Measurement of Sulfate on a Digital Microfluidic Lab-on-a-chip

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Abstract

An electrowetting-based digital microfluidic lab-on-a-chip (LoC) platform for automated trace sulfate measurement is presented in this paper. This platform was designed to be integrated with a digital microfluidic impactor for online ambient aerosol sampling and analysis. The LoC uses a discrete droplet format in contrast to the traditional continuous flow micro-fluidic systems. The traditional sulfate measurement method – methylthymol blue (MTB) colorimetric method is modified for its application on the LoC platform. An absorbance measurement system integrated with the chip was designed, consisting of a light emitting diode and a photodiode that detects the color change due to the reaction. The MTB colorimetric assay was modified to be compatible with the micro-fluidic platform and optimized with respect to reagent concentration. The LoC system provides a higher level of automation, consumes less reagent, and produces less waste than the conventional "macro" systems. It has a short analytical cycle of 30 seconds. The system has a limit of detection of 0.54 mg/L and provides a broader linear measurement range (up to 150 mg/L) than the traditional implementation of the methylthymol blue method.

1. Introduction

Atmospheric particulate matter (PM) contributes to adverse health effects [1, 2], visibility reduction [3] and global climate change [4], all with significant socio-economic implications. Despite the progress made in recent years, PM remains a poorly understood problem. Atmospheric aerosol originates from a wide variety of sources and exhibits strong temporal and spatial variation in size, composition and

concentration [5]. Knowledge of aerosol composition as a function of size is critical for understanding the origin, properties and health effects of particulate matter.

Size-segregated chemical composition of PM is usually measured with cascade impactors [6, 7]. In cascade impactors aerosol is collected on several stages with progressively smaller cut-off diameters. The aerosol is impacted on aluminum or Teflon filter substrates, which after the collection are extracted in ultra-pure water and analyzed. Because each of the preparation, sampling, extraction, and analysis steps is manual, impactors require a large amount of manual handling, hindering their application in monitoring networks. In addition, each handling step introduces a risk of contamination. Impactors have a low time resolution (several hours) which limits their use in studies of intra-day variations of aerosol properties. The relatively large size of these devices also prevents their use as personal monitors for assessment of individual exposure to ambient PM.

To answer the need for an automated instrument for size-segregated measurements of aerosol chemical composition, we are developing an instrument that utilizes a combination of the well-established principle of an impactor for aerosol collection and micro-fluidic device (or Lab-on-Chip, LoC) for automatic extraction and analysis of the collected aerosol [8]. The LoC devices have been demonstrated to be capable of replacing standard bench-top analytical systems [9] and are especially attractive for analysis of aerosol samples. In addition to their compact size, which opens the way for compact light-weight personal monitors, the micro-fluidic devices require smaller sample volumes, offer faster analysis, much less reagent consumption (and consequently waste production) relative to the conventional (macro) analytical instruments, and higher levels of throughput and automation.

In our approach towards micro-fluidics, which is often referred to as digital micro-fluidics, the liquid is manipulated as unit-sized discrete micro-droplets. An electro-wetting technique is used to actuate micro-droplets, which refers to the modulation of the interfacial tension between a conducting liquid phase and an insulated solid electrode by the application of an electric potential between the two. The use of electro-wetting for droplet dispensing, transport, merging, mixing and splitting has been demonstrated previously [10-15].

In our aerosol collection and analysis system the aerosol is impacted directly onto the surface of a micro-fluidic chip [8]. After a short collection phase a micro-droplet is digitally directed across the impaction surface extracting water-soluble aerosol components. The extraction droplet is then directed to the analysis part of the chip where it is mixed with reagents and an analyte is determined colorimetrically. Due to the very small volume of the extraction droplet (micro liter or less) the extract has a relatively high concentration and the limit of detection of most of the colorimetric methods can be achieved after a brief collection phase, allowing sampling times of the order of 1 minute [8]. The extraction of collected particles from a hydrophobic surface has been successfully demonstrated [8, 16].

Here we describe the adaptation to the digital micro-fluidic platform of the methyl-thymol blue (MTB) method for sulfate determination [17]. Sulfate is the major aerosol component in the atmosphere, comprising 30% to 80% of the fine aerosol mass [5]. Because of its abundance in the PM it controls a number of important aerosol properties and, thus, needs to be quantified. It should be noted also that the LoC system described here can be used standalone for the determination of trace sulfate levels in other aqueous environmental samples, for example, for water quality monitoring.

