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Study of geometrical effect of sample cell on nitrite determination

S. ABUBAKAR[a], N. ARSAD[a,b]*, M. S. AB-RAHMAN[a], A. A. EHSAN[b], S. SHAARI[b], R. OTHAMAN[c]
[a]*Department of Electrical, Electronic and System Engineering, The National University of Malaysia, Selangor, Malaysia*
[b]*Photonics Laboratory, Institute of Microengineering and Nanoelectronics, The National University of Malaysia, Selangor, Malaysia*
[c]*School of Chemical Sciences and Food Technology, The National University of Malaysia, Selangor, Malaysia*

The effect of cell geometry was studied for nitrite determination ~~by~~ using absorbance spectroscopy. Two cells with different geometry were used, one was flow injection cell in the form of a cylinder-shaped tube, and the other one was cuvette cell in the form of a tube with square cross section. Samples with varied concentration were formed in colorimetric using greiss reagent method. The maximum absorbance was found to be around 540nm for both methods. For overall comparison, flow injection exhibits higher absorbance, better linearity in the calibration curve and lower error as compared to the cuvette. This is due to the geometry of flow injection that can reduce the reflection and scattering of light. Both cells fall on moderate Savvin's sensitivity with flow injection has been more sensitive that cuvette.

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1. Introduction

Sample cell plays a vital role in spectrophotometry, especially at the part where light interacts with the sample, as shape or geometry of the cell can affect the performance of the instrument. There are two types of sample cells, namely cuvette and flow Injection. Cuvette is widely used for nitrite absorbance spectrometry analysis[1-7]. The geometry of the cell is in the shape of a cuboid tube, with square cross section, and typically used for static sample cell where during the experiment, sample is poured into the cell. Sample analysis is done using a double beam spectrophotometer. Flow injection on the other hand, comes in the shape of a cylindrical tube. It can be used for both dynamic and static samples, with the sample has been injected into the cell[8-14]. As both sample cells are widely used in spectrometric measurement, comparison between both cells utilizing different techniques would enable for selection of the more sensitive method for nitrite determination[15].

Nitrites (NO_2), consist of nitrogen and oxygen, are inorganic ions that occur naturally within the environment, and are ubiquitous in everyday life[16]. Being a part of the nitrogen cycle, nitrites are formed when microorganisms break down nitrogen-containing organic compounds in domestic or industrial wastes[1, 17]. Nitrites can also be found in preservatives, fertilizers, and curing agents for meat[18]. Nitrites can enter the human body by consuming food that use nitrite-based preservatives, or by drinking water contaminated with nitrites[19]. Overconsumption of nitrite can cause methemoglobinemia, where hemoglobin, which acts as the oxygen-carrying agent in blood changed into methemoglobin which cannot carry the oxygen[20]. Reaction between nitrites with secondary and tertiary amines in the body can result the formation of N-nitroso compounds which may cause the development of cancerous tumors. Consuming too much nitrite can also damage the nervous system and spleen liver[16, 21].

Nitrites' ubiquity was extended into the human biological system, where they are present as a part of reactive nitrogen species (RNS) in human saliva[22]. As nitrites of reaction with amines can trigger cancer development, high concentration of nitrites in saliva, becoming the potential to be used as a biomarker for the detection of oral cancer. And thus, salivary analysis can become a powerful tool for the diagnosis of oral cancer at an early stage [23-25].

Due to being widely pervasive in human life and largely negative effects on health, nitrite determination has been extensively researched. Some of the conventional methods have been developing include spectroscopic[26-30], spectrophotometric [2, 3, 31-34], electrochemical[35-37], chromatography[38-40], and capillary electrophoresis [41-43]. Among them, spectroscopy is the most appealing and widely used technique, due to its excellent detection limits and protocols of facile assay-type[28].

The aim of this study is to compare both sample cells using UV/VIS absorbance spectroscopy method. A quantitative absorbance spectroscopy was used to determine the sample concentration, based on the

measurement of the amount of light being absorbed by the sample [44]. The relationship involving absorbance (A) as represented in logarithmic manner of incident light (P_o) and transmitted light (P) is shown below,

$$A = \log(^{P_o}/_{P}) \quad (1)$$

The relationship between the absorbance and the concentration of the solution is known as Beer's Law, where A is absorbance, ε is molar absorptivity, b is path length, and c is solution concentration[45].

$$A = \varepsilon bc \quad (2)$$

2. Methodology

2.1 Experimental setup

The schematic diagram of the experimental system is illustrated in Fig. 1. A unit of UV-VIS-NIR Light Source (Model DH-2000-BAL) with deuterium tungsten halogen was used as a light source. All spectra measurements were done using an Ocean Optics spectrometer (Model HR4000), which was equipped with a 3648-element CCD-array Toshiba detector. The light was propagated into the sample cell and then the spectrometer using a silica fiber with a diameter of 400µm. SMA connectors were used to connect the fiber to the sample cell, spectrometer and light source. The path length of the sample cell was 1cm, and the data was displayed and recorded using Spectrasuite software.

