The Influence of Fiber-Probe Accessories Application on the Results of Near-Infrared (NIR) Measurements

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When several near-infrared instruments are used in a network and a common chemometric model is applied to spectral processing, comparison of the instruments is indispensable. Direct transferability often claimed by the producers should be treated with caution. It has been found experimentally that when measurements are performed with the help of a fiber optic probe, the main source of spectral discrepancy is related to probe sensitivity in contactless measurements. Here the influence of the probe-to-object distance on the acquired spectra is analyzed in detail. Special experimental setups are proposed to isolate various strongly influencing factors and to maintain stable measurement conditions. The application of an artificial standard instead of real-world objects helps to focus on the instrument/accessory characteristics.

Index Headings: **Diffuse-reflectance near-infrared measurements; Fiber probe; Probe-to-object distance; Instrument compatibility; Anti-counterfeiting.**

INTRODUCTION

Carrying out measurements by means of fiber optic probes (FPs) substantially speeds up routine near-infrared (NIR) spectrum acquisition in warehouses, in the field, and for various process analytical technology applications.[1–5] Such measurements are non-destructive and can be conducted through closed polymeric bags, glass and plastic ampoules, polyvinyl chloride (PVC) blisters, etc., without opening the packages. At the same time, the FP measurements possess specific features and bring additional distortion in the acquired spectra. The goal of the present study is to reveal the distinct features of such measurements. By analyzing the instrument-sample interaction, we tried to isolate the influence of various factors and to maintain stable measurement conditions. The application of an artificial standard instead of real-world objects helps to focus on instrument/accessory characteristics. At the same time we tried to bring experimental setups closer to real-world routine measurements.

The study is conducted in the framework of a state project aimed at medicine quality monitoring and anti-counterfeiting. A part of this project involves establishing and managing a special NIR network. The project poses the following conditions on spectra acquisition: (1) Intact tablets and capsules should be measured through PVC blisters. This is done in order to be able to return regular drugs to the drugstore. (2) Spectra should be acquired with the help of an FP, which is a common accessory for all laboratories in the network.

During routine measurements it is often difficult to maintain full contact between a tablet packed in a PVC blister and an FP tip, especially during field measurements in mobile laboratories. A routine field technique of tablet spectra acquisition consists of two steps. First, a background spectrum is measured using the internal Spectralon (Labsphere, Inc.) reference. Second, a tablet spectrum is acquired through the PVC blister using a handheld FP. The latter spectrum is recorded against the background spectrum obtained during the first step. A common inexactness in both steps, is a gap between the FP tip and the reference (in step 1) or the tablet surface (in step 2). In our opinion this is the main reason for spectral distortion or deterioration. There are few publications devoted to the study of probe-to-object distance influence on acquired spectra for the NIR defuse-reflectance measurements of solid samples. Most of such studies are devoted to in vivo measurements in biology and clinical practice.[6–8]

Another source of spectral distortion is dissimilarity in the instruments used and their accessories, that is, in FPs. The latter topic (also known as calibration transfer) has been extensively explored in the literature.[9–11] Special attention to the instrument standardization for NIR measurements with FPs is given by Sum and Brown.[12] Sahni et al.[13] concentrate on the problems related to the transfer of calibration models taking into account path length differences using fiber optic transmittance probes in in-line measurements. This problem is also addressed in the present paper with the focus on FP identity. We tried to separate the spectral distortions introduced by an experimental setup from those brought up by instruments' characteristics.

This study continues our investigation of the FP measurement features started in the work of Rodionova et al.[14]

MATERIALS AND METHODS

Materials. The NIST Traceable Extended Range Near-Infrared Wavelength Standard MRC-910-1920x (Middleton Research)[15] can be used for instrument performance testing. This commercial standard is made of four components: three rare earth oxides (Dy_2O_3, Er_2O_3, Ho_2O_3) and talc. The last is used to cover the region from 10 000 to 4000 cm^{-1}. The standard is made by sintering the oxides in spectrally neutral matrix. This has an advantage over SRM 1920 (no longer available from NIST), as the specular reflectance of the sapphire window in MRC-1920 is eliminated. All bands of the NIST standard and additional sharp and stable bands available

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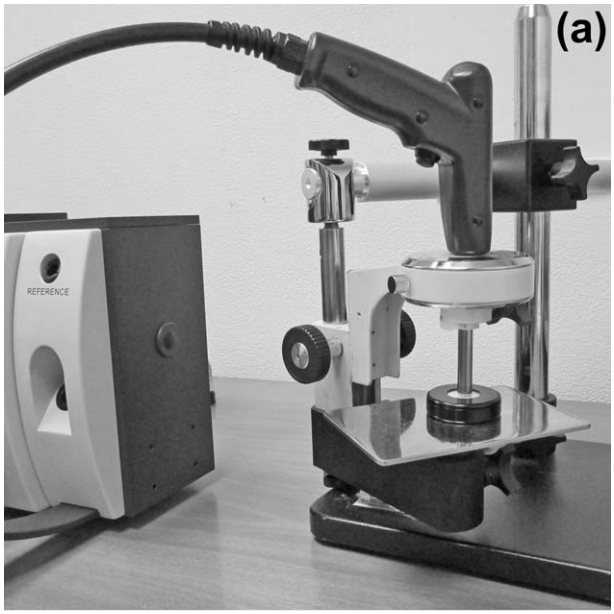