2. Experimental

2.1 Lab on a Chip and optical detection device

The digital microfluidic platform used in this study is similar to the one developed for colorimetric biochemical assays [9], and its theory of operation is described elsewhere [10-12]. The schematic of the LoC device is shown in Figure 1. The LoC consists of the electrowetting chip on which the droplet handling operations and the chemical processes occur, and a non-invasive optical absorbance measurement system. The electrowetting system consists of two parallel glass plates separated by a polymer spacer. The surface of the bottom glass plate contains an array of independently addressable electrodes patterned in a 200 nm thick layer of optically transparent indium tin oxide (ITO). The top glass plate is coated with a continuous layer of ITO and a 50nm layer of Teflon AF 1600 to form the ground electrode. Due to its transparent nature, ITO enables easy integration of optical measurement

techniques with the electrowetting system. The bottom plate is coated with Parylene C (800 nm) for insulation and a thin hydrophobic layer of Teflon AF 1600 (50 nm).

Electrowetting chips of two configurations were used in our study with their main difference being the size of the electrodes and the spacing distance (and thus the optical pathlength). The first configuration was a chip with an electrode pitch of 1.5 mm and a nominal gap spacing of 0.475 mm. This chip configuration will be referred to as Chip 1. The other chip configuration had the electrode pitch of 0.5 mm and a nominal gap spacing of 0.150 mm (Chip 2). The Chip 1 had an advantage of larger light pathlength. The other advantage of Chip 1 was that it had bigger electrodes, which transmitted more LED light and provided a larger detection area for the photodiode. Chip 2, had on-chip reservoirs, unlike Chip 1, allowing on-chip droplet dispensing and mixing as well as simultaneous optical detection of multiple droplets, if necessary. Chip 1 was mostly used as a basic test-bed, and thus had no on-chip reservoirs, and therefore the droplets had to be premixed and deposited onto the chip manually.

A custom electronic controller was built to address and switch each electrode independently. By applying a voltage differential between adjacent electrodes, the sample or reagent droplet can be dispensed from the on-chip reservoirs and moved between the two plates of the chip. To avoid evaporation of reagent droplets, which would change the concentration of the reagents and the analyte, the droplet movement was performed in a silicone oil medium that filled the gap between the plates. 1 cSt silicone oil was used in both chip configurations.

The optical detection system was set up perpendicular to the main plain of the microfluidic device (Figure 1). It consisted of an orange color light emitting diode (LED) with a peak emission at 609 nm (model 404-1091-ND, Digikey) and a photodiode (TSL257, Texas Advanced Optoelectronic Solutions, TX, USA) which was a light-to-voltage converter that combined a photodiode and an amplifier on the same monolithic device. The voltage output of the photodiode V_t was optionally amplified and logged on a computer using a 12-bit analog data acquisition board (PCI-DAS08, Measurement Computing, Middleboro, MA, USA), with custom written software. The analog data acquisition board had a measurable range of 0.05 to 4 V and an analog-to-digital (A/D) resolution of 2.44mV.

Because the LED diameter was much larger than the size of one electrode, the LED was equipped with a cap to focus the light through the analyte droplet. The cap had an orifice at the top center with diameter of 1.5mm, which was the same size as the electrode of Chip 1.

The absorbance was calculated by the following equation:

$$A_t = \ln\left(\frac{V_0 - V_{dark}}{V_t - V_{dark}}\right)$$

where V_0 corresponds to zero absorbance, V_t is the voltage output of the photodiode and is directly proportional to the light intensity incident on the photodiode, and V_{dark} corresponds to the voltage output of the photodiode under dark conditions.

2.2 Sulfate colorimetric method

The colorimetric detection of sulfate was based on the traditional method by Mudsen and Murphy [17] with some modifications to the reagent composition as described in a separate section below. The method consists of the following steps. First, the sample containing sulfate was reacted with an alcohol solution of barium chloride and Methylthymol blue (MTB) at a pH 2.5-3.0 to form barium sulfate. The combined solution was then raised to a pH of 12.5-13.0, so the excess barium reacted with MTB. The reactions are shown below:

$x\ SO_4^{2-} + y\ BaCl_2 \leftrightarrow x\ BaSO_4 + (y\text{-}x)\ Ba^{2+}$ (excess) pH=2.5-3.0 y>x

$(y\text{-}x)\ Ba^{2+} + z\ MTB \leftrightarrow (y\text{-}x)\ MTB{\cdot}Ba + (z\text{-}y\text{+}x)\ MTB$ pH=12.5-13.0 z>y

The sulfate concentration was determined indirectly based on the competitive reaction of sulfate and MTB with barium in solution based on absorbance measurements of either uncomplexed MTB or the MTB-barium complex. The uncomplexed MTB has a maximum absorbance at 460 nm while for the MTB-barium complex it is at 608 nm. Mudsen and Murhy reported that the absorbance decrease at 608 nm due to the MTB-barium complex was about 3 times greater than the corresponding absorbance increase at 460 nm due to the uncomplexed MTB [17]. Therefore, we used measurements of absorbance at 608 nm for sulfate detection.

