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RESEARCH ARTICLE

A preliminary study of identification halal gelatin using quartz crystal microbalance (QCM) sensor

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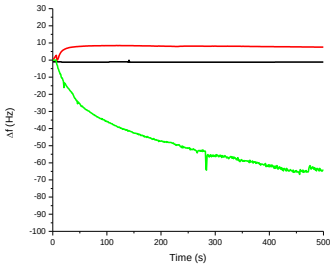
Graphical abstract



Abstract

Gelatin has been widely used as an additive in pharmaceutical, cosmetic, and food industry. The similar physical appearance between bovine and porcine gelatin causes an issue for some communities like a Muslim due to awareness of halal food. A Muslim community consider porcine gelatin is non-halal material which must be avoided. So there is a demand to distinguish and labeling the origin source of the gelatin in any products. In turn, it lead to development of a method to identify the source of gelatin. In this study, performance of a modified Quartz Crystal Microbalance (QCM) sensor to identify halal gelatin has been investigated. A QCM sensor was modified by depositing polyaniline/nickel compound on the surface of gold electrode QCM carried out by Layer by Layer (LbL) deposition technique. Bovine and porcine gelatin were measured in demineralized water at pH 9. This modified QCM sensor shows good frequency response to distinguish bovine and porcine gelatin. The measurements gave a negative frequency shifts for bovine gelatin and a positive frequency shifts for porcine gelatin. The modified QCM sensor also worked well in the real sample. This indicates that a modified QCM sensor is very useful and effective technique to distinguish bovine gelatin (halal) from porcine gelatin (non-halal).

Keywords: Gelatin, halal, quartz crystal microbalance (QCM), sensor

INTRODUCTION

Gelatin is a heterogeneous mixture of polypeptides which is obtained through partial hydrolysis of collagen from bones, hide and skins, and connective tissues of animal by acidic or alkaline treatment (Zhang et al., 2008; Nhari et al., 2012). Based on the origin of the raw material and hydrolysis process, gelatin is classified as type A gelatin and type B gelatin. Type A gelatin with isoionic point of 7 to 9 is produced using an acid pretreatment. This acidic hydrolysis is mainly used for pigskin, marine fish skin, and sometimes bone raw material. In the other hand, type B gelatin with isoionic point of 4 to 5, is produced using an alkaline pretreatment. This process is mainly used on bovine hide (Baziwane and He, 2007). During the hydrolysis, the breakdown of fibrous structure of collagen occurs irreversibly to form gelatin. The production of gelatin involve several steps i.e. pretreatment of raw material (controlled acidic or basic hydrolysis), temperature extraction, sterilization, and drying (Demirhan et al., 2012). The extraction temperature process was started from 55°C and gradually increased to 60, 70, and 80-90°C consecutively to get maximum result (Baziwane and He, 2007).

Gelatin contains a high amount of amino acid with a repeated of tripeptide unit. This unit consists of glycine, proline, and 4-hydroxyproline. It makes gelatin has a similar properties to collagen. The softness, elasticity, and formation a reversible gel of the gelatin depends on temperature (Yilmaz et al., 2013). Generally the commercial gelatin is found in the form of capsules and powders. But gelatin is also widely used in various field e.g. pharmaceuticals, photography, cosmetics, and the food industry (Hanani et al., 2012). It is because this material is easy to be formed. Especially in the pharmaceutical field gelatin is used as a soft and hard capsule shell, tablet manufacture, and dietary supplement. In the food industry, gelatin is used as emulsifier, gelling agent, thickener, and stabilizer in the manufacture of marshmallows, ice cream, candy, jelly, and meat processing (Cai et al., 2012). There are several sources of gelatin available for industrial uses, e.g bovine gelatin treated with alkali (BA gelatin), bovine gelatin treated with hydrochloric acid (BHA gelatin), porcine gelatin treated with alkali (PA gelatin), porcine gelatin treated with hydrochloric acid (PHA gelatin), and fish gelatin (F gelatin). But from all of those sources, the BA gelatin and the PHA gelatin are the most commonly used (Demirhan et al., 2012).

