore, a comparison between ESAW and QCM sensors was made. The fundamental frequency of the SAW sensor (315 MHz) with higher frequency than QCM (10 MHz) can be expected to exhibit more sensitive than QCM (10 MHz) crystal sensor [4,15,20]. As expected, the ESAW detector showed much more sensitive than QCM for glucose, as shown in Fig. 11.

Furthermore, the detection limit of glucose by using the ESAW detection system obviously is below 10^{-3} M as shown in Fig. 11. However, the range of glucose concentration in biological samples such as blood is within 10^{-2} to 10^{-3} M . This result seems to indicate that the ESAW detection system can be employed to detect glucose in some biological samples such as blood.

3.4. Analysis various ions in aqueous solution

The C-type electrodes were used to detect the concentration effects under the range of 10^{-3} M of different nitrate compounds. Alkaline metals, alkaline-earth metals and transition metals were investigated as shown in Figs. 12–14, respectively. For alkaline-metal and ammonium nitrates, the frequency shifts increase as the radii of the alkaline-metal cations increase in the same concentration and the regression equations of K^+ , Rb^+ , Cs^+ and

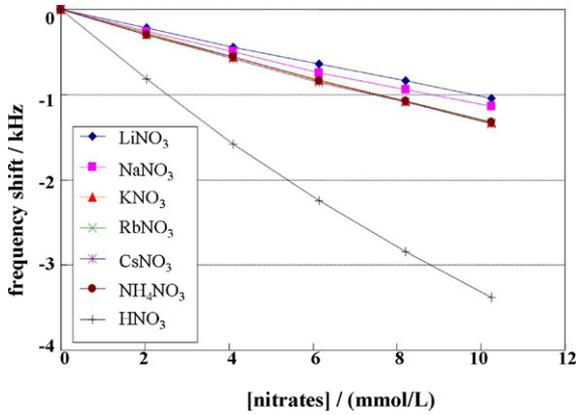


Fig. 12. Frequency responses of alkali metal/ammonium nitrates with the ESAW detection system.

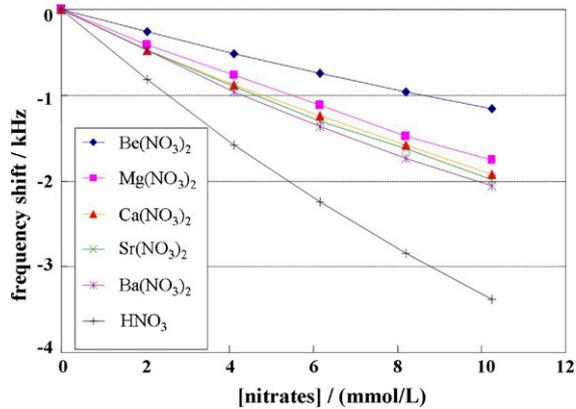


Fig. 13. Frequency responses of alkali earth metal nitrates with the ESAW detection system.

NH_4^+ are almost the same. The concentration effect of H^+ ion was also determined and it was obviously larger than any other cations. The alkaline-earth-metal cations reveal similar tendency that the frequency shifts increase as the cations' radii increase. The frequency responses of transition-metal cations with different charges (Ag^+ , Zn^{2+} , Cu^{2+} , Cr^{3+}) were also determined. Among all the cations, the frequency shifts increase as the charge

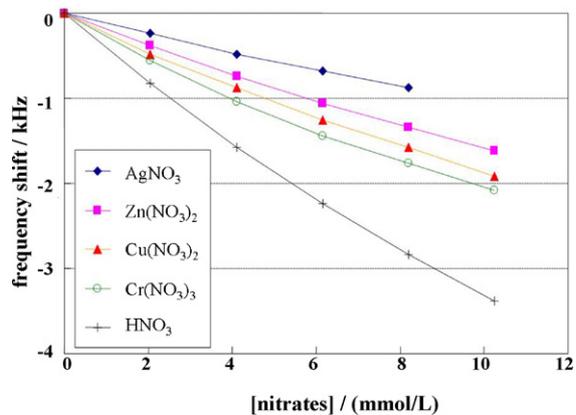


Fig. 14. Frequency responses of transition-metal nitrates with the ESAW detection system.