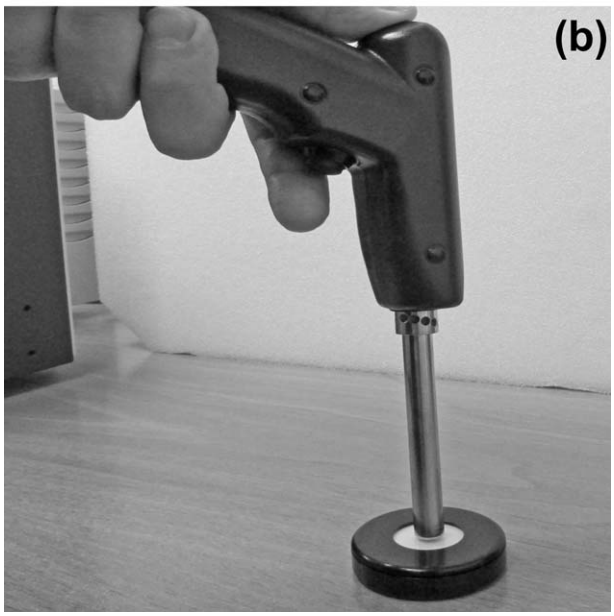

FIG. 1. Experimental setups (**a**) Setup 1. Measurements with in-house FP holder. (**b**) Measurements with handheld FP.

in the 5000–4000 cm^{-1} wavenumber region are presented by the MRC standard. The reasons for employing the commercial standard instead of a real-world sample are as follows:

- It is impossible to choose a universal standard among the drugs. First, there are varieties of pills and capsules containing different active pharmaceutical ingredients and excipients. Second, the drugs age, usually in about 2 or 3 years.
- The MRC standard meets the United States Pharmacopeia wavelength calibration requirements and covers a wide NIR range.
- Application of the standard helps to avoid additional sources of errors such as object heterogeneity and instability.

All background measurements are performed using the external 99% Labsphere standard, Spectralon disk. This is done on purpose, to avoid the influence of instruments' individual internal reference standards, which can be of diverse quality, that is, of different cleanness.

Experimental Setups. The diffuse reflection measurements were carried out using two identical Fourier transform near-infrared (FT-IR) multipurpose analyzer (MPA) spectrometers (Bruker Optics), labeled MPA-1 and MPA-2, equipped with two identical diffuse reflectance probes (labeled FP-1 and FP-2). Both probes have identical fiber optic length of 1.5 m and are equipped with Bruker Quick Connect, which allows changing the probes between the two instruments easily. Additional experiments were conducted when the fiber probes were exchanged between the MPA instruments, that is, MPA-1 with FP-2 and vice versa. The experiments were carried out under two various setups:

- In Setup 1, we used an in-house FP holder that made it possible to control and reproduce the distance between the probe and the object (Fig. 1a).
- In Setup 2, we used a handheld measurement technique with full contact between the probe and the object (Fig. 1b). Each time, five replicated readings were obtained to control the reproducibility. The background spectra were acquired before each measurement.

All measurements were conducted with 64 scans and 2 cm^{-1} resolution. The Unscrambler 10.2 software was used for the multivariate data analysis.[16] Common spectral pre-processing transformations used in the study are presented in detail elsewhere in literature, viz., Savitzky-Golay smoothing (S-G smoothing), standard normal variate (SNV), multiplicative scatter correction (MSC), and Savitzky-Golay first and second derivatives.[17,18]

Notation. For convenience the abbreviation RGap for the gap between the FP tip and the Spectralon reference is used. The external reference Spectralon is used for background measurements. The notation will be used for the gap between the FP tip and the measured object. In our case the measured object is the MRC-1920 standard.

Data Sets. ***Data Set 1.*** All spectra were acquired by means of MPA-1 and FP-1. Ten spectra were collected with gradually increasing OGap. The OGap length (the distance between the FP tip and the standard) was changed from 1 mm to 10 mm with a step of 1 mm. This subset is called ST11 and includes spectra ST11_1, ST11_2, . . ., and ST11_10m. The background was measured with the full contact (RGap = 0) between the FP and the external Spectralon disk.

Another 10 spectra were acquired in a similar way, with OGap kept at zero (full contact with the object) and the RGap (the distance between the FP tip and Spectralon reference) increasing. This subset is called B11 and includes spectra B11_1, B11_2, . . ., and B11_10. An additional 5 spectra were collected as replicas in the Setup 2 experiment, with OGap = 0 and RGap = 0, that is, at full contact between the FP tip, the Spectralon and the object. These spectra comprise subset R11. Overall Data Set 1 consists of 25 spectra divided into subsets ST11, B11, and R11.