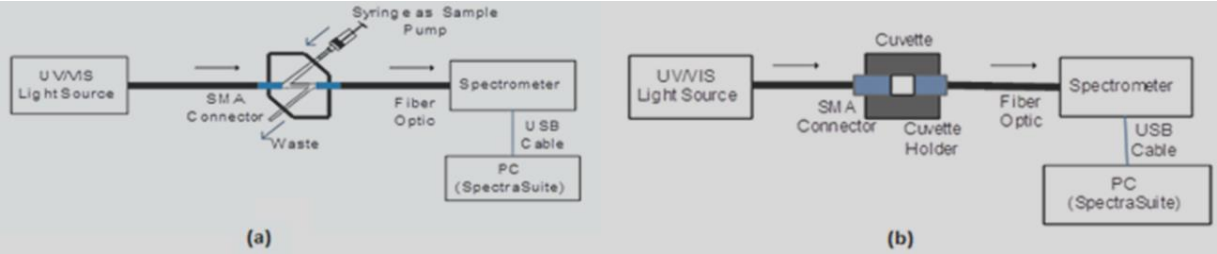

Fig. 1. Experimental setup: (a) Z-flow injection (b) Cuvette.

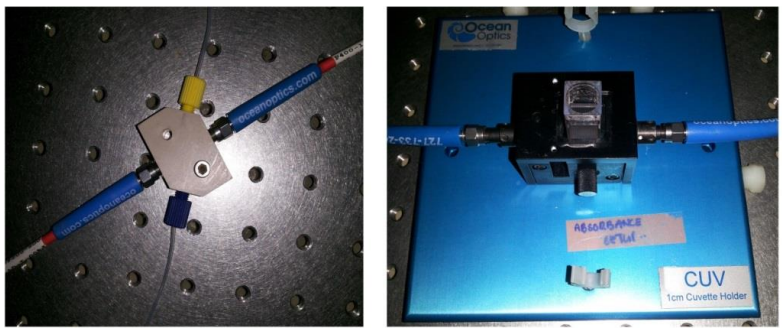

Fig. 2. Experimental setup: (a) Z-flow injection (b) Cuvette.

2.2 Solution and reagent

This experiment was done using deionised water as a solvent and sodium nitrite as the solute. Greiss reagent was prepared from sulphanilamide, N-1-napthylamine and phosphoric acid. All chemicals were bought from Sigma-Aldrich.

2.3 Recommended Procedure

Three solutions were prepared for the experiment, one acted as the sample solution and the other two solutions will be treated with Greiss reagent. The sample was prepared by diluting 0.0138g of sodium nitrite into 200mL deionised water using a volumetric method. The solution had 1mM concentration of the nitrite. The concentration of the nitrate was reduced by diluting the original solution with deionised water to give out another 5 samples with different nitrate concentrations, 50µm, 25µm, 12.5µm, 6.25µm, and 3.125µm. The greiss reagent consist of two different solutions, one having 0.0581M of sulphanilamide in 5% acid and another one having 0.0038M of N-1-napthylamine.

Colorimetric analysis of nitrite was performed by diluting each concentration with sulphanilamide acid and 1mL N-1-napthylamine was added for each solution. The solution was produced reddish-violet color after all reagents were added. Each sample had a total volume of 3ml where 1.5ml was used for cuvette and the remaining 1.5ml for flow injection.

In the absorbance measurement using flow injection, a syringe was used to inject the sample into the cell while for cuvette sample cell, the sample was simply poured into the cell.

3. Result and Discussion

3.1 Spectrum Absorbance

The spectrum absorbance of chemical species is a specific fingerprint that can distinguish particular species at a certain wavelength, and the absorbance at that wavelength varies proportionally according to the concentration of the species.

