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Near IR spectroscopy of the solutions of a bacteriochlorin derivative as a quantitative method for the quality assurance of liquid products

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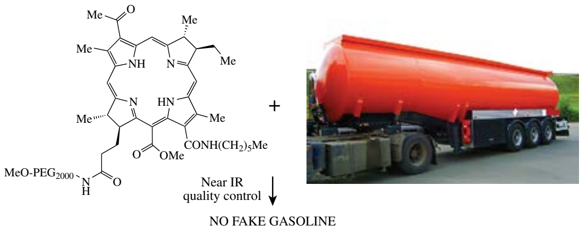
The measurement of the near IR spectra of strongly diluted and concentrated solutions of bacteriochlorin derivative in liquid products (hydrocarbons, industrial alcohols) indicated the absence of aggregation to show the prospects of its use under the inconstant optical path conditions through containers as an efficient method for checking the authenticity or degree of dilution with enhanced possibilities for simultaneous analyses of traditional quality parameters.

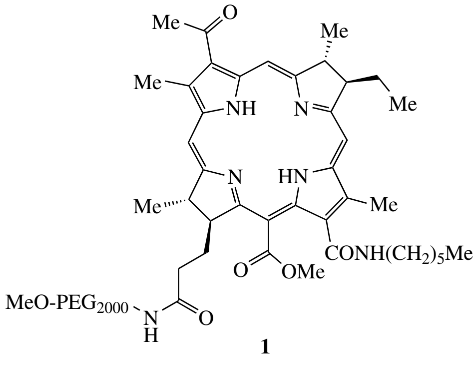


Near IR (NIR) spectroscopy is widely used for the quality control of different products. In this connection, different fiber optical probes are applied to monitor processes in reactors or industrial product streams. Statistical methods of multifactor analysis calibrated on reference data of a large variety of standard techniques make it possible to predict a large number of product quality parameters in real time. Portable NIR spectrometers provide possibilities for not only quality control but also product identification even without opening the package including the cases when comparable amounts of active and excipient components are mixed.[1] However, the low sensitivity of NIR spectra at small concentrations limits the identification potential of this method, particularly, in the differentiation of synthetic *vs.* biotechnological ethanol, small amount of API containing drugs *vs.* placebo, and in other cases comparatively to potential of *e.g.* electronic absorption spectra.

The insertion of various dyes, resonance labels, optical sensors and other taggants as indifferent and stable internal markers is an efficient approach to control the stability and authenticity of products by optical spectral methods. The application of NIR spectroscopy to a combination of corresponding taggants can be regarded as the most advantageous approach due to the possibility of the simultaneous use of three types of spectral characteristics: Raman, fluorescence, and IR overtones.[2]

In the NIR region, Fourier transform Raman spectroscopy was shown reliable for studying polymeric systems free of background fluorescence of impurities (or with resonance RS of NIR dyes),[3] while fiber-optic Raman, NIR absorption or diffuse reflectance spectroscopy[4–6] became one of the most rapidly developing methods for the quality control of industrial products and processes. Although modern FT-NIR spectrometers cover a broad range from 15000 to 3500 cm^{-1}, the sample path is frequently optimized for a more intense and informative long-wavelength part of the spectra, whereas the weakly attenuated short-wavelength part of the NIR range, which is practically blank, is not often used. The short-wavelength edge of the NIR range can be applied more widely when chromophores of the short-wavelength range being used. An approach for property prediction improvement under variations and mixing sources of hydrocarbons was demonstrated[7] with the help of coding signals of several NIR markers. In addition, unlike traditional UV labels, the NIR chromophores are often more photostable. The above stimulates an intensive search for active NIR taggants.

Here, we describe the applicability of bacteriochlorin derivative **1**[8] as a quantitative NIR marker implementing few functions in the same industrial NIR analyzer. Compound **1** possesses unique spectral characteristics due to the presence at the edge of the NIR region of sharp intense peak of electronic Q-transition, which is not overlapping with analyzed vibrational overtones of typical organic compounds. The goals of our study included: (i) identification and quantitative control of the integrity (the absence of dilution) of liquid products like hydrocarbon and/or



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alcohol mixtures media of different polarity on the basis of absolute electronic absorbance; (ii) evaluation of the use of compound **1** for the rapid quality control of liquid products under the conditions of inconstant optical path (such as transmittance through cylindrical and other forms of glass containers without their opening) using non-absolute vibrational band intensities of product and electronic bacteriochlorin band ratio as a criterion for the detection of suspicious articles.