Data Set 2. Data Set 2 is comprised of spectra measured with the help of one instrument, MPA-1, and two probes, FP-1 and FP-2. It combines subsets ST11, R11, described above, and two new subsets, ST12 and R12. The spectra in subset ST12 were measured using the same technique as in ST11 collection, i.e., moving the standard from the FP tip, but in this case FP-2 was used. The spectra in ST12 are called ST12_1, ST_12_2, . . . , and ST12_10. Five replicate measurements carried out

TABLE I. Data set description.

Subset description	Subset name	Plot mark	Instrument	Fiber optic probe
Setup 1. Increasing OGap	ST11	◆	MPA 1	FP 1
	ST12	□	MPA 1	FP 2
	ST21	+	MPA 2	FP 1
Setup 1. Increasing RGap	B11	■	MPA 1	FP 1
Setup 2. Replicate measurements	R11	○	MPA-1	FP-1
with OGap = RGap = 0	R12	▲	MPA-1	FP-2
	R21	X	MPA-2	FP-1

with FP-2 and conducted in line with Setup 2 comprise subset R12. Thus, Data Set 2 totals 30 spectra and includes subsets ST11, R11, ST12, and R12. Subsets ST11 and R11 are common for Data Set 1 and Data Set 2.

Data Set 3. For Data Set 3 we collected spectra using only one probe, FP-1, attached to MPA-1 and MPA-2, alternately. Subset ST21 was acquired using MPA-2 and FP-1 with increasing OGap. ST21 consists of 10 spectra labeled ST21_1, ST21_2, . . ., ST21_10. Replicated measurements under Setup 2 experiment compose subset R21. In total, Data Set 3 consists of 32 spectra divided into subsets ST11, R11, ST21, and R21. Subsets ST11 and R11 are common for Data Set 1, Data Set 2, and Data Set 3. The data sets are constructed to investigate the following problems. Data Set 1 is used to study the gap influence, Data Set 2 helps to compare measurements taken by different FPs attached to the same instrument, and Data Set 3 is used for the comparison of different instruments with the same FP.

The summary of various data sets and subsets used in the study is presented in Table I.

The baseline offset is the first effect that comes to the fore among various effects of spectral deformation caused by moving away the standard or reference disk. However, this effect can be easily eliminated by various spectral transformations, such as baseline shift, SNV, etc. The baseline offset is eliminated for all spectra, and it is not a part of the present study.

RESULTS AND DISCUSSION

Data Set 1. This data set is used for the exploration of the influence of the OGap and RGap sizes on spectra discrepancy. One instrument, MPA-1, and one fiber probe, FP-1, are used for all spectra acquisition.

Explorative PCA analysis performed after the S-G smoothing and SNV correction shows a distinct separation into three groups (Fig. 2a). The first principal component (PC1) explains 52% of total variance. PC1 accounts for separation of the spectra measured with and without OGap. The plot (Fig. 2b) of the first loading versus wavenumber shows that it is low-frequency noise, that is, the non-linear baseline deformation that is mostly responsible for subsets' grouping. The highest loading coefficients are in the range 4500–4000 cm^{-1}. The second principal component (PC2) explains 47% of total variance. It reflects the change in size of OGap (set ST11) and RGap (set B11). In Fig. 2a, and in all similar plots below, the labels near the plot markers correspond to the gap size. For example, a diamond marker with label 1 corresponds to OGap of 1 mm, label 2 corresponds to OGap = 2 mm, . . . , and label 10 corresponds to OGap = 10 mm. The same notation is used for RGap represented by the square markers in Fig. 2a. Markers without labels denote replicated measurement without gap, that is, OGap = RGap = 0.

