

Measuring and Application of NIR Light Absorption Coefficient of Bacteria

Pavel Krepelka¹, Fernando Camara Martos²,
Guiomar Denisse Posada-Izquierdo², and Fernando Pérez-Rodríguez²

¹Department of Theoretical and Experimental Electrical Engineering
Brno University of Technology, Technická 12, Brno 616 00, Czech Republic

²Departamento de Bromatología y Tecnología de los Alimentos, Ed. Darwin-Anexo
Campus Rabanales s/n, Universidad de Córdoba, Córdoba 14014, Spain

Abstract— Near infrared spectroscopy is a widespread technique in analytical chemistry. However, recently, there has been a growing interest in the usage of the NIR spectroscopy in microbiological analysis. Due to the improvement of the experiment setup and an increase in the efficiency of the evaluation methods, the knowledge of the absorption coefficient of a sample can be very beneficial. The purpose of this study is to introduce problems that relate to the measurement and handling of absorption coefficients.

The molecular bonds presented in the examined sample cause absorption of the incident light. When the energy quantum from a source of light equals the energy necessary for the transition of a bond to a higher vibration level, the light is absorbed. Considering this effect, it is possible to identify the chemical composition of a sample. Recent studies [1] have proved that the chemical composition of different bacteria species is sufficiently diverse, and thus the bacteria can be identified using a NIR spectrum. The absorption coefficient is mostly used in the Lambert-Beer-Bouguer law. This equation expresses how the optical intensity of a light wave is exponentially reduced along the beam's path through the sample. In the case of NIR spectroscopy, the output light intensity is measured. The multiplicative scatter and other unwanted influences of the incoming radiation make direct measurement difficult. For correct computing of the absorption coefficient, it is necessary to separate the physical light-scattering effect from the chemical light absorbance constituted by vibrations of a molecule's bonds. The absorption coefficient is a function of the energy of photons (wavelength), and its value depends on the position and amplitude of the fundamental, overtone, and combination types of molecular bond absorption.

Knowledge of the absorption coefficient is important due to optimizing the measurement technique and statistics methods. Because any straightforward measuring of bacteria cells requires a special approach, prior knowledge of the absorption coefficient can help to reduce costs in terms of money and time.

1. INTRODUCTION

The growing demand for rapid methods of bacterial identification accelerates the development of alternative techniques including Near infrared spectroscopy. Traditional methods are mostly based on immunoanalysis, gram staining, mass spectroscopy, DNA sequencing, and other biochemical testing approaches. In contrast with the above-listed common methods, Near infrared spectroscopy is a cheap, fast, and simple technique.

The ability to determine bacterial species is based on their different chemical composition, which leads to different ratio between IR active molecule bonds (C-H, N-H, O-H). The membrane structure of a bacterial cell and the ratio of lipids, proteins, and polysaccharides depend on the bacterial species. Unlike the mid-IR spectrum, the NIR spectrum is difficult to interpret because of the wide and overlapping combination and overtone peaks. Therefore, multivariate statistics need to be performed to provide information from the NIR spectrum. Applied procedures for signal pre-processing, classification and validation exert essential influence on the final identification efficiency.

The determination of a NIR absorption coefficient is a non-trivial procedure because of light scattering, spurious absorption, non-homogeneous sample, and other effects. To suppress these factors, the statistical parameters and experiment setup must be optimized.

2. ABSORPTION COEFFICIENT

The relation between the sample concentration and light absorption is described by the Lambert-Beer-Bouguer Law. The equation defines the amount of light absorbed by specific component in a

sample. The law assumes that the absorbances of multiple components are of additive character (at the same wavelength) and the samples are homogeneous and nonscattering

$$-\frac{dI_x}{I_x} = \frac{\sigma CSdx}{S}. \quad (1)$$

Consider the light incident on a material with a pre-determined sample concentration (C). The incoming light with intensity I_x illuminates $CSdx$ molecules, where S expresses the size of the illuminated area and dx is the light path length. The above-shown formula (1) expresses the probability of beam absorption in a material with thickness dx . The Lambert-Beer-Bouguer Law arises from integration of the Equation (1)

$$\int_{I_0}^I -\frac{dI_x}{I_x} = \int_0^x \sigma C dx \quad (2)$$

$$I = I_0 e^{-\sigma C x}. \quad (3)$$

The light intensity decreases exponentially with the length of penetration. To simplify the equations, linear attenuation coefficient (Eq. (4)) and absorbance (Eq. (5)) are used. Absorbance is defined as the inversed ratio of light intensity in front of and behind the sample

$$\mu = \sigma C \quad (4)$$

$$A = \log \left(\frac{I_0}{I} \right). \quad (5)$$

The extinction coefficient is defined as $\varepsilon = \log(e)\sigma$. The coefficient is used for expressing the light absorbance unit — see Eq. (6). In electromagnetic theory, extinction coefficient can be derived from the imaginary part of complex refractive index

$$A = \varepsilon C x. \quad (6)$$

Absorbance is linearly dependent on the concentration, pathlength, and absorption (extinction) coefficient of the investigated sample. The advantage of this notation lies in the possibility of summing up the absorption effects from particular components.