Among the whole variety of highly sensitive labels, the bacteriochlorin derivatives are distinguished by the very intense and narrow absorption band in the NIR range (about 750 nm) and have some region, which overlaps the latter with a luminescence band (at 809 nm).[9] This raises the possibility of utilizing fluorescence resonance energy transfer (FRET) between given chromophores and applicability for control of enzyme aggregation. One of the polarity-sensitive donor–acceptor pair systems showing aggregation-induced emission was described previously.[10] Measurements of fluorescence label bands in NIR might be performed using an FT-NIR spectrometer with a fluorimeter (Raman) module or with a fiber-optical CCD spectrograph under 90° illumination-detection scheme.

Starting from bacteriopheophorbide available from natural sources, we obtained modified bacteriochlorin **1**.[8] The presence of a poly(ethylene glycol) residue in the molecule of **1** (molecular weight, ~2000) makes it possible to prepare true solutions of this derivative in various solvents with a wide polarity range. This fact along with such properties as neutrality, stability, nontoxicity, environmental safety, high detectability is a necessary requirement for the applicability of this molecular system[11] as a NIR marker (note that a neutral medium is required to be kept during any spectroscopic measurement).

We studied the NIR transmission spectra of compound **1** with a nonuniform or varying optical path length with the variation of the concentration. More than a hundred of hydrocarbon fuels and ethanol- and butanol-containing samples as well as their mixtures were measured in standard and cylindrical cuvettes, which were made in a special arrangement with optically condensed NIR beam. The performance of this analysis and quality of calibrations were checked simultaneously with the analysis of the spectral features of compound **1** because the absorption bands of the chromophore could be broadened and shifted due to the possible aggregation of the molecules on going from dilute to concentrated solutions.

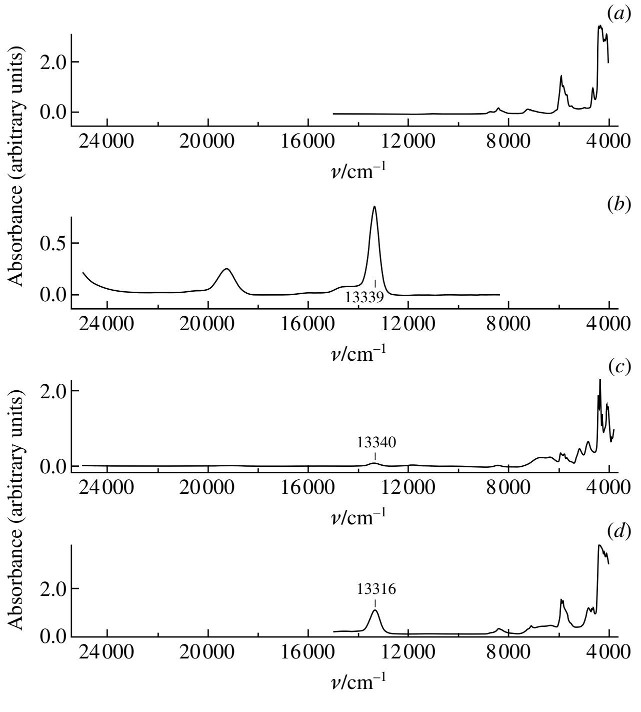

Figure 1 Absorption spectra in visible and NIR regions: (*a*) AI95 gasoline (optical path, 2 mm, compound **1** concentration $c = 0$), (*b*) compound **1** in ethanol ($c = 30$ μg cm^{-3}; optical path, 1 cm), (*c*) FT-NIR merged with visible absorption spectrum of compound **1** in ethanol ($c = 30$ μg cm^{-3}) and (*d*) butanol–AI95 gasoline mixture ($c = 400$ μg cm^{-3}, tube with an internal diameter of 3 mm).

