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Surface Acoustic Wave Resonators for Milk Analysis †

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Abstract: The analysis of milk samples includes both identification and quantification of specific components and the determination of physical parameters. The former covers both the normal ingredients of milk and unwanted components, such as veterinary drug residues and pathogenic microorganisms. The latter includes, for instance, the determination of liquid parameters, such as the viscosity as indicator of the freshness of the milk. Taken together, the parameters allow a comprehensive statement about the quality of the milk tested. Acoustic sensors, such as quartz crystal microbalances (QCMs) and surface acoustic wave (SAW) sensors, can basically be used for both types of analysis. Since acoustic devices are typically used as label-free detectors for sensors and biosensors, the functionalization of the device surface must ensure both analyte-specific binding and reduction of non-specific binding on the sensing layer to avoid interference with the results. The latter is also important when the devices are used for the determination of liquid parameters. This is particularly challenging when complex sample media, such as milk, are to be analyzed. In this work, SAW resonators are used as acoustic devices, since they are most promising regarding small and disposable sensor setups and arrays, including economic operating and read-out electronics. The SAW resonators are coated with a dicarboxy-terminated poly(ethylene glycol) (DC-PEG). This hydrogel layer is efficient enough to reduce non-specific adsorption of milk proteins to a minimum, leaving a reversible signal response characteristic for changes in the liquid parameters only. By this, the basic suitability of the SAW resonators for determining physical liquid parameters in milk samples is demonstrated.

Keywords: biosensors; drug residues; label-free; milk; physical liquid parameters; resonators; surface acoustic wave; viscosity

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1. Introduction

Milk and dairy products are an important dietary part for many people. In addition to the main components water, fat, proteins, and carbohydrates, milk contains vitamins and minerals, making it particularly valuable for the diet. However, like other foods, milk may also contain contaminants, such as pathogenic microorganisms or their toxins and veterinary drug residues. Besides the ingredients, the physical properties of the liquid milk are also of interest. The electrical conductivity, for instance, can be used to detect mastitis in the milked cow. The viscosity, on the other hand, is a commonly used quality parameter for milk samples, since it is influenced by age and composition of the milk and by the mechanical and thermal treatments to which the milk was subjected. In consequence, milk analysis includes both the detection of milk components, be it ingredients or contaminants, and the determination of physical parameters of the milk in total [1,2]. Biosensor setups are principally suitable for both types of analytical problem. Biosensors

with label-free detection are particularly advantageous with regard to fast and specific determination of analyte concentrations with low experimental effort. Most biosensor detectors use electrochemical, optical, or acoustic transduction principles [3,4]. Following the definition of biosensors as integrated receptor-transducer devices, they enable specific analyte detection, as was used for the detection of a variety of contaminants in milk [5-12].

In complex sample matrices, such as milk, it is important for the biosensing surfaces that they do not only capture the analyte but do also prevent non-specific binding from other sample components, since the latter may lead to false-positive results, especially when label-free biosensors are used. This shielding ability is also to be considered when the biospecific part of the biosensor ("receptor") is excluded so that the transducer can be used for the sole determination of physical sample parameters. An example for such a parameter is the viscosity, which can be determined by means of acoustic transducers [13,14]. Those mainly include QCM and SAW devices, where the latter are typically designed as delay line or as resonator devices. SAW resonators are particularly suitable for the design of small and disposable sensor setups and arrays. Furthermore, they deliver sharp resonance frequencies, which can easily be read out by simple and low-cost electronic setups, such as oscillators [15,16].

SAW resonators have successfully been applied for the label-free detection of penicillin G in milk in concentrations below the maximum residue limit of 4 ng/mL given by the European Commission [11]. In the following, it is shown that hydrogel-coated SAW resonators can basically be used for the measurement of physical liquid parameters in milk.

2. Materials and Methods

2.1. Milk Samples and Measurements of Physical Liquid Parameters

Skim milk containing 0.05% fat was prepared by skim milk powder. Lactose-free ultra-high temperature (UHT) milk containing 1.5% fat was obtained from the local supermarket. Skim milk samples were centrifuged at 13 000 rpm for 15 min to remove interfering fat. The remaining fluid is also called milk serum, i.e., the liquid phase of milk after removing fat and casein [2]. Sample conductivities were determined with a conductometer (WTW, type LF 539, Weilheim, Germany). Conductivity values were measured three times per sample. Sample densities were determined by weighing specific volumes. Density values were assessed five times per sample. Sample viscosities were determined with a rheometer (Haake, type Rheostress 300, customized from Thermo Fisher Scientific, Karlsruhe, Germany) at 22 °C. Viscosity values were measured four times per sample.