It has been reported that gelatin is yearly produced nearly 326,000 tons from pig-skin derived gelatin (46%), bovine hides (29.4%), bones (23.1%) and other sources (1.5%) (Karim and Bhat, 2009; Yilmaz et al., 2013). These various gelatin sources become an important issue for Muslim communities due to their awareness of halal food since gelatin is also widely used in their food products. Muslim Halal (purification) law requires any kind products (for both food and non-food) which are free from pork and its derivatives (Amqizal et al., 2017). Today, Muslim consumers rely on certification and labeling to ensure that the products used are manufactured by halal production process (Spiegel et al., 2012) since this "halalness" products is not easily verifiable (Ahmad et al., 2017). For instance in gelatin case, the

similar physical appearance of bovine and porcine gelatin make both of them can not be barely distinguished. Most of commercial halal gelatin is extracted from cow skin and bone, and its derivatives. The commercial fish gelatin products are also available, but they are not commonly used because their rheological properties is inferior to mammalian gelatin which in turn will affect the product quality (Cho et al., 2005; Amqizal et al., 2017). So the demand to distinguish and labeling the origin of the gelatin in any kind of products in Muslim world lead to developing methods to identify the gelatin source.

Several methods and studies have been reported for gelatin identification. Those methods are including based on chemical precipitation, chromatography, spectroscopic, and immunochemical techniques. The precipitation method for identifying the type of gelatin was done by Hidaka and Liu (2003). They reported that calcium phosphate precipitation method could detect gelatin which was derived from cow bone until concentration of 0.5 mg/mL and 4.0 mg/mL for gelatin which was derived from pig skin. More selective gelatin detection was performed by protein identification. Several methods such as High Performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS), Enzyme-linked immunosorbent assay (ELISA), and Polymerase Chain Reaction (PCR) were applied. Zhang et al., (2008) reported that the source of gelatin can be distinguished using HPLC-MS method. The mass spectrum obtained was compared to the available collagen database. Since the gelatin was obtained from hydrolysis of collagen which contains amino acids, so it is predicted that both of bovine and porcine gelatin will have similar peptide sequences with the collagen type I. But we still can find several different peptides in it between both of the gelatins which can be used as a marker to differentiate them. Enzyme-linked immunosorbent assay (ELISA) is also the common technique which is used in determination of food analysis because of its specificity and sensitivity (Nhari et al., 2012). Some specific antibodies are applied in this technique. It could detect the presence of gelatin in food (Venien and Levieux, 2005). Sandwich ELISA is the established ELISA method which could determine the bovine and porcine gelatin in processed food (Doi et al., 2009). In the other hand, they still have some disadvantages, such as complicated analysis, time consuming, high cost, and analytical error because of the possibility of protein denaturation during the food production process. Spectroscopic methods were also used to identify the origin source of gelatin, e.g. Fourier Transform Infrared (FTIR) spectroscopy method (Hashim et al., 2010; Cebi et al., 2016). However, these spectroscopic methods for the determination of gelatin need some repetition observation and high purity of samples. It is difficult to differentiate the source directly by comparing the FTIR spectra of gelatins. The markers were found at around 1700-1600 cm^{-1} and 1565-1520 cm^{-1}. They showed the identical peaks but with different signal intensity for fish, bovine and porcine gelatin (Cebi et al., 2016). The results obtained need to be analyzed using a chemometric method, principal component analysis (PCA), to distinguish these three types of gelatins.