2.3 Reagents

All reagents were prepared using reagent grade chemicals and doubly-deionized water. Methylthymol Blue (3,3-bis[N,N-bis(carboxymethyl)amino methyl] thymolsulfonephthalein, sodium salt) was obtained from Aldrich Chemicals (Milwaukee, WI). Barium Chloride dihydrate ($BaCl_2 \cdot 2H_2O$), sodium hydroxide, ethanol (95%) and methanol were obtained from Fisher Scientific (Fair Lawn, NJ). Sulfate standard solution (1004mg/L) was from Alltech Associates (Deerfield, IL, USA). 1cSt Polydimethylsiloxanes, Trimethylsiloxy Terminated silicone oil (DMS-T01) was from Gelest (Morrisville, PA, USA).

Ethanol is the traditional solvent for MTB reagent. However, it proved incompatible with our digital microfluidic platform due to its solubility in the silicone oil. After testing several alternative solvents the best results were obtained with methanol. Based on the reaction sensitivity and the compatibility with the LoC platform, as described in the Results section, the optimized MTB reagent for the LoC platform was prepared as follows: 0.1812 g of MTB was dissolved in a solution which was prepared by mixing 0.6 ml of 1.0M HCl, 21.84 ml of a 1.526g/L $BaCl_2 \cdot 2H_2O$ solution, and 70 ml of methanol, and then diluted with doubly-deionized water to 100 ml. The MTB reagent was prepared daily. A 0.036 M NaOH solution was prepared by using 70% methanol as solvent. Sulfate standards of different concentration were prepared by quantitative dilution of the 1004 mg/L standard sulfate solution in 40% methanol solution.

3. Results and discussion

3.1 Chemical composition modification

In the digital microfluidic platform, silicone oil was used both to prevent evaporation and contamination of both sample and reagent droplets, and to lower actuation voltages required to move droplets. The actuation voltage should not exceed 50V to prevent damaging the electrodes. In selecting the silicone oil, two factors must be considered: transparency and viscosity. The selected silicone oil should be transparent to allow absorbance measurements. Also, the silicone oil should have proper viscosity. If its viscosity is too high, the sample and reagent droplets will be

hard to move. On the other hand, oils with too low viscosity evaporate quickly. Based on these criteria, the 1.0 cSt viscosity DMS-T01 silicone oil satisfied the selection criteria and was used in all of the tests described below.

The traditional MTB assay uses ethanol [17]. The ethanol content was reported to influence the method sensitivity, possibly because of the more complete reaction at low concentrations of barium and sulfate in the non-aqueous environment [17]. However, ethanol is soluble in the 1.0 cSt DMS-T01 silicone oil, which causes the reagent droplet to shrink quickly in the silicone medium during the droplet transport through the chip, which is detrimental to both the droplet movement and the assay accuracy.

We used methanol as an alternative to ethanol because it was not soluble in the selected silicone oil, and it had similar chemical properties to ethanol. The MTB reagents prepared with the same concentration of ethanol and methanol were compared during measurements of sulfate standard solutions using a bench-top HACH DR/2000 spectrometer equipped with 1cm path length (HACH comp., Loveland, CO, USA) at 608 nm. The bench-top spectrometer was used in these tests instead of the on-chip detector for convenience reasons. The MTB reagent was prepared with 0.0116g MTB, 0.6 ml of 1.0M HCl, 8ml of deionized water, and 1.4 ml of a 1.526g/L $BaCl_2 \cdot 2H_2O$ solution and diluted with 95% ethanol or methanol to 100 ml. 0.036 M NaOH solution was prepared using 45% of ethanol or methanol according to the traditional colorimetric method.

The results of the comparison are shown in Figure 2. Although both the ethanol and methanol solutions show linear relationships with sulfate concentration and show a similar slope, the ethanol solution has a lower intercept. This results in a modest increase in the Limit of Detection (LOD) for the methanol-based solution. The LOD is determined as three times the standard deviation of the blank (sulfate concentration is zero) measurements. Because sulfate concentration is determined by the absorbance difference between the blank and the sample, and because the noise is proportional to the absorbance signal, a higher intercept results in a higher LOD. However, the drop in sensitivity (an increase in the LOD) is rather modest relatively to the ethanol solutions. Given this fact and the compatibility with the microfluidic platform, methanol was selected as a substitute for ethanol in the MTB reagent.