2.2. SAW Resonator Measurement Setup

2.2.1. SAW Resonator Device and Electronic Setup

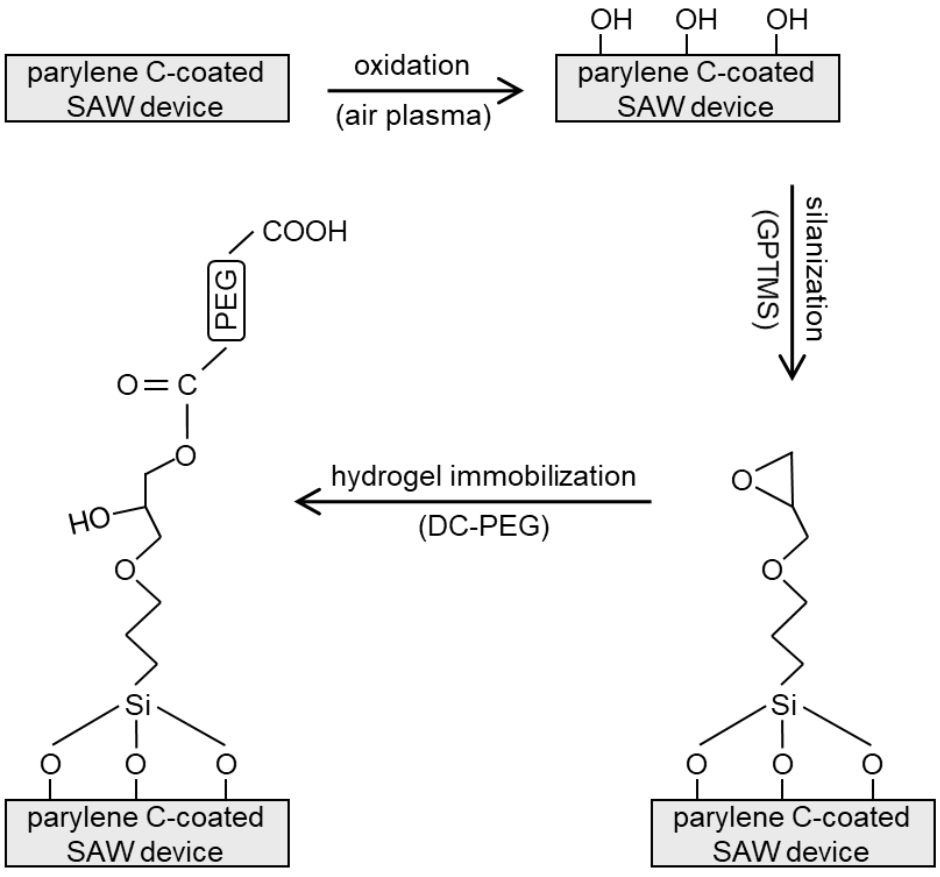
Shear horizontal two-port SAW resonators type SR062 with an operating frequency of 426.4 MHz (in buffer) were delivered by SAW Components, Dresden, Germany. The SAW resonator was operated as frequency-determining element in an oscillator circuit. Resonator frequencies were determined as difference frequencies relative to a reference resonator oscillating at 433.9 MHz. Therefore, processes leading to a frequency decrease of the SAW device, such as mass increase, resulted in increasing difference frequencies. Physical liquid properties (e.g., conductivity σ, density ρ, viscosity η) affect the SAW simultaneously in varying degrees, making it difficult to predict the actual (difference) frequency response of a liquid sample. However, as long as two liquids displace each other completely, the effect is fully reversible. Details of the device and the measurement setup have been described earlier [17,18].

2.2.2. Fluidic Setup and Measurement

A flow cell was designed allowing both electric and fluidic coupling of the SAW resonator. Sample handling and sampling of the device was performed by a flow injection analysis (FIA) system switching between load and inject modes. In the load mode, carrier medium was driven through the flow cell while the sample was loaded into the sample loop. In the inject mode, the content of the sample loop was driven by the carrier medium across the SAW resonator in the flow cell. Details of the flow cell, the fluidic setup and the measurement procedure have been published earlier [11,17].