Quartz crystal microbalance (QCM) has been used as a high sensitive mass sensor which allows the measurement of mass changes at the surface of it with nanogram resolution (Casero et al., 2010). It showed a good sensitivity performance for both in gas and liquid phase. When the analyte target attached on the coating surface of QCM (adsorbent), the mass will be increase. As the result occurs the resonant frequency change. According to Saurbrey's equation, the increase mass on the surface of QCM lead to frequency decrease linearly (Sharma et al., 2014). Today, QCM has been used frequently as a sensor due to its rapid respond and high sensitivity. Moreover, QCM is relatively easy to use, more stable result during long term operation, selective on detection, and simple sample preparation. QCM has been widely applied for determination of clinical targets (Mao et al., 2002), environmental pollutants (Kurosawa et al., 2006; Özgür et al., 2013), oxidative stress investigation (Ersöz et al., 2009; Say et al., 2009), protein identification (Sener et al., 2010), and investigation of biomolecular interaction (Svedhem et al., 2003).

In this work, we present a novel method to identify the halal gelatin (bovine) from non-halal gelatin (porcine) using modified QCM sensor. We fabricated QCM sensor by electrodepositing polyaniline /nickel compounds on the surface of QCM sensor using layer by layer (LbL) deposition technique. This enhanced the sensor performance to provide selective and sensitive analysis of halal gelatin. The performance of our method is also compared to FTIR analysis method which has been reported from other works.

EXPERIMENTAL

Materials

Sodium hydroxide [NaOH], hydrochloric acid [HCl], and aniline [$C_6H_5NH_2$] were purchased from Merck. The aniline was purified before used by distillation technique. Porcine and bovine gelatins were purchased from local retail store in Surabaya, East Java, Indonesia. Quartz Crystal Microbalance (QCM) used a commercial 5 MHz AT-cut quartz crystal (diameter 25.4 mm) which was purchased from Renlux Crystal (Shenzhen, China). Nickel compound was synthesized in Instrumentation and Analytical Sciences Laboratory, Chemistry Department, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia (Budipramana et al., 2016). Demineralized water was used for all cleaning, preparation, and measurement. All chemicals were used without further purification unless mentioned. Commercial hard capsule from local drugstore that has gelatin source information was used as a real sample.

FTIR gelatin characterization

Fourier Transform Infrared (FTIR) (Shimadzu Instrument Spectrum One 8400S) spectrophotometer was used to characterize for both bovine and porcine gelatins. All spectra were recorded within a range of 4000-500 cm^{-1}. All measurements were performed in a dry atmosphere at room temperature (25 ± 0.5°C). A single beam spectrum was obtained for all samples. These all sample spectrums were subtracted against a background air spectrum.

Pretreatment of QCM sensor

The gold QCM sensor was cleaned in demineralized water ultrasonically for 10 min. Afterwards, the QCM sensor was immersed into piranha solution (concentrated H_2SO_4 : 30% H_2O_2 = 1:3 v/v) for 20 seconds, followed by rinsing thoroughly with demineralized water. The clean gold QCM sensor was kept in desiccator prior to use.

QCM sensor modification

The gold QCM sensor that has been treated as mentioned above was electropolymerized in 0.1 M aniline solution at pH 1.5 (the pH was adjusted using HCl solution). The electropolymerization was conducted using cyclic voltammetry technique with three-electrode cell system, which gold QCM sensor as working electrode (WE), Ag/AgCl (3M KCl) as reference electrode (RE), and platinum as counter electrode (CE). It was carried out at scan rate of 50 mV/s over potential -0.5V to 1.0 V for forty cycles. The deposition of nickel compound on the surface of the working electrode (Budipramana et al., 2014; Zulkarnain et al., 2016) was conducted using Layer by Layer (LbL) deposition technique (Ivanov et al., 2009; Fitriyana and Kurniawan, 2015; Kurniawan et al., 2017). Briefly, the QCM sensor was immersed in nickel solution for 15 min then dried in room temperature. The treatment was repeated once again before the QCM sensor soaked into nickel solution for 24 h. The surface of gold QCM sensor with and without modification were monitored using optical microscopy (Olympus BX60) to justify that all the modification process worked well (Fitriyana and Kurniawan, 2015; Kurniawan and Madurani, 2015).

Sample preparation

30,000 ppm of bovine and porcine gelatins stock solution were prepared by diluting 3 grams of each gelatins in 100 ml volumetric flask using demineralized water. 100 ppm porcine and 100 ppm bovine gelatins solution was prepared by diluting stock solution. Real sample was prepared by dissolving commercial hard capsule with demineralized water.