3.2 Reagent optimization

The reagent composition had to be modified for the LoC device for two reasons: 1) ethanol was substituted with methanol to make it compatible with the electrowetting environment; 2) the pathlength of the LoC devices is shorter (0.15 mm or 0.475 mm) than in the original method (1 cm).

The effect of methanol content of the reagent on the method sensitivity was tested. The reagent and other chemicals were prepared as in Section 3.1. Absorbances of samples with the same concentration of sulfate mixed with reagents having different methanol content were tested with the HACH DR/2000 desktop spectrometer with a 1 cm light pathlength.

The results are shown in Figure 3, in which each point represents the average of five measurements and the error bars represent one standard deviation. The results show that a higher content of methanol increases the absorbance difference and, thus, the sensitivity of the method. This is similar to the findings with the standard ethanol-based method, which recommends high solvent contents[17]. However, we found it is not possible to use a reagent with such a high methanol content (85-95%) in the LoC system, because reagent droplets could not be dispensed from the on-chip reservoirs without substantially increasing the actuation voltage. This is probably a result of a substantially lower surface tension of the reagent with ~~a~~ high methanol content. Our tests showed that only solutions with methanol content no higher than 75% can be dispensed at normal actuation voltage from the reservoir.

To solve this problem without sacrificing the sensitivity of the assay due to a low methanol concentration, we have reduced the concentration of methanol in the MTB reagent to 70%, but added methanol to the NaOH solution and sulfate standards. The methanol content in NaOH solution was increased to 70%, and the sulfate standard solution was made to contain 40% of methanol. The methanol concentration of the droplet obtained by mixing these three droplets is 60%, which is higher than the original traditional method with ethanol (final ethanol content 43%). In the aerosol collector the extraction of the aerosol deposit will be made using a droplet of 40% methanol solution, which quantitatively dissolves inorganic aerosol deposit and can be easily actuated.

The reagent composition also needed ~~also~~ to be modified for the LoC application due to the much smaller pathlength in the LoC platform. Figure 4 shows the effect of reagent concentration on the absorbance signal measured on Chip 1 for the premixed sulfate liquid samples. Each data point is the average value of about 20 data points for the specific measurements. Higher concentrations of the reagent allowed for a broader sulfate measurement range. It is interesting to note that on the LoC platform the measurement range can be extended beyond that of the traditional bench-top method (up to 6 mg/L [17]). This is probably due to the lower path-length on the LoC, which prevents saturation of the absorbance signal at high MTB concentrations, and the higher solvent content in the modified method. The concentrations of MTB and $BaCl_2 \cdot 2H_2O$ were selected as 2.145 mM and 1.364 mM, respectively (0.1812g per 100ml and 21.84 ml of a 1.526g/L $BaCl_2 \cdot 2H_2O$ per 100ml).

3.3 Droplet movement test on the LoC platform

After the optimal reagent composition was found, droplet movement tests were done to characterize the assay performance on the electrowetting chip. The test process involved droplet dispensing, mixing, moving, light detection, and finally removing droplets to the waste reservoir. Laboratory tests showed that a droplet can only be dispensed from the reservoir at 1Hz or lower within the selected silicone oil environment. Factors that limit the droplet dispensing from the reservoir include the chemical components, liquid viscosity, surface tension of the liquid sample within the reservoir, as well as the droplet actuation voltage. Once dispensed, droplets can move evenly at rates up to 8Hz, but droplet actuation does not perform normally at rates higher than 8Hz. For the absorbance detection and data recording, a droplet needs to remain on the specific electrode for at least one second to ensure proper data recording (data recording rate of 1 to 4 Hz). Using these frequencies, the whole analysis process takes less than 30 seconds, which includes 8s for dispensing, 4s for mixing, 4s for moving, 5s for absorbance measurement (data recording rate of 2 Hz, droplets remain on the specific electrode for 5 seconds), and 8s for removal to the waste reservoir. Due to the small volume of the droplet, the mixing is quick and the color formation is very fast, taking only one second after the mixing. Figure 5 shows an example that illustrates variation of the observed voltage signal with the reagent-

sample premixed droplet moving in/out of the detection region of the chip. Both the droplet movement and the signal detection rates in this example are 1Hz.