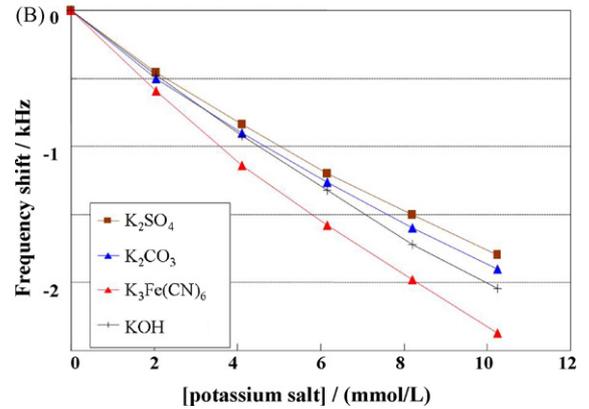
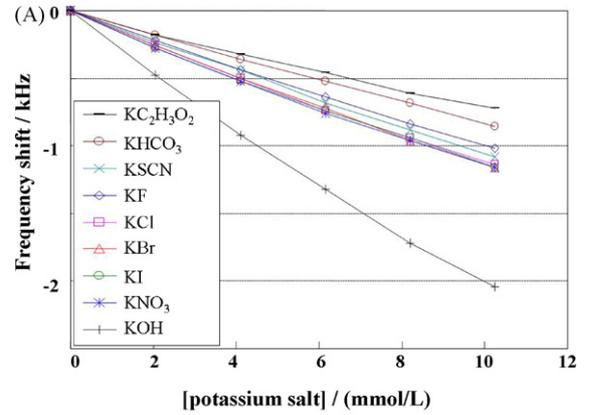


Fig. 15. Anion effect of potassium salts on the frequency shifts of the FESAW detection system.

of the cations increase except for H^+ ion. Fig. 15 shows the concentration effects under the range of 10^{-3} M of the potassium compounds with different anions. Among all the anions, the frequency shifts increase as the charge of the anions increase except for OH^- ion. For potassium halides, the regression equations of Cl^- , Br^- , and I^- are almost the same. Most one-charge anions reveal similar frequency shifts as one-charge cations in the same concentration.

3.5. Detection limits of various metal ions detected by the ESAW system

The ESAW system was applied to detect different kind of electrolytes solutions. As shown in Fig. 14, the frequency shifts for the concentrations of various metal ions reveal good linearity under the range of 10^{-3} M. The detection limits (D.L.) of various metal ions with ESAW system were also studied. IUPAC [23] recommends that the detection limit of a detector (C_L) can be estimated as follows:

$$\text{D.L.} = k \frac{S_b}{m} \quad (3)$$

where S_b is the standard deviation of the blank signals obtained in pure water with 20 measurements, m the analytical sensitivity which can be estimated as the slope of the working curve for the detection range less than 3.0 mmol/L, and k is the numerical factor chosen in accordance with the desired confidence

Table 1
The detection limits, D.L., of various metal ions with the ESAW detection system

Analyte	D.L. (mol/L)	D.L. (ppm)	Analyte	D.L. (mol/L)	D.L. (ppm)	Analyte	D.L. (mol/L)	D.L. (ppm)
H ⁺	7.0×10^{-5}	0.1	Be ²⁺	2.2×10^{-4}	2.0	NH ₄ ⁺	1.9×10^{-4}	3.4
Li ⁺	2.6×10^{-4}	1.8	Mg ²⁺	1.4×10^{-4}	3.4	Ag ⁺	2.4×10^{-4}	25.9
Na ⁺	2.2×10^{-4}	5.1	Ca ²⁺	1.2×10^{-4}	4.8	Zn ²⁺	1.5×10^{-4}	9.8
K ⁺	2.0×10^{-4}	7.8	Sr ²⁺	1.2×10^{-4}	10.5	Cu ²⁺	1.2×10^{-4}	7.6
Rb ⁺	2.0×10^{-4}	17.1	Ba ²⁺	1.1×10^{-4}	15.1	Cr ³⁺	1.0×10^{-4}	5.2
Cs ⁺	1.9×10^{-4}	25.3						

level. As suggested by Long and Winefordner [24], the use of $k=3$ allows a confidence level of 99.86% for a normal distribution of the blank signal. Therefore, the detection limit (D.L.) of Cu²⁺ ion with ESAW system was estimated to be approximately 1.2×10^{-4} mol/L (7.6 ppm), from the analytical sensitivity (m) of 2.55×10^4 Hz/(mol/L) and the standard deviation of the blank signal (S_b) of 10 Hz with $k=3$. The detection limits of various metal ions with the ESAW detection system are shown in Table 1. The ESAW detection system obviously showed good sensitivity with the detection limit of <10 ppm for most of metal ions.

4. Conclusions

The utilities of ESAW system for chemical analysis in solutions provide the successful development of UHF electromagnetic waves. The ESAW detection system can be applied as a biosensor to detect bio-species, e.g., glucose. The ESAW detection system with enzyme has been demonstrated as a useful detection system for quantitative analysis for glucose with the detection limit of $<10^{-3}$ M. The advantages of ESAW detection system are very low cost, easy fabrication and long-distance-wire-equipped detection without quick energy-loss. The total cost of oscillation-circuit and SAW device is less than 100th the cost of other liquid SAWs. Furthermore, the quantitative analysis for metal ions in electrolyte solution with the ESAW system was available. The ESAW detection system exhibited good sensitivity with the detection limit of <10 ppm for most of metal ions.

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