Sample measurement

All the measurement were performed under alkaline conditions (pH 9, adjusted using NaOH solution) with QCM system (QCM 200, SRS, USA) as shown in Fig. 1.

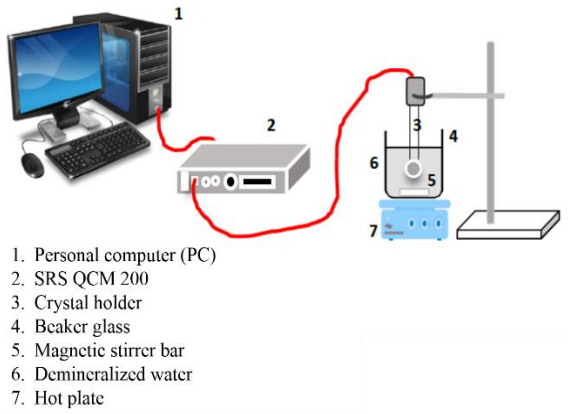


Fig. 1 Experimental set-up schematic diagram of Quartz Crystal Microbalance (QCM).

The response of the unmodified and modified gold QCM sensor toward bovine and porcine gelatins solution were monitored by immersing the QCM sensor into the sample solution under stirring condition. The response of modified gold QCM sensor was compared unmodified gold QCM sensor. All measurement used 100 ppm of each bovine and porcine gelatin solutions. The modified QCM sensor was also tested to the commercial hard capsule which is available in the local market after dissolving it to demineralized water.

RESULTS AND DISCUSSION

Characterization of gelatin

The characteristic of both bovine and porcine gelatin were identified using Fourier transform infrared (FTIR) spectroscopy. This characterization aims to identify the functional group of both gelatins. The FTIR spectra of bovine and porcine gelatins are shown in Fig. 2a and Fig. 2b, respectively. Both spectra show almost similar characteristic. There are four major peaks, i.e. wavenumbers at 3500-2300 cm^{-1}, 1656-1644 cm^{-1}, 1560-1335 cm^{-1} and 1240-670 cm^{-1} which correspond to Amide A, Amide I, Amide II, and Amide III regions, respectively. The similar results were also reported in the other works (Hashim et al., 2010; Cebi et al., 2016).

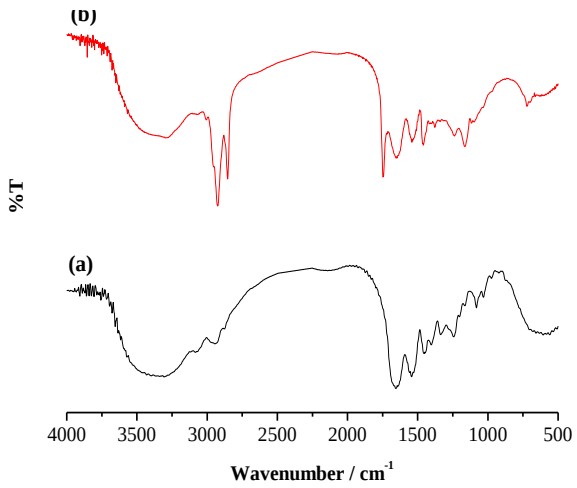


Fig. 2 FTIR spectra of bovine gelatin (a) and porcine gelatin (b).

Amide-A represents N-H stretching coupled with hydrogen bonding and free O-H, while Amide I contains of C=O stretching vibration with contribution of C-N bond stretching vibration. The Amide I band between 1600 and 1700 cm^{-1} is the most useful peak for FTIR analysis of secondary protein structures (Muyonga et al., 2004; Pranoto et al., 2007; Nur Hanani et al., 2012). Amide II arises from N-H bending vibration and C-N stretching vibration. Amide III represents vibration in the plane of C-N and N-H groups of bound amide or vibration of CH_2 group.