3. MATERIAL AND METHODS

In the first experiment, the absorption spectrum of bacteria in an aqueous solution was measured. Three different common food pathogen bacteria (*Listeria ivanovii*, *Escherichia coli*, *Salmonella*) were grown in tryptic soy broth, shaken, and measured in a saline solution. The near infra-red spectra were acquired via a diffuse-reflectance integrating sphere within the region of 1100 nm–2500 nm using a PerkinElmer Spectrum One NTS instrument. Figure 1(a) shows the setup of the design. In most cases, spectra variations caused by the investigated component are negligible; thus, multivariate statistics need to be performed. In chemistry, statistics is called chemometrics and comprises three main parts: Exploratory analysis (Principal component analysis), Regression (Partial least square regression), and Classification (Soft Independent Modeling of Class Analogy; Canonical variate analysis). Generally, bacterial identification is a classification task. The spectrum-processing techniques were carried out using Mathworks Matlab 8.1a. It is clear from Figure 1(b) that the peaks caused by water are dominant and the spectra variations caused by the bacteria are negligible. The absorption coefficient of the solution varies from 0–120 cm⁻¹. From the known bacteria concentration verified by a laboratory method, it is possible to compute the number of bacteria in the measured area. Approximately 17 10⁵ bacteria were placed on the mirror.

Despite the application of advanced statistics, the validated correct classification rate is low (see Table 1). It follows from this level of accuracy that the analysis of bacteria in an aqueous solution is impossible to perform. A proof based on the absorption coefficient will be introduced later.

The total of 60 samples were included in the second experiment. To remove the effect of the supernatant, the samples were centrifuged and resuspended in distilled water. Further, the samples were filtered with a glass fiber filter and dehydrated to eliminate the absorption effect of water. On each filter, five measurements were carried out at different places. To ensure the same level of concentration, the sample absorbance of wideband (420 nm–580 nm) light was maintained at

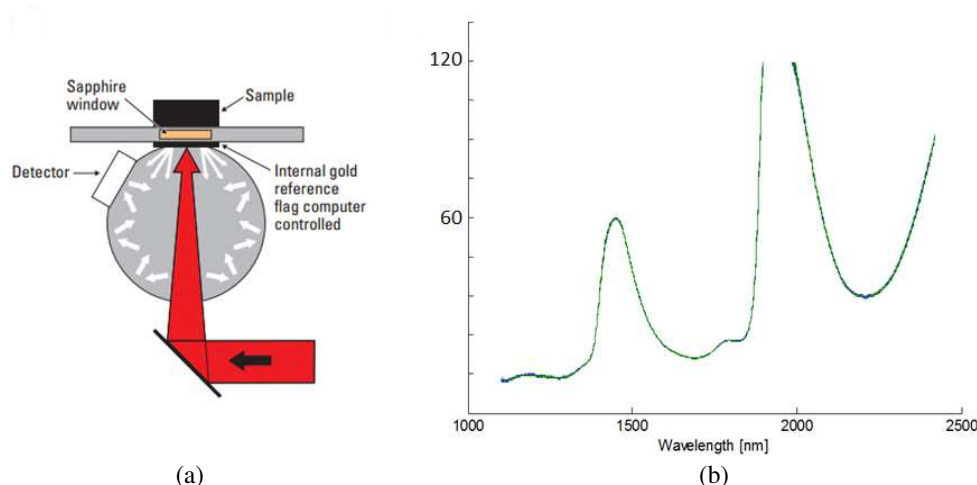


Figure 1: Design of the first experiment and the NIR spectrum of aqueous solution.

Table 1: The models performed in the first (first row) and the second (second row) experiment. Performance is expressed correct classification rate. CVA — canonical variate analysis, PLS-DA — partial least square-discriminant analysis, SIMCA — soft independent modeling class analogy, ANN — artificial neural network.

CVA	PLS-DA	SIMCA	ANN
40	45	34	35
100	90	100	95

approximately 0.85 (concentration aprox. 10^7 CFU/ml). The whole experimental design is shown in Figure 2. By this procedure, the pure spectrum of bacteria can be acquired. Although the spectra variations are more pronounced, we have to apply suitable statistics to facilitate correct computation of the absorption coefficient. For separate chemical absorption and light scattering, the EMSC algorithm [3] (Extended Multiplicative Scatter Correction) was performed. This algorithm can modify the NIR spectrum and make it applicable to the Lambert-Beer-Bouguer law, which considers only chemical light absorption (see Eq. (7)).

$$Z_i = a_i I + b_i m + h_i p + d_i \lambda + e_i \lambda^2 + \varepsilon_i, \quad (7)$$

where I is the identity matrix, m is the mean spectrum, p constitutes the first canonical component, and is the vector of the wavelengths. This equation is minimized using the method of least squares. Table 1 presents the high correct classification rates of the models. It is clear that the identification of highly concentrated bacteria suspensions was successful.

