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A FLEXIBLE EXPERIMENTAL SET-UP FOR DEVELOPMENT OF SPECTROPHOTOMETRIC ANALYSERS OF FOOD

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Abstract – A spectrophotometric analyser of food (SAF) is a spectrophotometric instrument dedicated to measuring physical and/or chemical parameters characterizing a pre-defined class of chemical or biochemical substances which appear in food raw materials or products. It is usually composed of a spectrophotometric transducer and a digital processor. The latter is transforming raw measurement data, provided by the transducer, into the final result of measurement, *i.e.* estimates of concentrations of selected compounds of the analysed sample. Thus, the R&D works related to SAFs require tools for development of algorithms of measurement data processing and tools for acquisition of measurement data to be processed by those algorithms. In this paper, a low-cost and flexible experimental set-up for the latter purpose is proposed, and practical examples of its application are given.

Keywords: Spectrophotometry, food analysers, measurement data acquisition

1. INTRODUCTION

A variety of measurement methods and techniques, which are currently applied in food science and technology, is encompassing both simple tools and procedures for measuring mass or temperature and very sophisticated ones, such as computer-based measuring systems of chromatography combined with mass spectrometry. Somewhere in the middle of this variety, spectrophotometric analysers of food may be spotted – measurement instruments getting recently more and more important for industrial applications. This trend is a response to a growing demand for adequate methods of food analysis, driven by several factors – first of all to:

- a growing demand for this kind of tools, the demand implied by the advancement of standards related to environment protection, health care, individual and collective security, as well as by the widespread use of optical means for inspection of industrial procedures;
- a growing need for technical support of legal efforts aimed at protecting consumers and ensuring fair trade by counteracting infringements related to pesticide residues, veterinary drug residues, endocrine disruptors, processing contaminants, packaging materials, natural toxins, *etc.*

The term "spectrophotometric analyser of food" (SAF) is used here for various spectrophotometric devices, instruments, probes and testers dedicated to measuring physical and/or chemical parameters characterizing a pre-defined class of chemical or biochemical substances which appear in food raw materials or products. A SAF is usually composed of a spectrophotometric transducer and a digital processor. The latter is transforming raw measurement data, provided by the transducer, into the final result of measurement, *i.e.* estimates of concentrations of selected compounds of the analysed sample. A general methodology of spectrophotometric data processing, as well as an exhaustive (including more than 200 references) review of numerical methods that may be applied for this purpose, is provided in the authors' papers [1] and [2]. Most recent advancements of the authors' algorithmic works are published in [3], [4] and [5]. Here, a laboratory infrastructure is shown which enables their experiment-based implementation.

2. PROPOSED SOLUTION

A block diagram of the proposed solution is shown in Fig. 1.

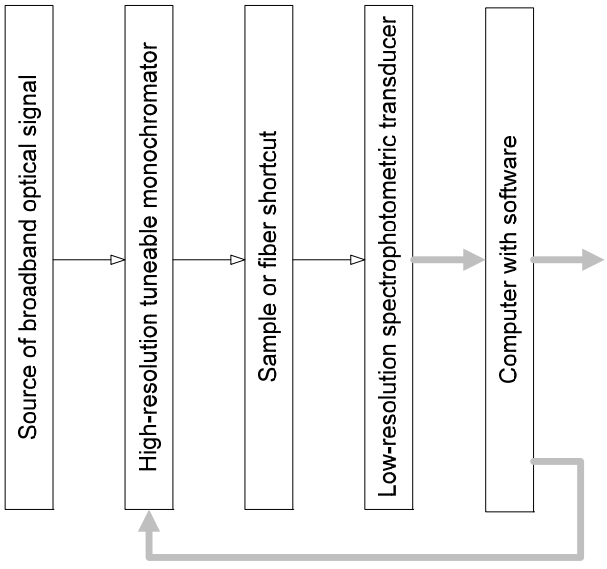

Fig. 1. Proposed experimental set-up.