The obtained FTIR spectra (Fig. 2a and Fig. 2b) show that both bovine and porcine gelatins contain identical functional groups. This makes both gelatins can not be distinguished by FTIR technique. Nevertheless we still can see insignificant different from the spectra intensity in its every single peak. Especially it is found at wavenumber around 2900 cm^{-1} and 700 cm^{-1}. Absorption at around 2900 cm^{-1} comes from C-H group vibration of protein. This group commonly is found in any organic compounds. So this peak can not be used to distinguished porcine and bovine gelatins which have a lot of C-H group in their molecules. Due to some reasons this peak has a low intensity at Fig 2b. It is probably because of the free water which is contained in the gelatin overlaps with the absorption spectrum of C-H. The absorption of O-H group from water suppresses the absorption of C-H group from gelatin. In the other side, FTIR spectrum at around 700 cm^{-1} is in the fingerprint area. Small changes in the sample environment will affect the absorption spectrum. Thus, in our opinion this result also can not be used to distinguish porcine and bovine gelatins. It is reported from previous works that the FTIR spectra of bovine and porcine gelatins show relatively different in intensity from our work.

QCM sensor modification

The electropolymerization was performed by cyclic voltammetry in 0.1 M aniline at pH 1.5. The voltammogram of aniline polymerization was shown in Fig. 3. It shows that the voltammogram peak is gradually increased for further cycles. This indicates that polyaniline were successfully attached on the surface of gold QCM sensor layer by layer along with the cycles.

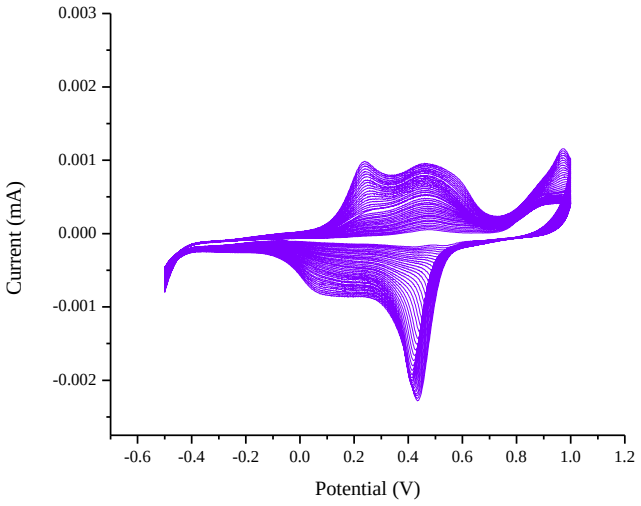
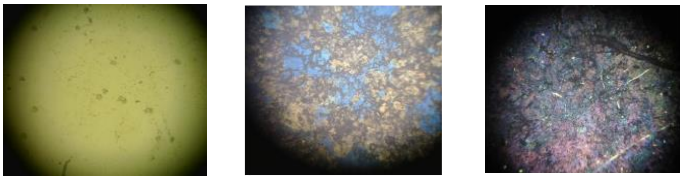


Fig. 3 Voltammogram of aniline electropolymerization on the surface of gold QCM sensor.

There are two peaks in anodic sweep and two peaks in cathodic sweep observed from the voltammogram (Fig. 3). The first anodic peak was observed at +0.22 V with current response from +0.16 mA to +1 mA, consecutively. The second one was observed at +0.78 V with current response from +0.21 mA to +0.93 mA, consecutively. The both cathodic peaks arise at +0.04 V and +0.47 V with current response from -0.17 mA to -0.8 mA and -0.22 mA to -2.1 mA, respectively. The anodic peak at +0.22 V is marked as oxidation peak of leucomeraldine (polyaniline in fully reduce state) which had oxidized into emeraldine (polyaniline in half oxidized state). Emeraldine will undergo further oxidation into pernigraniline

(polyaniline in fully oxidized state) which is showed by the second anodic peak at +0.78 V.