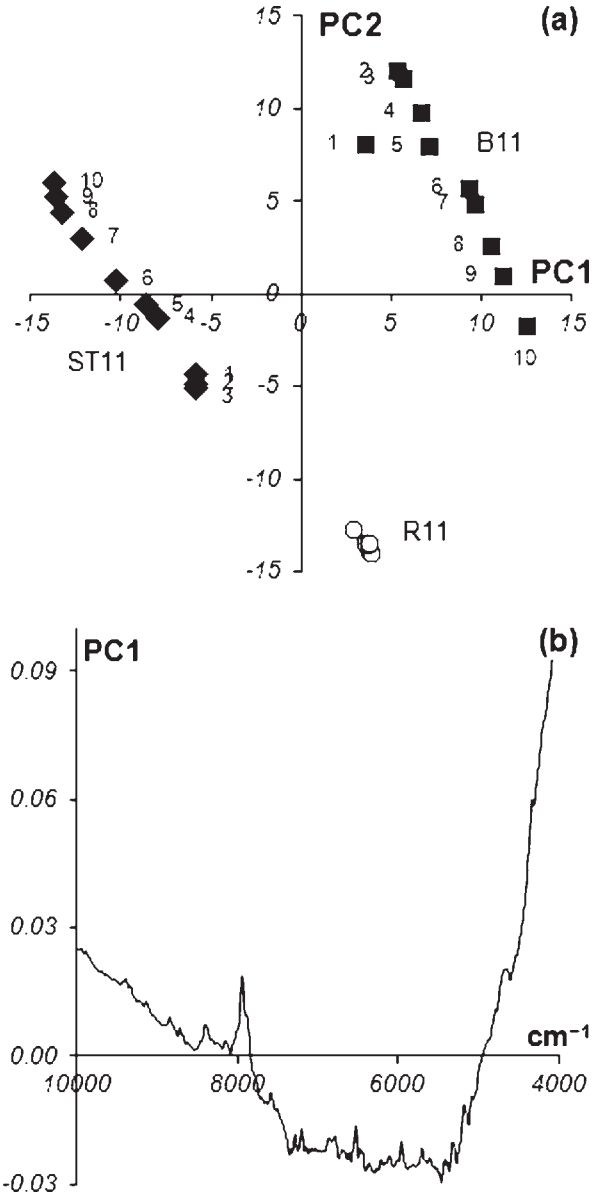

FIG. 2. PCA for Data Set 1: (**a**) PCA scores plot. Subset ST11 with increasing OGap; subset B11 with increasing RGap, subset R11 with replicated, measurements with no gap. The marker labels denote the gap size in millimeters. (**b**) PCA loading PC1 versus wavenumbers.

It is important to recall that the NIR spectra are presented in absorbance units defined as $A = \log(1/R)$, where R is reflectance. In practice, the sample reflectance is measured as a relative value,

$$R = I_s/I_r$$

where I_s stands for the intensity of a sample reflectance and I_r is intensity of the reference reflectance. When OGap is increased, the I_s value decreases. Similarly, increasing RGap, we decrease the I_r value. The most influence is observed in the region of 4500–4000 cm^{-1}, where both intensities I_s and I_r are low. The impact of the increasing OGap and RGap is illustrated in Fig. 3. It can be seen that the greater the RGap (the farther FP is from Spectralon), the steeper is the baseline slope, that is, the

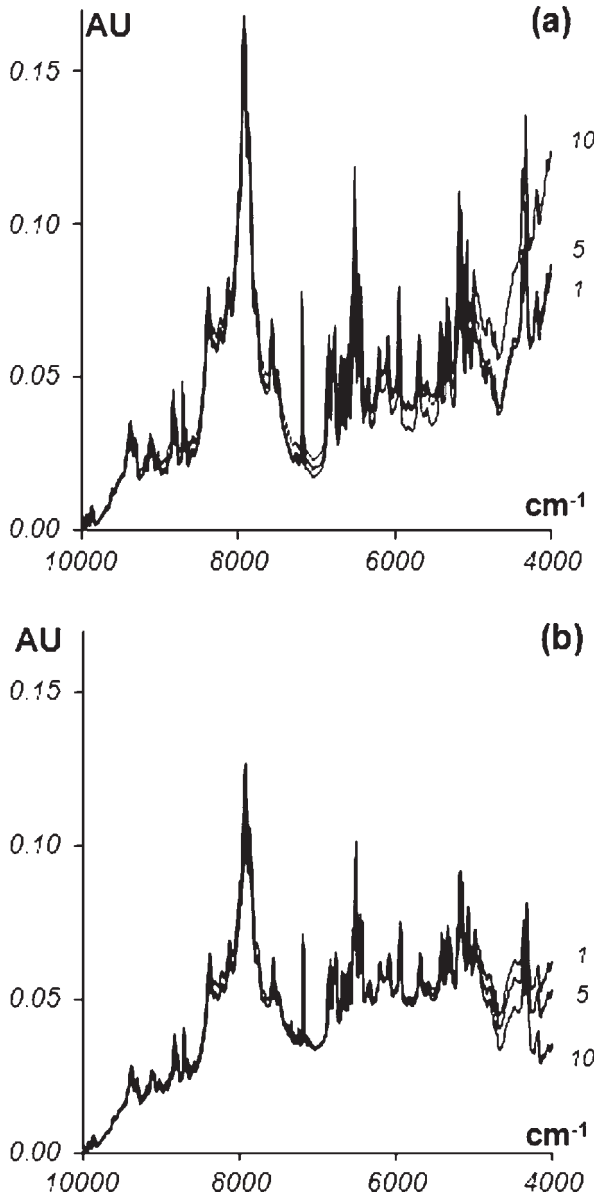

FIG. 3. Spectra after baseline offset correction (**a**) Subset B11. Moving FP away from the reference. (1) RGap = 1 mm, (5) RGap = 5 mm, (10) RGap = 10 mm. (**b**) Subset ST11. Moving FP away from the standard. (1) OGap = 1 mm, (5) OGap = 5 mm, (10) OGap = 10 mm.