Table 1 Positions of the absorption maxima and the extinction values of compound **1** Q-band in various solvents.

Sample	Absorption maximum/cm^{-1} (nm)	Extinction/ ml mg^{-1} cm^{-1}
Solution of **1** in *n*-butanol	13 334 (749.9)	34.54
Solution of **1** in *n*-butanol–AI95 mixture (1:1)	13 324 (750.5)	8.24
Solution of **1** in AI95	13 324 (750.5)	2.80

The comparison of the NIR spectra of used organic liquids and solutions of compound **1** presented in Figure 1[†] reveals that the regions of intense absorption of the chromophore near 750 nm (about 13 330 cm^{-1}) and the solvents show almost no overlap.

Figure 1 clearly demonstrates that the absorption region of the medium above 12 000 cm^{-1} can be used for the development of chemometric models and quantitative analysis of objects, for instance, their traditional quality parameters without interference from inserted compound **1**. In contrast, the high-frequency region of the NIR spectrum, which usually is not used, makes it possible to quantitatively analyze the bands of compound **1** even at very low concentrations. As can be seen in Table 1, in the polar media few times larger extinction facilitates the detection of compound **1** absorption band. Thus, its addition to synthetic ethanol makes easier the NIR differentiation of original and synthetic ones in alcohol beverages and other products. The parameters remained unchanged upon a prolonged (more than a month) storage of a solution of compound **1** in the light, indicating its stability. The NIR spectra of compound **1** were studied in various solvents in a wide concentration interval in three repeated entries at turns of tubes filled with the solution to simulate polar properties from alcohols to nonpolar hydrocarbon products. The spectra of **1** in solutions of an equivolume mixture of *n*-butanol and AI95 gasoline with concentrations from 400 to 53 μg cm^{-3} (detected in a 3-mm tube) and from 53 to 1.26 μg cm^{-3} (recorded in a 1-cm cell) are shown in Figure 2.[†] The shift of the baseline to higher

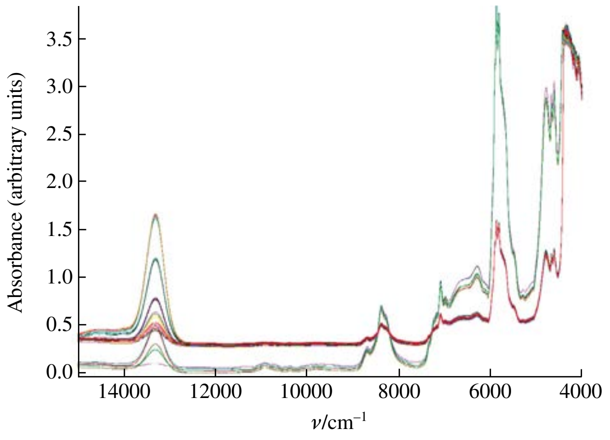

Figure 2 FT-NIR absorption spectra of compound **1** in a butanol–AI95 gasoline mixture at concentrations from 400 down to 1.26 μg cm^{-3}. The cylindrical tubes with a 3-mm internal diameter were used to record spectra of solutions of 400, 268, 178, 119, 79 and 53 μg cm^{-3} (high background level) while more diluted solutions (52.7, 35.1, 23.4, 6.28 and 1.26 μg cm^{-3}) were studied in a 1-cm quartz cell.

[†] For details, see Online Supplementary Materials.

absorbance of the group of the spectra recorded in tubes is evidently due to a greater fraction of the light reflected from the cylindrical tubes, whereas the lower amplitude of the signals is a result of a smaller effective optical layer.