The electropolymerization of aniline was carried out at pH 1.5 because we would like to produce a conductive polymer. This conductive polymer can be obtained if the polymer is formed through head to tail coupling which can only be conducted under acidic pH conditions. While at higher pH (alkaline conditions), the deposited film consist of low chain oligomeric material (Stejskal et al., 2010), so the polymerization of aniline will form head to head coupling. The polymer that formed from head to head coupling does not have a conjugated double bond; hence the polymer obtained has non-conductive properties (Fitriyana and Kurniawan, 2015).



(a) (b) (c)

Fig. 4 Surface image of gold QCM sensor (a); polyaniline modified gold QCM sensor (b); polyaniline-nickel compound modified gold QCM sensor (c).

Fig. 4 shows the surface image of gold QCM sensor. It is observed that there is a color change of the QCM sensor before and after every step of modification. The surface color of the gold QCM sensor changes into dark blue (Fig. 4b) from bright yellow (Fig. 4a) after being electropolymerized with aniline. Further modification makes the surface of the QCM sensor becomes darker (Fig. 4c). This is an indication that nickel compound was successfully deposited on the surface of polyaniline modified gold QCM sensor.

The QCM sensor performance

The result obtained from the measurement of bovine and porcine gelatin using QCM sensor with and without modification were shown on Fig. 5 and 6, respectively. Fig. 5 shows the response of unmodified QCM sensor towards 100 ppm of bovine (halal) and porcine (non-halal) gelatin solution. It is observed that the QCM sensor shows negative frequency shift for both bovine and porcine solution. The same direction of frequency shift (negative) indicates that the unmodified QCM sensor can not be used to differentiate between porcine and bovine gelatin.

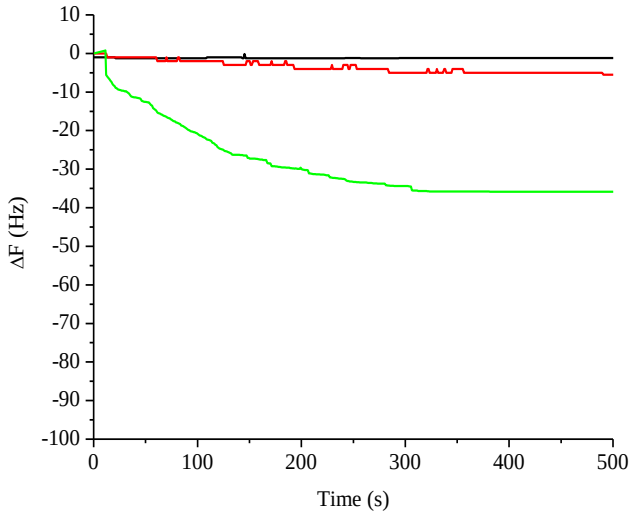


Fig. 5 Real time signal responses of unmodified gold QCM sensor in demineralized water (black), 100 ppm bovine gelatin (green) and 100 ppm porcine gelatin (red) solution. The experiments were performed at room temperature.

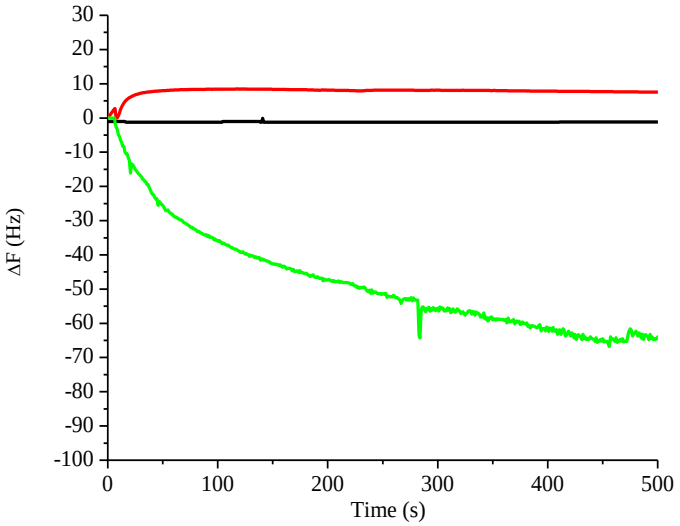


Fig. 6 Real time signal responses of polyaniline/nickel compound modified gold QCM sensor in demineralized water (black), 100 ppm bovine gelatin (green) and 100 ppm porcine gelatin (red) solution. The experiments were performed at room temperature.