Fig. 3 shows the absorption spectra of deionised water samples contained of nitrite at different concentrations in the visible wavelength of 400nm to 700nm using flow injection and cuvette sample cell, respectively. Maximum absorption for both figures was achieved at wavelengths at about 540nm[46]. The amount of absorption, increased with increasing concentration of nitrite samples.

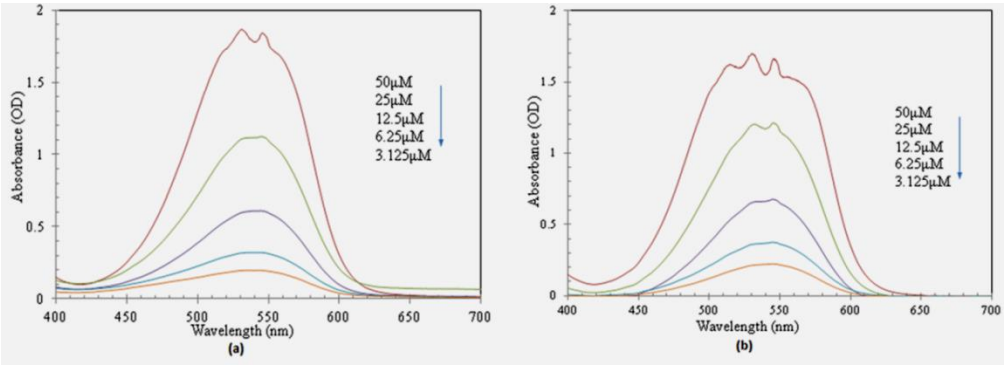

Fig. 3. Absorption spectra of water samples contained different nitrite concentration obtained from spectrophometer by using (a) flow injection cell and (b) cuvette sample cell.

The spectrum of nitrite absorbance in cuvette sample cell appears wider with noise quite prominent at a peak wavelength of 50µM and 25µM concentrations. Flow injection cell on the other hand has a narrower peak with noise occurrence only happening in the concentration of 50µM. In addition, the cuvette sample cell shows higher absorbance at lower concentration, whereas flow injection shows higher absorbance at high concentration.

3.2 Beer-Lambert Law Curve Plot

According to the Beer-Lambert law, absorbance is linear to concentration. Hence, in an ideal case where the concentration of nitrite increased, absorbance will increased in direct proportion with the concentration[45]. A calibration curve was plotted based on Beer-Lambert law to determine the performance of both sample cells. For this purpose, the peak absorbance for each concentration was measured seven times and the average results are used for curve plotting.

Table 1. Data for Beer-Lambert law's plot for nitrite.

Concentration (µM)	Absorbance* at 540 nm	
	SMA Z-Flow	Cuvette
3.125	0.19657	0.19543
6.25	0.32014	0.37729
12.5	0.60886	0.68214
25	1.12186	1.21243
50	1.86414	1.70414

*Average of 7 repeated measurements

Table 1 shows the tabulated data for Beer-Lambert law calibration curve plot with each data is an average, or mean, of 7 repeated measurements. The time taken for a single data acquisition was 2 minutes, thus 14 minutes in total were required for each sample to carry out the data spectrum acquisition.

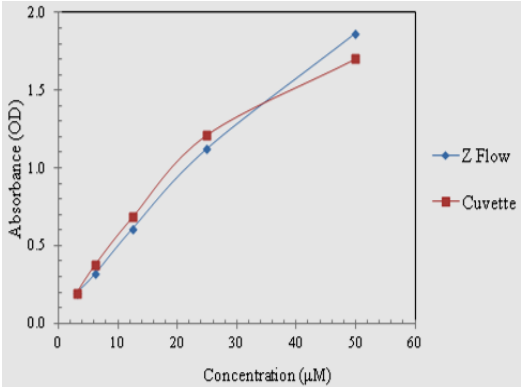

Fig. 4. Comparing calibration curve of absorbance of nitrite using Z-Flow and Cuvette.

Based on Fig. 4, it can be seen that the calibration curve of sample using flow injection cell appears to be more linear with constant increment, while cuvette cell calibration curve shows linearity at the outset but lags behind in increment at higher concentrations. Thus, it can be concluded that cuvette is more sensitive towards smaller concentration of the solution, while flow injection is equally sensitive at both high and low concentrations.