Approximately 10^{10} bacteria were placed on the filter. We can compute the corresponding volume of the highly concentrated solution of bacteria, which is necessary for correct identification.

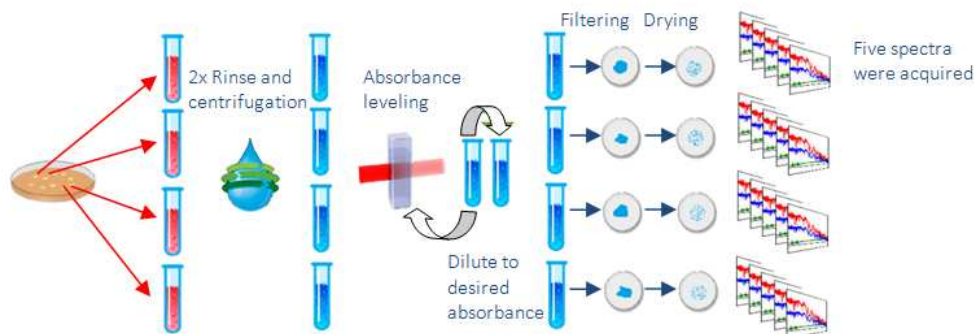


Figure 2: Design of the second experiment.

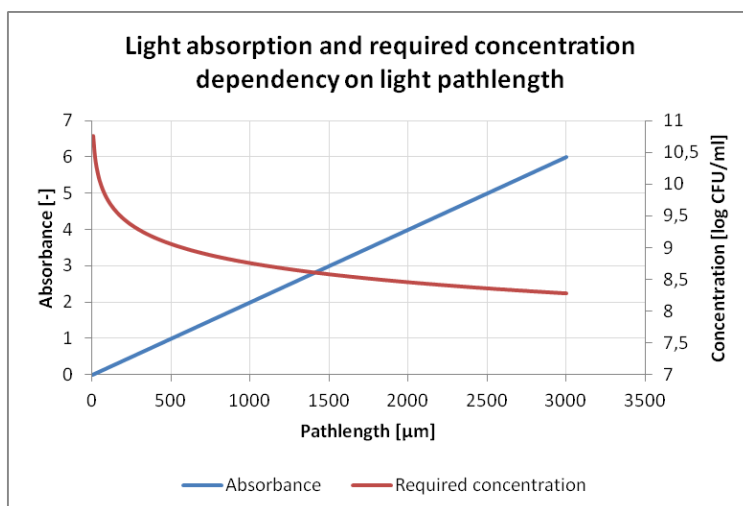


Figure 3: Graph of light absorption and required concentration dependency on light pathlength.

Since the absorption coefficient of the solution is known, a graph expressing the relation between the absorption of bacteria and the solution can be constructed.

4. CONCLUSION

In the first experiment, the impossibility of bacteria strain identification in the suspension was proven. In some cases, the composition of the sample can be predicted based on secondary chemical changes, but the biomass volume is not sufficient to facilitate determination. All the performed models exhibit very low correct classification rates.

The following experiment showed the ability of NIR spectroscopy to identify bacteria strains. A method for examining the pure spectrum of bacteria was developed. A bacteria suspension was placed on a glass fibre filter, and the water was evaporated. The samples prepared in this manner were identifiable via NIR using advanced statistical methods.

If the absorption coefficients of the bacterial cells and water are known, it is possible to define the minimal sample path length. Therefore, the possibilities of measurement in water can be theoretically determined, and the experiment setup can be optimized.

ACKNOWLEDGMENT

The research described in the paper was financially supported by project of the BUT science fund, No. FEKT-S-14-2545, and a project from the Education for Competitiveness Operative Programme, No. CZ.1.07.2.3.00.20.0175 (Electro-researcher).

REFERENCES

1. Boyer, A. E., M. G. Candela, R. C. Lins, Z. Kuklenyik, A. Woolfitt, H. Moura, S. Kalb, C. P. Quinn, and J. R. Barr, "Quantitative mass spectrometry for bacterial protein toxins — A sensitive, specific, high-throughput tool for detection and diagnosis," *Molecules*, Vol. 16, 2391–2413, ISSN 1420-3049, 2011.
2. Thermo Scientific, Smart NIR Integrating Sphere, Product Datasheet, online: http://www.thermo.com/eThermo/CMA/PDFs/Product/productPDF_7545.pdf.
3. Thennadil, S. N., H. Martens, and A. Kohler, "Physics-based multiplicative scatter correction approaches for improving the performance of calibration models," *Appl. Spectrosc.*, Vol. 60, 315–321, 2006.