Different responses are shown for polyaniline/nickel compound modified gold QCM sensor (Fig. 6). The bovine gelatin solution shows a negative frequency shift while the porcine gelatin shows a positive frequency. This different response could be explained through Sauerbrey's equation (1) below:

$$\Delta F = -C.\ \Delta m \quad (1)$$

where ΔF represents the frequency shifts of the QCM sensor, C is the sensitivity factor of the crystal used (it is 56.6 Hz μg^{-1} cm^2 for 5 MHz AT-cut quartz crystal at room temperature), and Δm is the mass variation per unit area (g cm^{-2}). When the analyte is attached to the surface of QCM sensor, the mass on the microbalance will increase so that the frequency will decrease (negative frequency shift). Meanwhile, the positive frequency shift means that the interaction between the sensitive layer and the analyte is relatively strong. The analyte takes off the sensitive layer from the surface of polyaniline/nickel compound modified gold QCM sensor into the solution. The free mobility of the analyte over the sensor layer results positive frequency shift called the anti-Sauerbrey behavior (Latif et al., 2012).

From the result, it can be said that the interaction between bovine gelatin and the nickel compound on the surface of modified QCM sensor is not strong enough to peel this nickel layer off into the solution. This causes the bovine gelatin molecule to be strongly attached to the nickel compound on the surface of QCM sensor. The opposite result occurs for porcine gelatin which give a positive frequency shift. The different response of polyaniline/nickel compound modified gold QCM sensor for bovine and porcine gelatin indicates that the sensor can be used effectively to distinguish bovine (halal) and porcine (non-halal) gelatin.

Repeatability of polyaniline/nickel compound modified gold QCM sensor

Fig. 7 shows the repeatability of polyaniline/nickel compound modified gold QCM sensor measurement. Three measurements 100 ppm of bovine (Fig. 7a) and porcine (Fig. 7b) gelatins solution using one modified gold QCM sensor were carried out. The polyaniline/nickel compound modified gold QCM sensor shows a good repeatability performance of frequency shift during the all measurements. It always shows decreasing real time signal responses to bovine gelatin and increasing real time signal responses to porcine gelatin.