3.4 Calibration and limit of detection

Calibration of sulfate measurements on the LoC device has been done for both chip configurations to asses the effect of the path length and the droplet size. The size of the electrodes on the chip determines the dispensed droplet size, which also determines the reagent consumption and the extraction droplet concentration. The calibration results for these two chips are shown in Figure 6. Each data point is based on about 20 data points for the specific measurements. Both configurations show a linear relationship for sulfate concentration ranging from the limit of detection to 150 mg/L, with r^2 higher than 0.97. For Chip 1, the absorbance is linearly correlated with the sulfate concentration with a slope of -0.0029 ± 2.27e-4 (the slopes and their uncertainty were determined using Origin 7 software, OriginLab Co). For Chip 2, the correlation coefficient is -0.0008 ± 6.91e-05 (one standard deviation). The limit of detection determined as 3 times the standard deviation of the blank measurements is 0.536 mg/L for Chip 1 and 1.154 mg/L for Chip 2. The ratio of the correlation coefficients for the two chip configurations is close to the ratio of their path lengths.

4. Conclusions

An electrowetting-based digital microfluidic LoC system for measurements of sulfate in aqueous solution in the part per million range has been developed and presented in this paper. The traditional MTB method has been modified and adopted to the LoC platform. This device allows for a much broader range (0.536 - 150 mg/L) of sulfate concentrations than the traditional colorimetric method (up to 6 mg/L), which is probably due to the lower path-length on the LoC, and the higher solvent content in the LoC method. Two chip configurations have been tested. The first configuration (Chip 1) was a chip with an electrode pitch of 1.5 mm and a nominal gap spacing of 0.475 mm. The second chip configuration (Chip 2) had the electrode pitch of 0.5 mm and a nominal gap spacing of 0.150 mm. At the selected optimal reagent composition and the droplet dispensing / actuation rates, the limit of detection for Chip 1 and Chip 2 configurations is 0.54 and 1.15 mg/L, respectively. The system

provides means for automated measurement with analytical cycle of 30 seconds. The reported digital microfluidic system and the analytical method provides a basis for the development of a digital microfluidic impactor for online automated measurement of sulfate in the ambient aerosols.

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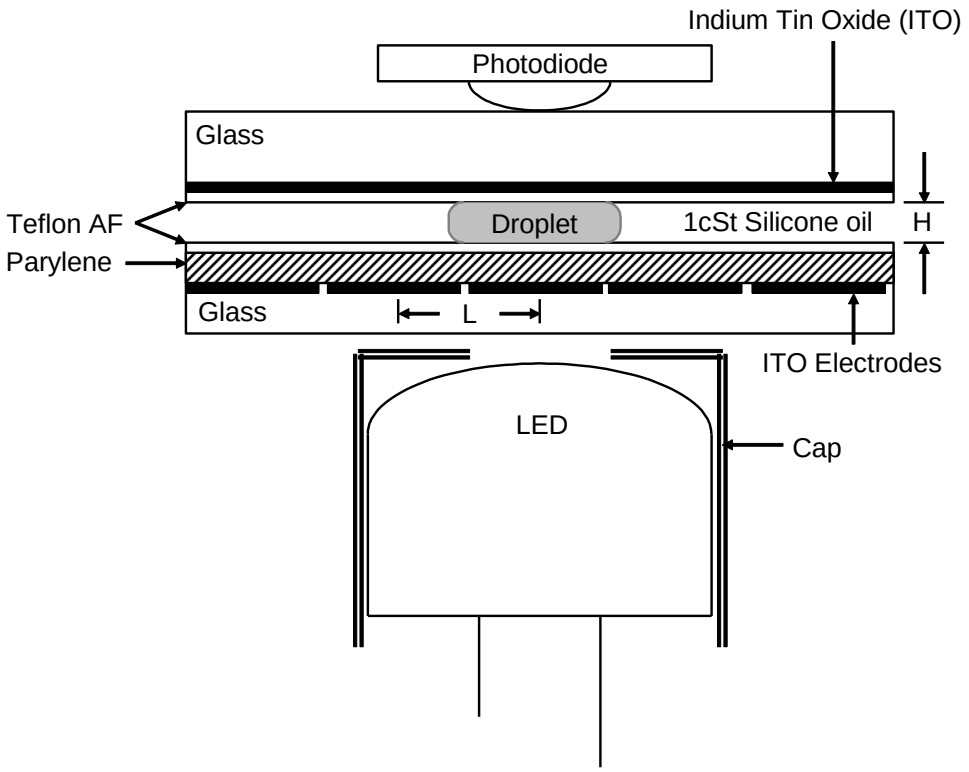

Figure 1. Side view of the electrowetting chip along with the optical detection system.