Note that at 1-cm thickness the third order overtones were observed in the range 11 800–9 080 cm^{-1}, the most intense amplitude of which at 10949 cm^{-1} (0.049) was comparable with the intensity of compound **1** band at 13 332 cm^{-1} (0.042) at a concentration of about 6.28 μg cm^{-3}. Optimal sample thickness in this region would be about 10 cm, which is comparable with a large number of liquid products containers, and well detectable compound **1** concentrations could be about 0.1 μg cm^{-3} (~100 ppb). A number of CCD based analyzers of 700–1100 nm (~14000–9090 cm^{-1}) spectral region are used *via* fiber optic networks to quality control of light hydrocarbon streams at refineries. Such portable CCD spectrographs are well suited as single instruments for the above purposes also in cases of inconstant optical pathways. Typically, NIR spectra are calibrated at constant thicknesses to predict different quality parameters. However, some chemometric models could be used independently of thickness to predict oxygenates, *e.g.* butanol content in gasolines. Simple oxygenate predicting model with a number of AI95 samples mixed with butanol at different concentrations measured in cuvettes and tubes was based on the variations of intensities in a region of 7000–6000 cm^{-1} (domination of 2nd OH stretch overtones) relatively to 8800–7800 cm^{-1} (domination of 2nd CH combinations). When AI92 gasoline was used for testing a sample mixed with butanol, the model showed outlier. At the same time, usage of 13 332 cm^{-1} as an internal standard showed good predictions of butanol content independently of thickness and gasoline type.

The construction of the calibration plots as predicted compound **1** concentration *vs.* true one on the basis of absolute and relative Q-band absorbance intensities is shown in Figure S1 (see Online Supplementary Materials). The analyses of absolute absorption intensities in the whole concentration range indicated that, when the optical layer thickness was specified to be equal to the internal diameter of the tube, the experimental points did not fall near one straight line and decomposed to two lines [Figure S1(*a*)]. In this case, when the optical length is specified to be equal to the tube diameter, the points for one solution (53 μg cm^{-3}) do not coincide with those for another solution showing that the effective optical path of focused on axes beam inside cylinder differs from the diameter value. The correlation coefficient for this model is $R = 0.966$ and the σ parameter in the error distribution is 0.037. After correction of absolute intensities of Q-bands measured in tubes for the ratio in 1-cm standard cuvette at the same concentration the quality of the calibration model is improved. However, this improvement is insufficient and outliers appeared because of some variations of pathway in different installations [Figure S1(*b*)] ($R = 0.985$ and $\sigma = 0.021$). However, if the Q-band absorption intensities were measured relatively to the absorption of liquid product in an interval of 9000–7600 cm^{-1} as an internal standard for the development of the model, the experimental points show no appreciable outliers at extreme concentrations and in the region of ‘cross-linking of experiments’ [Figure S1(*c*)]. The coefficients $R = 0.997$ and $\sigma = 0.010$ indicate the high quality of the calibration model, as well as high quality was found for reverse case model of variations of butanol concentrations in gasoline with Q-band as internal standard. This demonstrates the feasibility and reliability of internal standard enhanced predictive models for direct quality control through containers under variations of hydrocarbon batches and sources.

Note that the optimization of the calibration equation was specially chosen in the framework of the linear model of intensity *vs.* concentration functions in all cases, although the program allows one to use a third degree polynomial for optimization, which evidently would achieve the higher quality of the model. An analysis of the differential absorption spectra of compound **1** for the lowest and highest concentration points reveals no noticeable change in the band half-width and shift, indicating no aggregation of compound **1** in the medium.

The natural origin and metal-free environmentally safe structure of compound **1**, as well as the simplicity of its chemical preparation, makes this substance acceptable for practical use according to all official technological regulations on liquid fuel additives. Moreover, this product does not influence the color or any properties of fuels at working concentrations.

The experimental results show that compound **1** forms true solutions over a wide range of the concentrations and polarities of media corresponding to the liquid fuels, and the invariance of the spectral characteristics and the absence of overlapping of the bands with the absorption bands of the medium are also shown.

Thus, we found that the measurement of the NIR spectra of highly diluted solutions of bacteriochlorin derivative **1** can be used as an efficient method to control the authenticity, integrity or degree of dilution of liquid products without hindrances for the simultaneous analysis of the traditional quality parameters. At the same time, compound **1** as an internal standard can help one to make reliable chemometric models for quantitative predictions of quality parameters under the inconstant optical path conditions and batch or source variations.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2016.05.014.

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