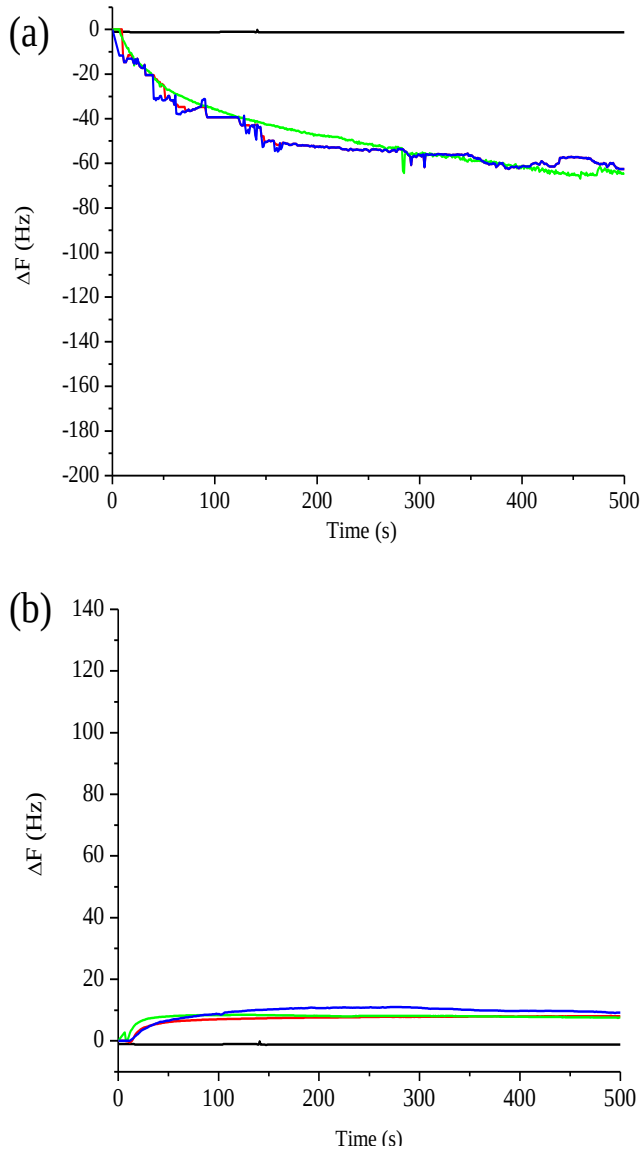


Fig. 7 Real time signal responses of polyaniline/nickel compound modified gold QCM sensor in 100 ppm bovine gelatin (a) and porcine gelatin (b). The experiments were performed at room temperature and repeated three times. Black lines in (a) and (b) are blank solution (demineralized water) responses. Red lines, blue lines, and green lines in (a) and (b) are the signal responses from the first, second, and third measurements, respectively.

Gelatin identification in commercial hard capsule samples

For further identification, the polyaniline/nickel compound modified gold QCM sensor was applied to identify non-halal (porcine) gelatin in the commercial hard capsule. The aim of this experiment is to know the performance of the polyaniline/nickel compound modified gold QCM sensor in the complex matrices (there are filler, plasticizer and dye). The result is shown in Fig. 8. The measurement gives a positive frequency shift for commercial hard capsule samples. This response indicates that the commercial hard capsule samples used in this study contain porcine (non-halal) gelatin. It is in a good agreement with the information label of the product. No significant effect of the hard capsule matrices is observed in the measurement.

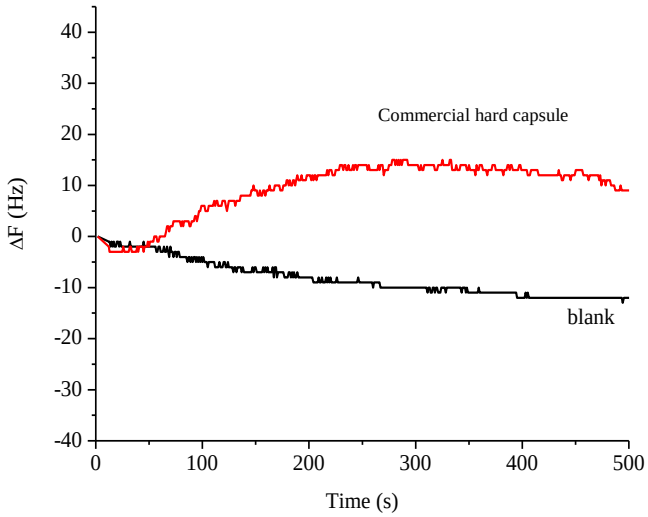


Fig. 8 Real time signal responses of polyaniline/nickel compound modified gold QCM sensor in commercial hard capsule sample solution.

CONCLUSION

A preliminary study of identification halal (bovine) gelatin from non-halal (porcine) gelatin using polyaniline/nickel compound modified gold QCM sensor has been studied. The result shows that bovine and porcine gelatins can be distinguished using polyaniline/nickel compound modified gold QCM sensor by seeing the direction of the frequency shift response. Bovine gelatin shows a negative frequency shift response while positive frequency shift response for porcine gelatin. The performance of polyaniline/nickel compound modified gold QCM sensor also gives a good result for the real sample. No significant interference is shown from the matrices of commercial hard capsule samples. It indicates that the polyaniline/nickel compound modified gold QCM sensor has a potential to be applied to distinguish halal (bovine) and non-halal (porcine) gelatin.

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