2.3. SAW Resonator Coating and Characterization

To obtain a chemically homogeneous surface, the SAW resonators were coated with 0.1 µm poly(2-chloro-p-xylylene) (parylene C) by chemical vapor deposition with a commercial parylene deposition system (type Labcoater 1, PDS 2010, purchased from Specialty Coating Systems, Indianapolis, IN, USA) as previously described in detail [19]. The parylene C-coated resonators were activated via plasma treatment and subsequent silanization with (3-glycidyloxypropyl)trimethoxysilane (GPTMS), as described earlier [11]. The freshly activated resonators were treated with a solution of a dicarboxy-terminated poly(ethylene glycol) (DC-PEG; M_r 2000) dissolved in acetone at 2 mg/mL, incubated at 70 °C for 2 h, rinsed and dried at room temperature. A schematic representation of the surface functionalization is shown in Scheme 1.



Scheme 1. Surface functionalization of parylene C-coated SAW resonators with GPTMS and DC-PEG.

Contact angles were determined by the sessile drop method using a contact angle measurement microscope (type 20668; purchased from Erma, Tokyo, Japan).

3. Results and Discussion

Figure 1 shows measurements performed with parylene C-coated SAW resonators. The carrier medium was adapted to the respective samples with regard to the electrical properties to reduce signal interference from this point.

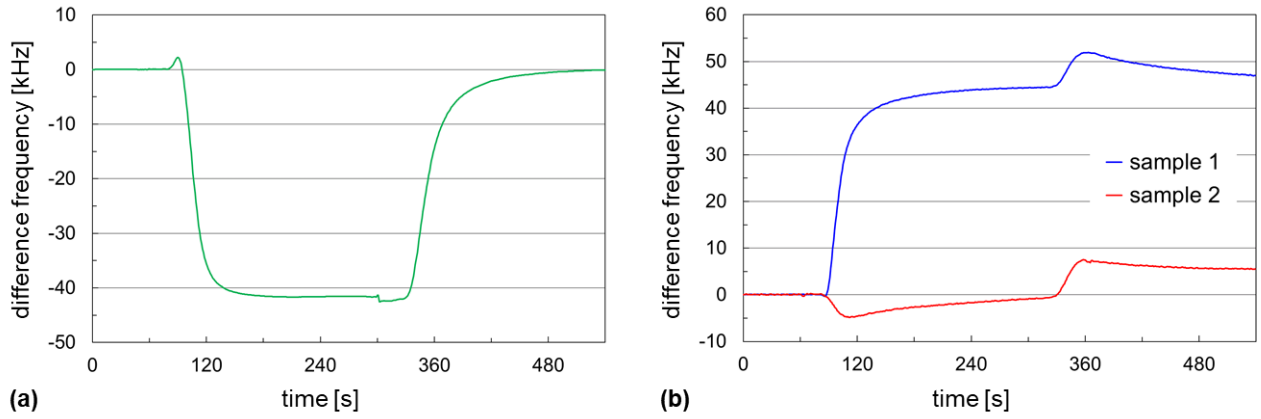


Figure 1. Frequency response curves obtained from measurements with parylene C-coated SAW resonators. Samples were injected into the running carrier medium at a time interval of 60–300 s. A new resonator was used for each carrier medium and sample type. (**a**) An aqueous solution containing 16% m/m ethylene glycol was injected into double distilled water as carrier medium. (**b**) Two skim milk serum samples were successively applied on the same SAW resonator; phosphate buffer, pH 6.8, was used as carrier medium.

The frequency curve shown in Figure 1a represents a measurement with an aqueous ethylene glycol solution as sample which is injected into double distilled water as carrier medium. None of the two liquids in the binary sample mixture adheres to the parylene C-coated resonator surface, leading to a completely reversible signal with a constant frequency level during the major part of the sampling, representing the changes in the physical liquid properties of carrier medium and sample. In contrast to that, when a skim milk serum sample (i.e., the liquid phase of skim milk after centrifugation) is applied on a parylene C-coated SAW resonator surface, mass loading on the sensor surface is added to the changes in the physical liquid properties of carrier medium (phosphate buffer pH 6.8, $\sigma = 2.50 \pm 0.08$ mS/cm; $\rho = 1.005 \pm 0.005$ g/cm³; $\eta = 1.09 \pm 0.05$ mPa·s) and skim milk serum ($\sigma = 8.45 \pm 0.01$ mS/cm; $\rho = 0.97 \pm 0.22$ g/cm³; $\eta = 1.48 \pm 0.04$ mPa·s) leading to an irreversible sensor response, as shown in Figure 1b. This is observed for two samples in a row, i.e., the non-specific adsorption is not completed by the first sample. Therefore, it is not possible to use this sample as a kind of blocking reagent to get a reversible signal in the following. Instead, the SAW resonator surface has to be adapted.