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In the authors' laboratory, it is implemented in the following way:

- The block "Source of broadband optical signal" is the lamp Horiba-Yvon-Jobin *LSH-T250*, covering the wavelength range $\lambda \in [250\text{ nm}, 2600\text{ nm}]$.
- "High-resolution tuneable monochromator" is the Horiba-Yvon-Jobin monochromator *Triax 320*, covering the range of wavelength $\lambda \in [250\text{ nm}, 3500\text{ nm}]$, and enabling the selection of the optical resolution in the range $\text{FWHM} \in [0.1\text{ nm}, 20\text{ nm}]$, where FWHM stands for *Full Width at Half Maximum*.
- The block "Low-resolution spectrophotometric transducer" is one of the following microspectrometers:
  - an Ocean Optics grating spectrophotometer *USB2000*, covering the wavelength range $\lambda \in [200\text{ nm}, 850\text{ nm}]$ with the optical resolution varying in the range $\text{FWHM} \cong 2\text{ nm}$;
  - an ArcOptics Fourier-Transform spectrophotometer *ARCspectro NIR*, covering the wavelength range $\lambda \in [1000\text{ nm}, 2600\text{ nm}]$ with the optical resolution varying in the range $\text{FWHM} \in [6\text{ nm}, 40\text{ nm}]$.
- The block "Sampler or fibre shortcut" is:
  - an optical shortcut during characterisation of spectrophotometric transducers;
  - a sampler during acquisition of calibration and validation data necessary for development of spectrophotometric analysers.
- The block "Computer with software" is a PC with two programs in *LabView* and *LabWindows/CVI* currently installed, *viz.*:
  - the program for systematic characterisation of spectrophotometric transducers;
  - the program for acquisition of calibration and validation data necessary for development of spectrophotometric analysers.

The main advantage of the proposed solution is its simplicity, universality and flexibility: it enables one to complete – at a relatively low cost – all the experimental tasks which are related to the development of spectrophotometric analysers:

- measurement characterisation of spectrophotometric transducers that may be applied in those analysers, necessary for their mathematical modelling;
- acquisition of spectrophotometric data of predefined resolution, necessary for the development of algorithms for estimation of concentrations.

3. APPLICATION EXAMPLE: CALIBRATION OF A SPECTROPHOTOMETER

A spectrophotometer working in the intensity-mode is converting an optical signal into a digital signal – the data $\tilde{\mathbf{y}} = [\tilde{y}_1 \ldots \tilde{y}_N]^T$ representative of the intensity spectrum $x(\lambda)$ of the input optical signal. Numerical processing of those data by computing means comprises all the operations necessary for transforming "meaningless" digital codes into "meaningful" representation of the spectrum $x(\lambda)$, *i.e.* its approximate values corresponding to selected discrete values $\{\lambda_n\}$ of wavelength λ such that:

$$\lambda_{\min} = \lambda_1 < \lambda_2 < \ldots < \lambda_{N-1} < \lambda_N = \lambda_{\max} \tag{1}$$

where N is the number of data provided at the digital output.

The intensity data, provided by a spectrophotometer, may be modelled using a white-box approach, a black-box approach, or a grey-box approach combining some advantages of white-box and black-box approaches [7]. 80 years of experience behind modelling of spectrophotometric data seems to support the conclusion that the approximation power of a superposition of a linear integral operator with a nonlinear algebraic operator (the so-called *Wiener operator*) is sufficient for adequate modelling of the relationship between the intensity spectrum $x(\lambda)$ and the corresponding vector of raw data $\tilde{\mathbf{y}}$. Let the variable $\hat{y}_n$ denote the mathematical model of the "noise-free" version of the data point $\tilde{y}_n$. Then this operator may be given the form:

$$\hat{y}_n = F\left(\int_{-\infty}^{+\infty} g_n(\lambda_n - \lambda)\, x(\lambda)\, d\lambda; \boldsymbol{\alpha}_n \right) \tag{2}$$

where:

- $g_n(\lambda)$ is the response of the spectrophotometer, recorded when it is excited with a monochromatic optical signal, *i.e.* a signal whose spectrum is close to $x(\lambda) \cong \delta(\lambda - \lambda_n)$ for $n = 1, \ldots, N$;
- $F(\bullet; \boldsymbol{\alpha}_n)$ is an *a priori* known function (*e.g.* an algebraic polynomial or a cubic spline) whose parameters are organised in a vector $\boldsymbol{\alpha}_n$.

Both $g_n(\lambda)$ functions and $\boldsymbol{\alpha}_n$ vectors have to be determined during calibration of the spectrophotometer.