The geometry of the sample cell comes into consideration when discussing the linearity of a sample. As shown in Fig. 5a, flow injection comes in the shape of a cylinder tube with the path length of 1cm, 0.8mm inner

diameter, and having a volume of 26µL. This configuration can reduce the reflection and scattering of light, and as a result the linearity of the measured sample increased. On the other hand, the cuvette sample cell is square in cross section, with the path length of 1cm wide and 3cm high, and having a volume of 1.5ml as shown in Fig. 5b. Chamber-like construction of the cuvette sample cell would encourage the scattering of light. Reflection of light also occurs, as a result of spacing at the slit for the wavelength between the cuvette and the end of the light source fiber.

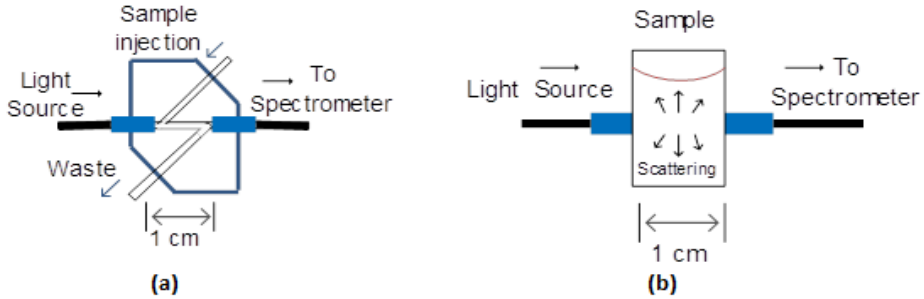

Fig. 5. Detail configuration of sample cell (a) Z-Flow injection and (b) Cuvette sample.

3.3 Experimental and Theoretical Comparison

Both the internal and external states of an experimental instrumentation can affect the experimental data. Hence, comparisons between experimental and theoretical data are done to measure how close the experimental data to the perfect results are. Theoretical data are obtained using (2), with a correlation coefficient of 1 expressed by a straight, perfectly linear curve.

The nonlinearity of the experimental data is mostly caused by stray light. These unwanted lights came from various external sources and the monochromator of the instrument, and are present in the system during the measurement[47].

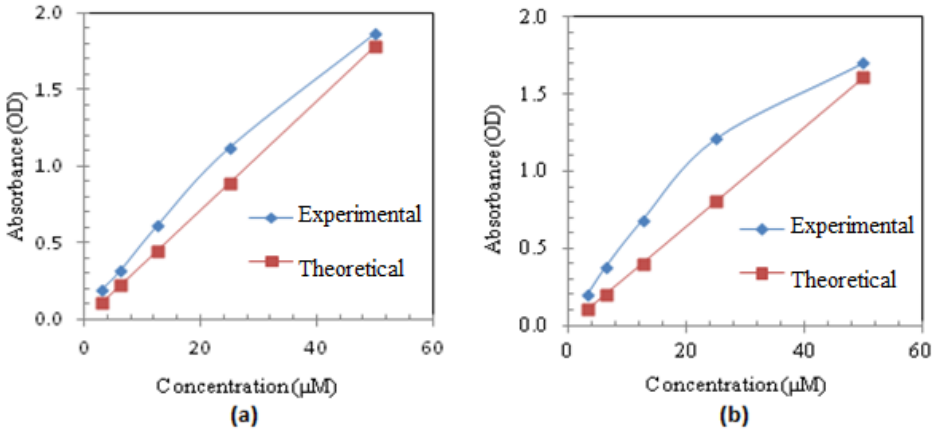

Fig. 6. Comparison between experimental and theoretical absorbance by using (a) Z-flow injection sample cell and (b) cuvette sample cell.

Fig. 6a shows the comparison between experimental data and theoretical calculation of sample absorbance using flow injection sample cell. The lowest absorbance difference was found to be at a concentration level of 3.125 and 50 µM, a difference of 8.54×10^{-2}. The highest difference of 2.325×10^{-1} was at 25 µM concentration. In 6.25 and 12.5µM concentration, the absorbance is 9.78×10^{-2} and 1.6×10^{-1}, respectively. The lowest and highest concentration showed no differences of the comparison. They are the smallest of all. The biggest difference is in the middle of variation concentration. The average of the difference is 1.3×10^{-1}.

Fig. 6b shows the comparison between experimental data and theoretical calculation of sample absorbance using cuvette sample cell. The lowest difference of 9.485×10^{-2} is at both 3.125 and 50 µM concentrations, while the highest difference with the concentration is 25 µM, an absorbance difference of 4.078×10^{-1}. At 6.25 and 12.5 µM concentrations, the absorbance 1.761×10^{-1} and 2.798×10^{-1}, respectively. There were no differences in the comparison to the (low and high) concentrations. The average difference was 2.11×10^{-1}.