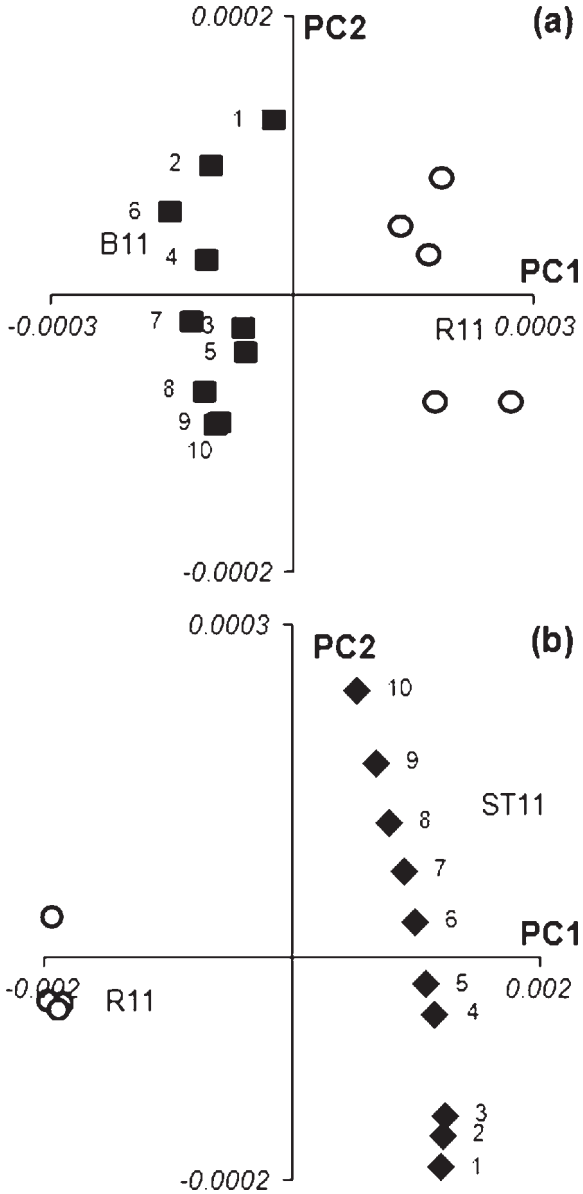

FIG. 4. PCA scores plots. Pre-processing by the S-G second derivative. The marker labels denote the gap size in millimeters. Markers without labels present replicated full contact measurements. (**a**) Moving FP away from the reference. Subset B11 with increasing RGap, subset R11with replicate measurements in the full contact. (**b**) Moving FP away from the standard. Subset ST11 with increasing OGap; subset R11 with replicated measurements in the full contact.

right side of a spectra rises (Fig. 3a). This distortion can be removed by the second derivative transformation, which eliminates the baseline slope.

PCA analysis of the joint subsets B11 and R11 after spectra transformation by the S-G second derivative with a 19-point window and the third order polynomial shows that objects are spread more or less homogeneously in the PC1-PC2 plot (Fig. 4a). Group B11 is very close to group R11, and the B11 spectra scattering is comparable with scattering among the replicated measurements in R11.

A more complex situation is observed in the case of an increasing OGap (the MRC standard is moving away from the FP). Ordinary pre-processing methods do not compensate the spectral discrepancy. The results of PCA analysis on the joint subsets ST11 and R11 performed after the spectra transformation by the S-G second derivate are shown in Fig. 4b. The objects from R11 are separated from ST11 along PC1, which explains 99% of the total variance. The second principal component (1%) is responsible for the OGap size. Other popular spectroscopic pre-processing methods, such as SNV, MSC, or their combinations, such as SNV plus the second derivative, MSC on the objects of subset R11, and subsequent application of the second derivative transformation, give similar results.

The analysis of Data Set 1 shows that both gaps between the object and the reference (OGap and RGap) deform spectral baseline (low-frequency noise). Spectra discrepancy caused by OGap is the most difficult to correct. One can anticipate the following spectra deformations (see Fig. 5a): (1) an increase in noise level (see Table II), which may be corrected by a smoothing transformation, and (2) a decrease in signal intensity, which may be corrected by normalization or SNV