In Fig. 2 and Fig. 3, five exemplary functions $g_n(\lambda)$ – obtained by means of the experimental set-up, described in Section 2, for the spectrophotometer *ARCspectro NIR* – are shown. They are normalized in two ways:

- $\int g_n(\lambda)\, d\lambda = 1$ in Fig. 2, which is convenient for mathematical processing of the model (2);

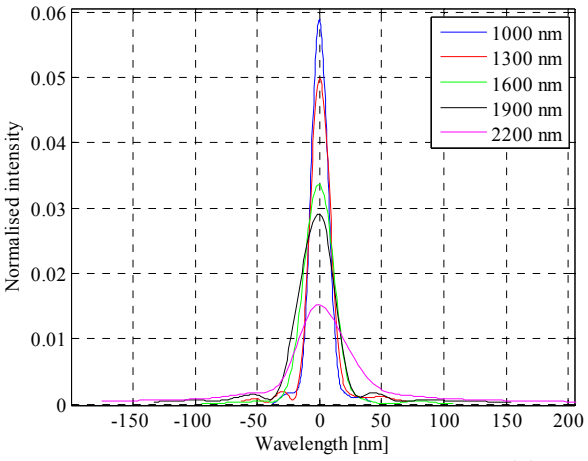

Fig. 2. The integral-normalised functions $g_n(\lambda)$.

– $\max\{g_n(\lambda)\}=1$ in Fig. 3, which is convenient for estimation of the optical resolution indicator FWHM.

The wavelength-dependence of the optical resolution indicator FWHM of the spectrophotometer *ARCspectro NIR* is shown in Fig. 4.

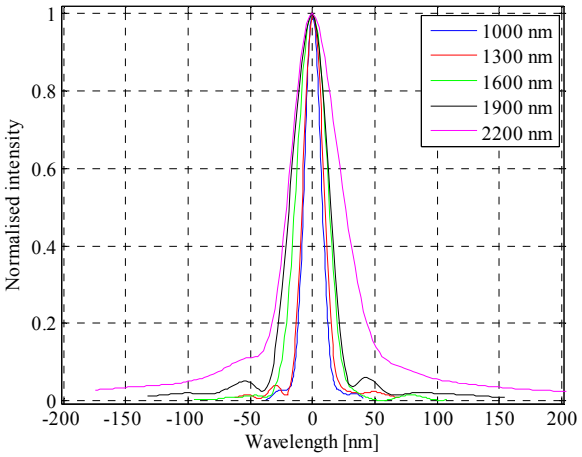

Fig. 3. The maximum-normalised functions $g_n(\lambda)$.

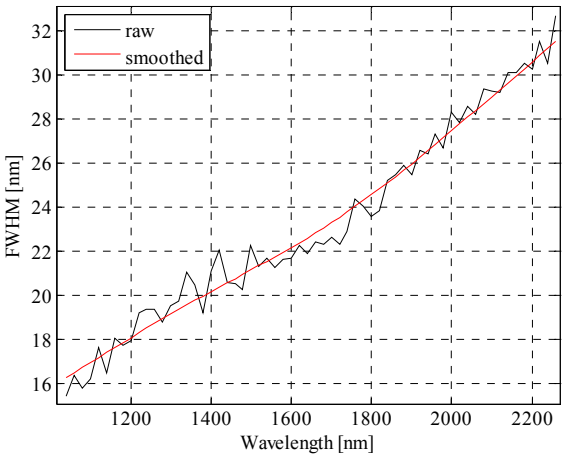

Fig. 4. The wavelength-dependence of the optical resolution indicator FWHM of the spectrophotometer *ARCspectro NIR*.

A sequence of exemplary vectors $\boldsymbol{\alpha}_n \equiv [\alpha_{0,n}\ \alpha_{1,n}]^T$ has been obtained by means of the experimental set-up, described in Section 2, for the spectrophotometer *ARCspectro NIR*, on the basis of its response to the zero signal ($\tilde{\mathbf{y}}_0$) and its response to the maximum signal ($\tilde{\mathbf{y}}_1$) from the optical source:

– The responses $\tilde{\mathbf{y}}_0$ and $\tilde{\mathbf{y}}_1$ have been determined on the basis of the average from $R = 10\,000$ interferograms, and normalised to the maximum component of the vector $\tilde{\mathbf{y}}_1$ (see the result in Fig. 5).
– The source spectrum $x(\lambda)$ has been normalised to its maximum (see the result in Fig. 5).
– The functions $g_n(\lambda)$ have been normalised in such a way that $\int g_n(\lambda)\,d\lambda = 1$.
– The functions $F(\bullet;\boldsymbol{\alpha}_n)$ have been assumed to have the form: $F(y;\boldsymbol{\alpha}_n) \equiv \alpha_{0,n} + \alpha_{1,n} y$.
– The sequence of vectors $\boldsymbol{\alpha}_n$ have been determined by smoothing the sequences of the solutions of the following equations:

$$\alpha_{0,n} + \alpha_{1,n}\tilde{y}_{0,n} \cong 0 \quad \text{and} \quad \alpha_{0,n} + \alpha_{1,n}\tilde{y}_{1,n} \cong x(\lambda_n) \qquad (3)$$

All the resulting values of $\alpha_{0,n}$ are close to $1.83\cdot10^{-4}$, the values of $\alpha_{1,n}$ are shown in Fig. 6.