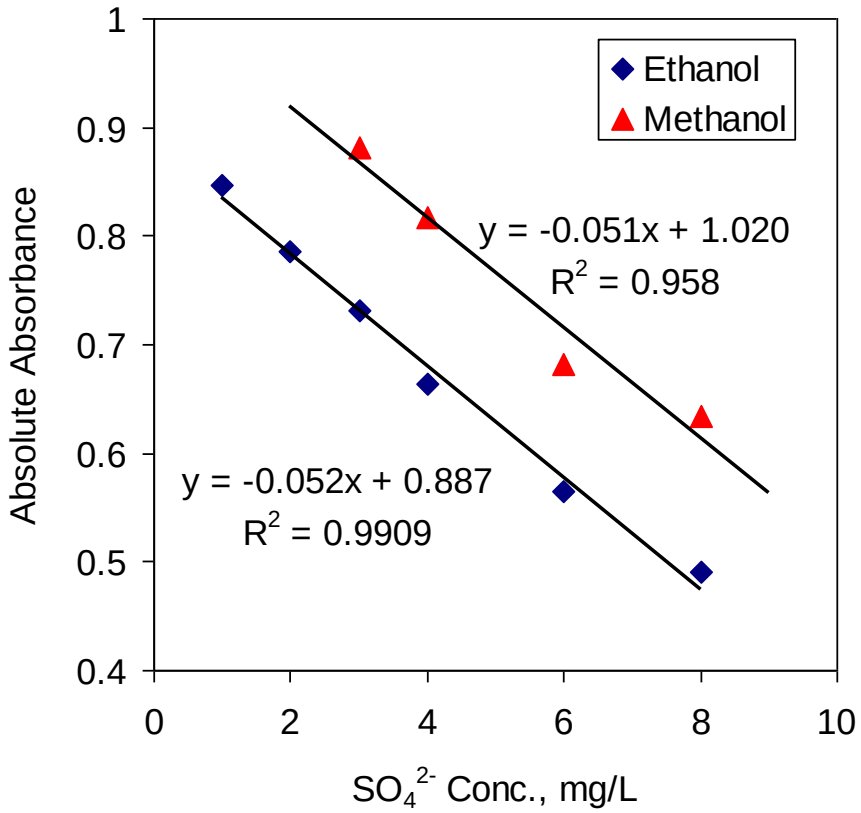

Figure 2. A comparison of the absorbance as a function of sulfate concentration for ethanol- and methanol- based MTB reagents measured on HACH DR/2000 spectrometer with a 1cm light pathlength.

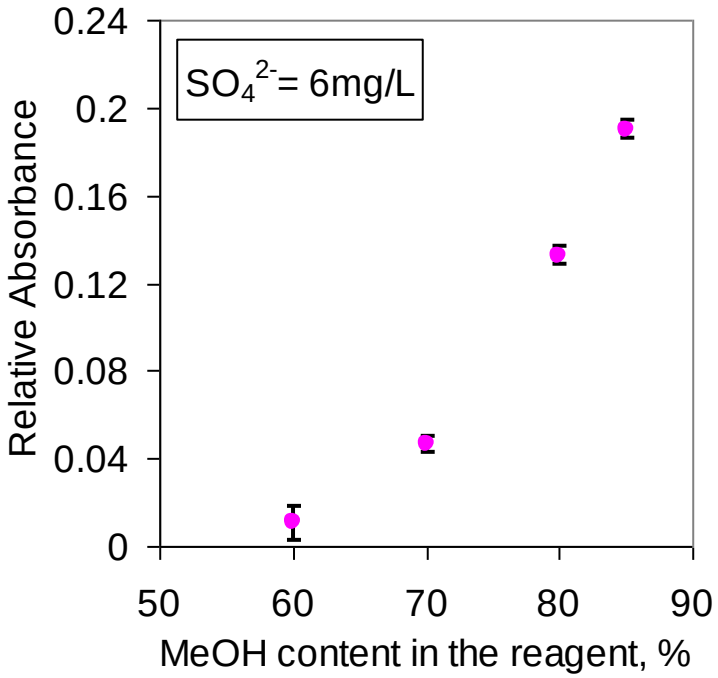

Figure 3. Effects of methanol content in the MTB reagent on the absorbance signal. Absorbance is measured with HACH DR/2000 spectrometer with a 1cm light pathlength. Relative absorbance is the absolute absorbance when sulfate concentration is zero minus the absolute absorbance measured at sulfate concentration of 6 mg/L.