The compounds adsorbing on the hydrophobic parylene C surface from the skim milk serum (Figure 1b) are mainly proteins. Non-specific protein adsorption can effectively be reduced by hydrophilic hydrogel layers as used, for instance, for label-free biosensor applications [3]. In the following, DC-PEG was used as hydrogel, resulting in contact angles around 20°, whereas the contact angle of the untreated parylene C-coated sensors was around 85°. Figure 2 shows the frequency responses of DC-PEG-coated SAW resonators when milk samples are applied.

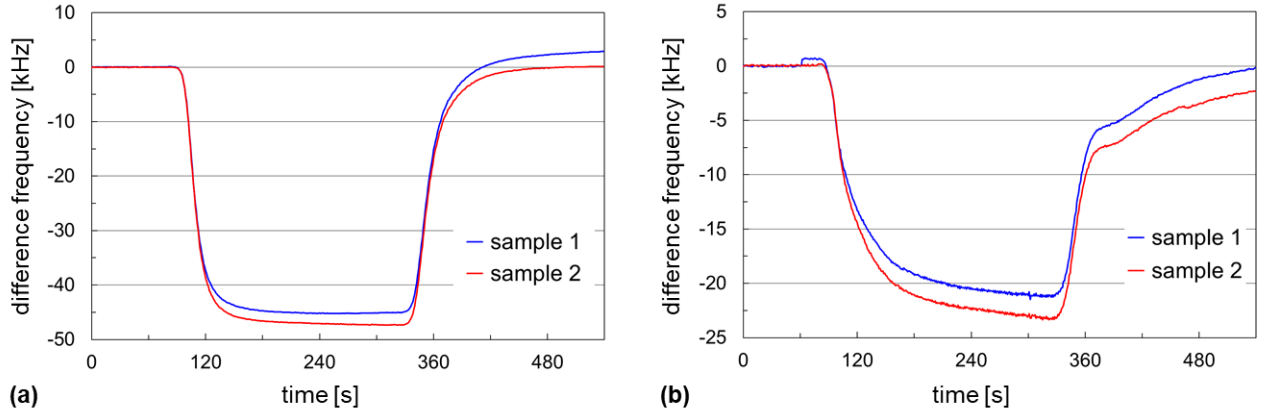


Figure 2. Frequency response curves obtained from measurements with DC-PEG-coated SAW resonators. Samples were injected into running phosphate buffer, pH 6.8, as carrier medium at a time interval of 60–300 s. A new resonator was used for each sample type. (**a**) Two skim milk serum samples were successively applied on the same SAW resonator. (**b**) Two UHT milk samples containing 1.5% fat were successively applied on the same SAW resonator.

In the measurement shown in Figure 2a, two skim milk serum samples are applied in a row on the same SAW resonator, i.e., except for the transducer surface, the measurement procedure is the same as shown in Figure 1b. However, with the DC-PEG surface, the mass increase by protein adsorption is successfully reduced, and similar frequency curves are obtained with the first and the second sample. Using two samples of UHT milk containing 1.5% fat (σ = 7.75 ± 0.01 mS/cm; ρ = 1.02 ± 0.02 g/cm³; η = 1.88 ± 0.02 mPa·s) on a DC-PEG-coated SAW resonator, similar results for the first and second sample are obtained again, as shown in Figure 2b. However, frequency decrease and increase are less steep than obtained before, leading to a less distinct range with the same frequency level. Furthermore, the frequency shift is smaller than obtained with the skim milk serum, indicating different liquid parameters of skim milk serum and UHT milk, as confirmed by the separate determination of the respective parameters. Another reason for both the delayed frequency response and the different frequency level is attributed to the sample fat, which is typically not prevented from depositing on the surface by a hydrogel layer [11]. Therefore, the fat may also affect the frequency response by surface deposition—even if only temporary—leading to mass increase and changes in the electrical environment of the acoustic transducer, to which especially SAW resonators are susceptible [17]. It has to be investigated in the future, whether a signal calibration considering the fat content can circumvent this problem.

4. Conclusion

SAW resonators can be used for quality analysis of milk samples, whereby the actual application depends on the surface coating and functionalization. As shown earlier by the example of penicillin G detection in milk, SAW resonators can be used as the transducer part of biosensors. In this work, preliminary experiments show that SAW resonators can basically be used for measurements of liquid propertied in milk serum or even milk samples. Calibration curves by means of physical liquid parameter measurements are still to be compiled; and it has to be investigated how to avoid problems due to the fat content (apart from centrifugation). When these issues are solved, a SAW resonator array could be designed allowing both analyte-specific detection of milk contaminants and physical characterization of the milk sample.

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