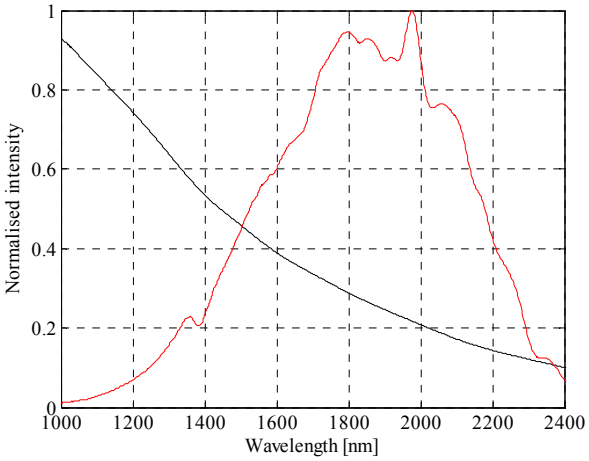

Fig. 5. The source spectrum $x(\lambda)$ (black line) and the response $\tilde{\mathbf{y}}_1$ (red line), both normalised to their maxima.

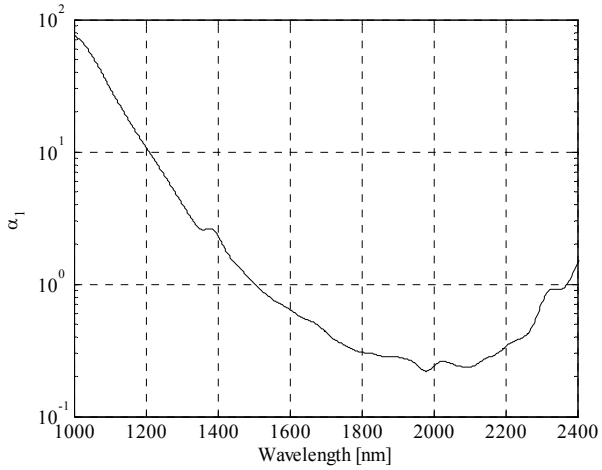

Fig. 6. The dependence of $\alpha_{1,n}$ on wavelength.

4. APPLICATION EXAMPLE: ACQUISITION OF DATA AT A PREDEFINED OPTICAL RESOLUTION

In order to make possible the acquisition of spectral data at a predefined optical resolution, a special procedure for control of optical resolution has been designed. It is based on a mathematical model of the interdependence of four quantities: groove density, wavelength, slit width and resolution. That model, provided by the manufacturer of the monochromator in an implicit numerical form, has been approximated by means of polynomials of order 2–7 in order to guarantee the stabilization of FWHM (in the domain of wavelength or wavenumber) at the level of 10 %.

Such performance of the designed procedure has been confirmed in the range of wavelength 1000–1700 nm by means of the spectral analyser Advantest *Q8384* set to the resolution 10 pm. The deviation of the actual optical resolution indicator FWHM from its predefined value, along the wavelength axis, is shown in Fig. 7.

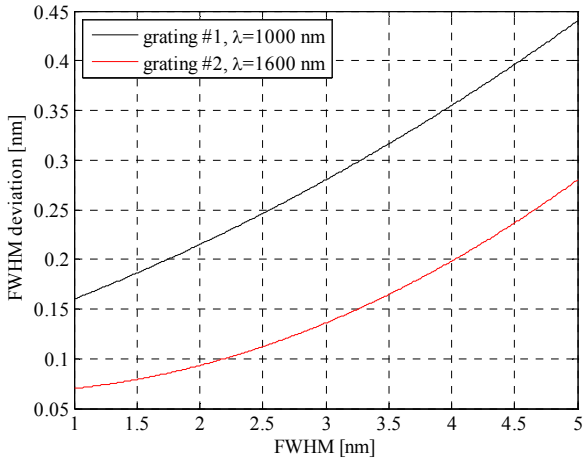

Fig. 7. The deviation of the actual resolution from its predefined value.