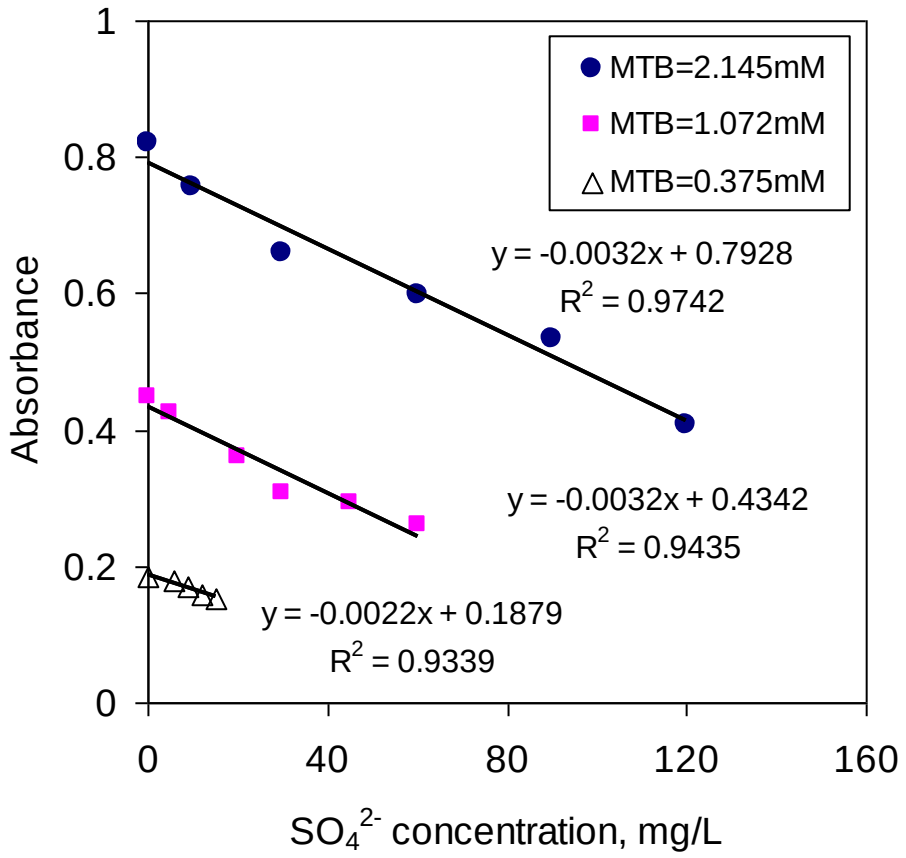

Figure 4. Effects of reagent concentration on the absorbance signal measured on Chip 1 for premixed sulfate liquid samples. $BaCl_2 \cdot 2H_2O$ concentration is increasing with MTB at fixed MTB/Barium stoichiometric ratio of 1.57. Each data point is the average value of 20 data points.