At a glance, both sample cells showed similar characteristics with the highest and lowest absorbance difference occurring at identical concentrations. The Z-flow indicated low difference due to less effect of stray light in the flow compared to the cuvette. The stray light reduced the linearity of absorbance.

3.4 Optical Parameter

The sample displayed the same characteristics in both sample cells in terms of color, Beer's law range, maximum absorbance wavelength and Savvin's sensitivity. The concentration range of the sample, symbolizing the Beer's law range is between 3.12 and 50 µM, as to limit the application to the low concentration range. The maximum absorbance was found to be on a wavelength 540nm for both sample cells. Molar absorptivity for sample in Z-flow injection was found to at 3.5 x 104 Lmol-1cm-1, and 3.2 x 104 Lmol-1cm-1 for sample in the cuvette.

Based on Savvin's criteria of sensitivity that related between molar absorptivity and sensitivity, both sensor setups fall into moderate sensitivity, with Z-flow sample having higher value of absorptivity to be described as more sensitive in comparison to cuvette sample[48]. The maximum absolute error achieved by Z-flow injection is also significantly lower than cuvette, with 0.137 differences in value.

Correlation coefficient signifies the linearity of a sample under investigation, providing a range of value to determine how close is the sample to a true positive linearity (+1) or true negative linearity (-1)[49]. Based on the table, the correlation coefficients of 0.9911 for Z-flow sample and 0.9536 for cuvette sample both show strong positive linear correlation and approaching +1, proving that both samples have an almost linear trend and do not deviate far from the Beer-Lambert law.

Table 2 shows the optical parameter that compares the sample characteristics and optical responses in both cuvette and Z-flow injection sample cells.

Table 2. Optical parameter.

Parameter	Z-flow injection	Cuvette
Color	Red-Violet	
Beer's law range (µM)	3.125 - 50	3.125 - 50
λ_{max} (nm)	540	540
Savvin' sensitivity	Moderate	Moderate
Molar absorptivity ($Lmol^{-1}$ cm^{-1})	3.5×10^4	3.2×10^4
Maximum absolute error	0.134	0.271
Regression equation[a]		
Correlation coefficient (R^2)	0.9911	0.9536
Slope (*a*)	0.0357	0.0318
Intercept (*b*)	0.1314	0.2184

However, Z-flow sample exhibits a higher value of coefficient compared to cuvette sample, thus having a stronger linearity. Z-flow sample produces a steeper graph with a slope value of 0.0357 and intercepts the axis at 0.1314 absorbance, while cuvette sample has a slope of 0.0318 and intercepts at 0.2184 absorbance.

4. Conclusion

In a nutshell, an experiment to study the effect of sample cell geometry on the detection of nitrite samples with varying concentration has been carried out, comparing between flow injection and cuvette sample cells. Both sample cells achieved maximum absorbance wavelength at about 540nm, with noise presents at higher concentration of nitrite sample, and more frequent occurrence for cuvette sample cell. The Beer-Lambert law calibration curve for flow injection cell exhibits better linearity at all concentrations, while cuvette cell shows linearity at lower concentrations of the samples. The geometry of the cells attributes to their linearity as cylinder-shaped cell can reduce the scattering of light and increased the linearity. Cuvette cell with square cross section on the other hand causes reflection and scattering of leading, leading to decrease in absorbance, increase in noise, and thus lowering its linearity.

Comparison between experimental and theoretical data of both cells shows a greater deviation of experimental data from theoretical results in cuvette sample cell, with the greatest difference of absorbance to be at 0.40778 and 0.23249 for cuvette cell and flow injection cell, respectively. Molar absorptivity for flow injection sample cell is at 3.5 x 10^4 $Lmol^{-1}cm^{-1}$, and 3.2 x 10^4 $Lmol^{-1}cm^{-1}$ for cuvette sample cell, both presenting moderate Savvin sensitivity with flow injection having higher sensitivity. The correlation coefficient for both cells shows strong positive linear correlation approaching +1, with flow injection cell having the highest value of 0.9911 and cuvette at a value of 0.9356. For overall comparison, flow injection cell presents a more reliable performance and produces more sensitive measurements as compared to using cuvette cell.

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*Corresponding author: norhana@eng.ukm.my
sabiranmuslim@gmail.com