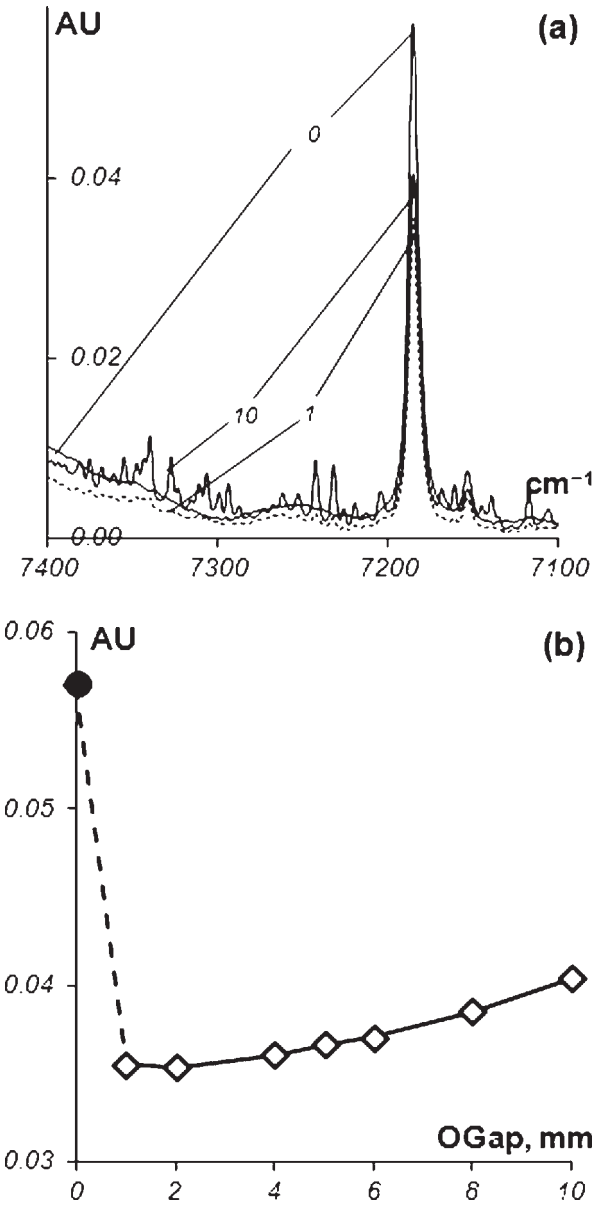

FIG. 5. Subset ST11. (**a**) The range around the certified talc peak (7185.5 cm^{-1}). Spectra of R11 (0, OGap = 0), ST11_1 (1, OGap = 1 mm), ST11_10 (10, OGap = 10 mm) after baseline alignment. (**b**) Talc peak intensity versus OGap.

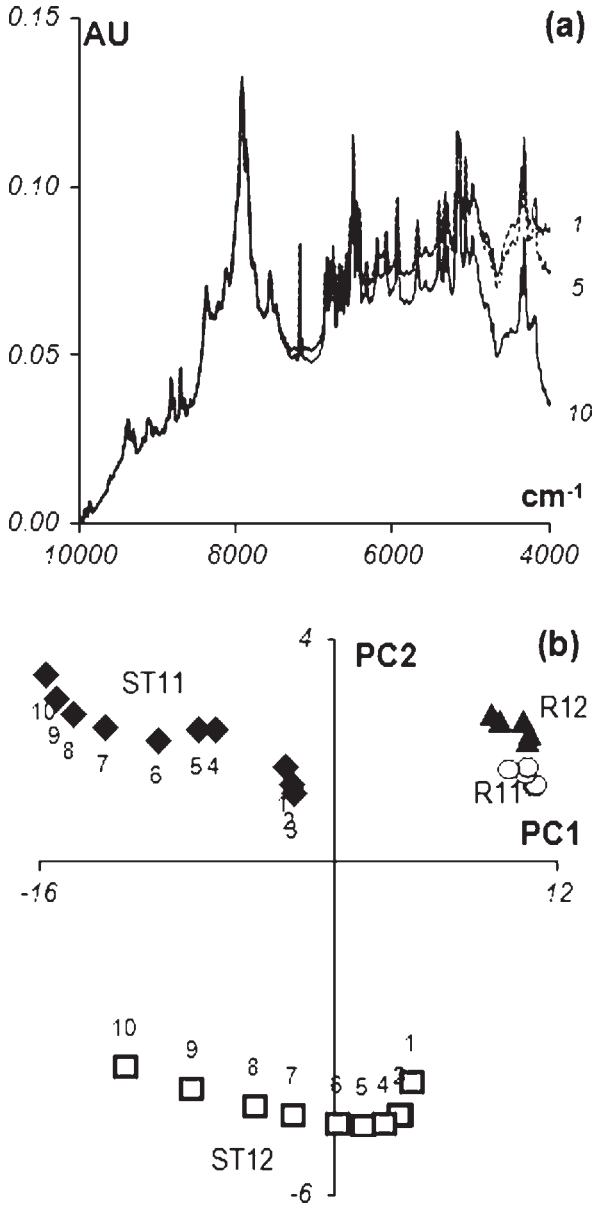

FIG. 6. (**a**) Subset ST12 spectra. Moving FP away from the standard. (1) OGap = 1 mm, (5) OGap = 5 mm, (10) OGap = 10 mm. (**b**) PCA score plot for Data Set 2. Subsets ST11 and ST12 with increasing OGap; subsets R11and R12 with replicated measurements in the full contact. The marker labels denote the gap size in millimeters. Markers without labels present replicated full contact measurements.

filters. Interestingly, a material decrease in signal intensity (about 40%) is observed already when the probe tip is moved just 1 mm away from the sample. At the same time, with the OGap increasing, the illumination area is enlarged and the signal intensity slightly increases. This phenomenon is illustrated in Fig. 5 for the talc peak.