For a selected wavelength value, λ_n, the procedure for control of optical resolution enables the automatic choice of a grating and of a slit width guaranteeing the required resolution. The response of the "Low-resolution spectrophotometric transducer" in the intensity domain:

$$\ldots, \tilde{y}_{n-2}, \tilde{y}_{n-1}, \tilde{y}_n, \tilde{y}_{n+1}, \tilde{y}_{n+2}, \ldots \tag{4}$$

is recorded, and used for computation of the resolution-corrected measure of intensity according to the following formula:

$$I(\lambda_n) = \sqrt{\ldots, \tilde{y}_{n-2}^2 + \tilde{y}_{n-1}^2 + \tilde{y}_n^2 + \tilde{y}_{n+1}^2 + \tilde{y}_{n+2}^2, \ldots} \tag{5}$$

The applicability of the developed tool for acquisition of spectral data at a predefined optical resolution has been demonstrated using a sample of olive oil. The absorbance spectrum has been determined according to the formula:

$$A(\lambda_n) = -\log_{10}\left(\frac{I_1(\lambda_n)}{I_0(\lambda_n)}\right) \tag{6}$$

where $I_1(\lambda_n)$ is the intensity measure calculated after Eq.(5) using the data acquired for a sampler filled in with olive oil, and $I_0(\lambda_n)$ is the intensity measure calculated after Eq.(5) using the data acquired for the empty sampler. The result, obtained by means of the system under study (set to the resolution 2 nm), is shown in Fig. 8. It is compared there with the corresponding result obtained by means of the spectrophotometer *ARCspectro NIR* and by means of the reference spectrophotometer Perkin Elmer *FT-IR System 2000* set to the resolution 0.4 nm.

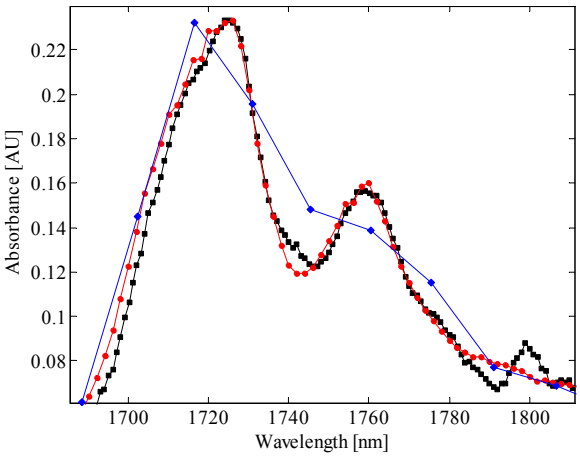

Fig. 8. The data, representative of the absorbance spectrum of olive oil, obtained by means of the system under study (black squares), the spectrophotometer *ARCspectro NIR* (blue diamonds) and spectrophotometer Perkin Elmer *FT-IR System 2000* (red circles).

4. CONLUSION

The cost-efficient experimental set-up, proposed in this paper, enables one to effectively solve numerous problems related to the design and development of spectrophotometric analysers; in particular:

- to complete calibration of low-resolution spectrophotometers;
- to acquire spectrophotometric data with a predefined optical resolution, the data necessary for the development of algorithms for estimation of analyte concentration or other spectrum-related parameters of analysed substances.

The flexibility of the developed experimental set-up consists not only in its applicability to all standard tasks that may appear during the design of a spectrophotometric analyser, but also in the programmability of metrological parameters. In particular, its user may select or predefine:

- the required resolution in the range $\mathrm{FWHM} \in [1\ \mathrm{nm}, 5\ \mathrm{nm}]$;
- the number of interferogrames to be averaged (and thus the level of noise in the data);
- the wavelength values of interest.

The most important limitation of the developed experimental set-up is related to the insufficient power of the source of optical signal and/or insufficient sensitivity of the applied spectrophotometer. As a consequence the signal-to-noise ratio is diminishing very quickly with the increase of the required resolution. To keep it at a satisfactory level, one has to average very many interferograms – thus, to accept a significantly increased measurement time (up to several minutes per point of spectral data).

Disclaimer: the commercial names of the instruments and equipment, used in the authors' experimental set-up, are provided for illustration purpose only and cannot be considered as an authors' recommendation for re-implementation of the described solution.

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