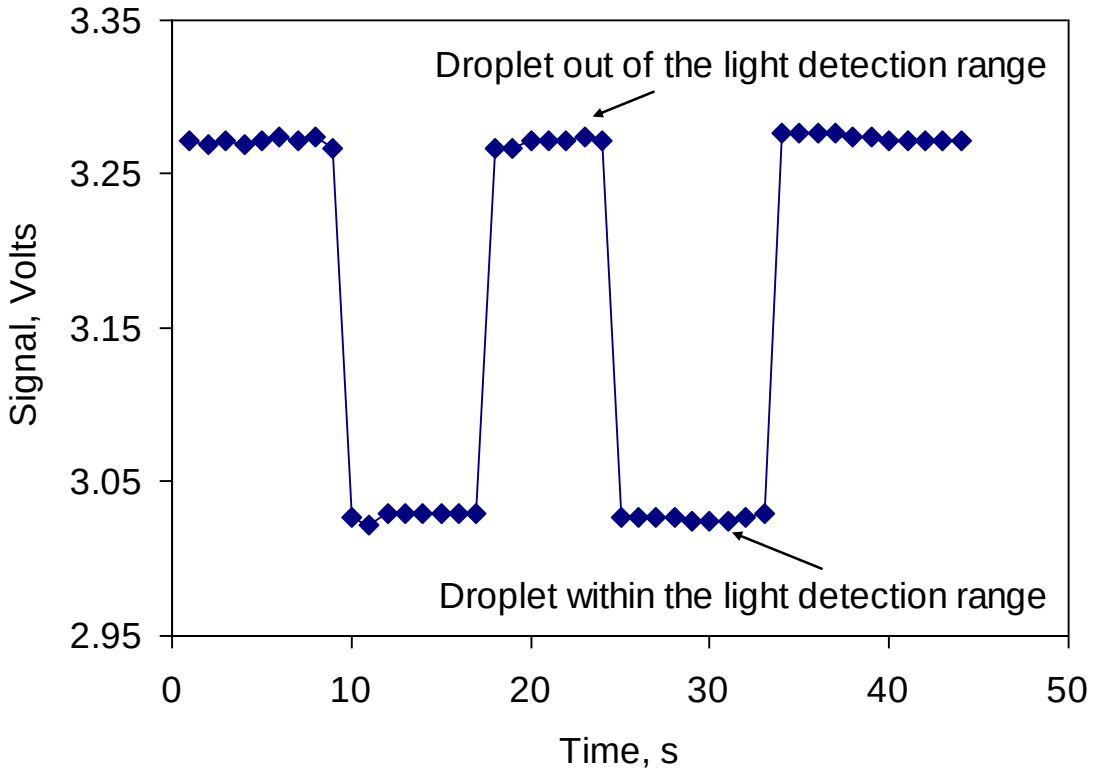

Figure 5. Variation of the observed voltage signal with the mixed reagent-sample droplet moving in/out of the light detection electrode. Droplet movement and signal detection rates are 1Hz. One standard deviation of the noise is 0.003V.

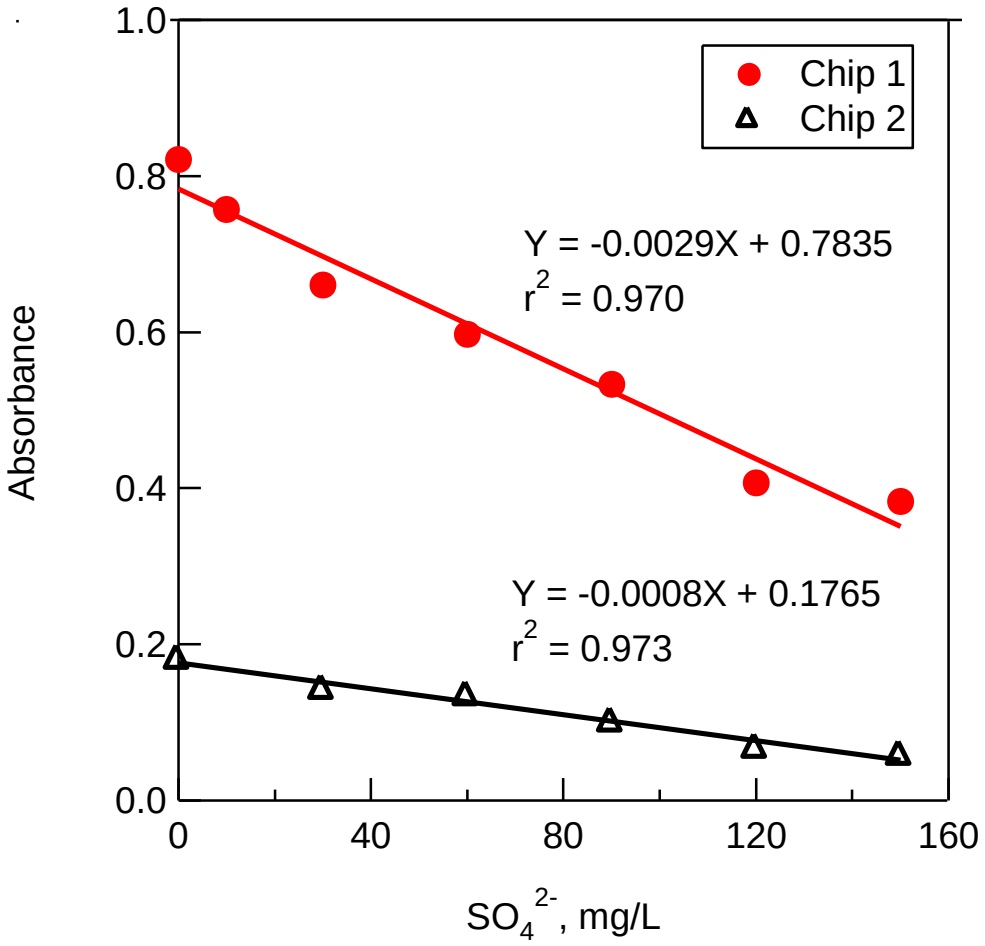

Figure 6. Sulfate calibration on the two LoC platforms. Chip 1 configuration has the electrode pitch of 1.5 mm and the nominal gap spacing of 0.475 mm. Chip 2 configuration has the electrode pitch of 0.5 mm and the nominal gap spacing of 0.150 mm.