In addition, the nonlinear changes in baseline were observed. This deformation was unexpected and required some special spectra transformations.

TABLE II. Root mean square noise for measurements with various OGap values.

OGap = 0 mm	OGap = 1 mm	OGap = 10 mm
4.09	10.6	26.4

Data Set 2. The data set is used to assess the influence of FPs on measurements by a NIR instrument. In these experiments all spectra were acquired by the same instrument, MPA-1, and two fiber probes, FP-1 and FP-2, claimed to be identical by the producer. When the measurements are conducted without gaps (RGap = OGap = 0), the spectra in subsets R11 and R12 are very similar. Spectra smoothing and the SNV correction remove the differences in the two groups. PCA analysis on the joint subsets R11 and R12 shows that after pre-processing all objects are spread homogenously in the PC1-PC2 plot (not shown here). No object grouping is observed.

When OGap is increased (the objects are moving away from FP), the signals measured by FP1 and FP2 diverge substantially. The most impact is observed in the range of 4500–4000

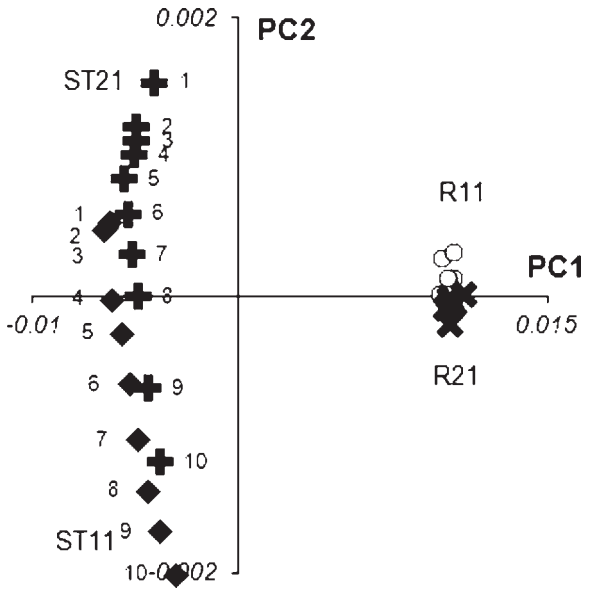

FIG. 7. PCA scores plot for Data Set 3. Subsets ST11 and ST21 with increasing OGap; subsets R11 and R21 with replicate measurements in the full contact. The marker labels denote the gap size in millimeters. Markers without labels preset replicated full contact measurements.

cm^{-1}, where the spectra baseline drops for FP2. Moreover, for spectra ST12_9 and ST12_10, two certified talc peaks at 4018 and 4053 cm^{-1} disappear (Fig. 6a).

PCA analysis for Data Set 2, after smoothing and SNV pre-processing, reveals three groups (Fig. 6b), namely, a collection of compact groups of replicated measurements by FP1 and FP2 (subsets R11, R12), a separate group reflecting measurements with growing OGap for FP1 (subset ST11), and a group of spectra corresponding to growing OGap for FP2 (subset ST12). The first PC explains 88% of the total variance. It accounts for the OGap increase. The second PC explains 11%; it is responsible for the separation of ST12 subset from all other spectra. Other pre-processing methods, such as second derivative, or smoothing and MSC correction, yield similar results.

The case study above demonstrates that a difference in FPs that cannot be revealed by regular measurements without gaps may seriously distort the spectra shape when objects are located at some distance from the FP.

Data Set 3. The data set is used to assess the influence of comparable NIR instruments on diffuse reflectance measurements carried out with the same fiber probe. In order to do so, all spectra acquisitions are conducted using two instruments, MPA-1 and MPA-2, and one fiber probe, FP-1. Replicated full contact (OGap = RGap = 0) measurements are very similar. PCA analysis of the joint subsets R11 and R21, after pre-processing by the S-G first derivative, confirms this. The objects are spread homogeneously in the PC1–PC2 score plot (not shown here).

PCA performed on the whole Data Set 3 shows a clear distinction between the compact group in the right side of the score plot (Fig. 7), which consists of R11 and R21 subsets, and the rest of the spectra that present measurements with an increasing OGap. The first PC explains 97% of the total variance. The second PC2 explains only 1% and is responsible for the OGap size. A systematic dependence on OGap is seen along PC2 from marker 1 (OGap = 1 mm) until marker 10 (OGap = 10 mm). It can be seen that the ST11 and ST21 subsets spectra are merged in 1 group.

The analysis of Data Set 3 shows that ceteris paribus (all other conditions being equal), the difference between the spectra measured by comparable instruments may be easily eliminated by a common spectra pre-processing such as the first derivative transformation.

CONCLUSIONS

1. When NIR measurements are conducted by means of FPs, a substantial impact on spectral quality is caused by the distance between the probe and the object. Such spectra discrepancy is hardly compensated by common spectroscopic pre-processing methods. Spectral distortions include signal attenuation, a low-frequency effect such as nonlinear baseline shift, and high-frequency noise, mostly noticeable in the range where absorption is low. In some cases even a loss of peaks is observed (when object and reference have low intensity).
2. When measurements are carried out in full contact with the object/reference, the differences in spectra measured by various similar instruments and/or FPs are easily neglected by ordinary pre-processing methods such as the first/second derivative transformation, or SNV correction.
3. The performance of just two FT-NIR spectrometers equipped with FPs was analyzed in detail. However, the experiments conducted with six other instruments from the NIR network (four MATRIX-F instruments by Bruker Optics and two MPA instruments) have revealed similar problems. Hence, the detected FPs differences are not attributable to the specific properties of the two tested probes. The problem is of a general nature.
4. As a rule, the producers of modern FT-NIR instruments claim high compatibility of the instruments of the same product line. However, the general instrument consistency does not guarantee accessory compatibility in various experimental setups. Our finding shows that the main reason for experimental discrepancy is the sensitivity of various FPs to contactless measurements. This issue should be taken into account when several FT-NIR spectrometers are consolidated in one network.

1. A.L. Pomerantsev, O.Y. Rodionova. "Process Analytical Technology: A Critical View of the Chemometricians". J. Chemometrics. 2012. 26(6): 299-310.
2. C.B. Zachariassen, J. Larsen, F. van den Berg, S.B. Engelsen. "Use of NIR Spectroscopy and Chemometrics for On-Line Process Monitoring of Ammonia in Low Methoxylated Amidated Pectin Production". Chemometr. Intell. Lab. Syst. 2005. 76(2): 149-161.
3. A.L. Pomerantsev, O.Y. Rodionova, M. Melichar, A.J. Wigmore, A. Bogomolov. "In-Line Prediction of Drug Release Profiles for pH-Sensitive Coated Pellets". Analyst. 2011. 136(22): 4830-4838.
4. M. Andersson, O. Svensson, S. Folestad, M. Josefson, K.-G. Wahlund. "NIR Spectroscopy on Moving Solids Using A Scanning Grating Spectrometer—Impact on Multivariate Process Analysis". Chemometr. Intell. Lab. Syst. 2005. 75(1): 1-11.
5. P. Geladi, B. Sethson, J. Nystrom, T. Lillhonga, T. Lestander, J. Burger. "Chemometrics in Spectroscopy: Part 2. Examples". Spectrochim. Acta, Part B. 2004. 59: 1347-1357.
6. Y. Ti, W.-C. Lin. "Effects of Probe Contact Pressure on in vivo Optical Spectroscopy". Opt. Express. 2008. 16(6): 4250-4262.
7. W. Chen, R. Liu, K. Xu, R.K. Wang. "Influence of Contact State on NIR Diffuse Reflectance Spectroscopy in Vivo". J. Phys. D: Appl. Phys. 2005. 38(15): 2691-2695.

8. H. Arimoto, M. Egawa. “Non-Contact Skin Moisture Measurement Based on Near-Infrared Spectroscopy”. Appl. Spectrosc. 2004. 58(12): 1439-1446.
9. Y. Xiea, P.K. Hopke. “Calibration Transfer as a Data Reconstruction Problem”. Anal. Chim. Acta. 1999. 384(2): 193-205.
10. T. Fearn. “Standardization and Calibration Transfer for Near Infrared Instruments: Review”. J. Near Infrared Spectrosc. 2001. 9(4): 229-244.
11. F. Stout, J.H. Kalivas. “Tikhonov Regularization in Standardized and General Form for Multivariate Calibration with Application towards Removing Unwanted Spectral Artifacts”. J. Chemometrics. 2006. 20(1–2): 22-33.
12. S.T. Sum, S.D. Brown. “Standardization of Fiber-Optic Probes for Near-Infrared Multivariate Calibrations”. Appl. Spectrosc. 1998. 52(6): 869-877.
13. N.S. Sahni, T. Isaksson, T. Næs. “Comparison of Methods for Transfer of Calibration Models in Near-Infrared Spectroscopy: A Case Study Based on Correcting Path Length Differences Using Fiber-Optic Transmittance Probes in In-Line Near-Infrared Spectroscopy”. Appl. Spectrosc. 2005. 59(4): 487-495.
14. O.Y. Rodionova, Y.V. Sokovikov, A.L. Pomerantsev. “Quality Control of Packed Raw Materials in Pharmaceutical Industry”. Anal. Chim. Acta. 2009. 642(1–2): 222-227.
15. Middleton Research. http://www.middletonresearch.com [accessed May 29 2013].
16. The Unscrambler. http://www.camo.com/rt/Products/Unscrambler/unscrambler.html [accessed May 29 2013].
17. T. Naes, T. Isaksson, T. Fearn, T. Davies. Multivariate Calibration and Classification. Chichester, UK: Wiley, 2002.
18. A. Rinnan, F. van den Berg, S.B. Engelsen. “Review of the Most Common Pre-Processing Techniques for Near-Infrared Spectra”. Trends Anal. Chem. 2009. 28(10): 1201-1222.