Near-Infrared-Spectroscopic Investigations of Solid Pharmaceutical Formulations

S. Borchert[3], J.-O. Henck[2] and H. W. Siesler[1]

[1] Department of Physical Chemistry
University of Duisburg-Essen, Schützenbahn 70, D 45117 Essen, Germany

[2] Bayer HealthCare, BHC-PH R&D-PD-P, D 51368 Leverkusen, Germany

[3] Uhlmann VisioTec GmbH , Uhlmannstr. 14-18, D 88471 Laupheim, Germany

Abstract

In this study near-infrared (NIR) transmission and diffuse reflection spectra of tablets with two different pharmaceutical active substances in variable concentrations were recorded. The purpose of these tests was to correlate the spectra with the content of active ingredient, hardness and moisture content in order to implement a fast, non-destructive quality control of solid drug formulations in an industrial environment.

Keywords: FT-NIR spectroscopy; diffuse reflection, transmission, solid drug formulation, active ingredient, hardness, moisture, quality control

1. Introduction

1.1 NIR-spectroscopy

Near-infrared spectroscopy is a valuable analytical tool with many applications in the chemical, pharmaceutical, agricultural and food industry.[1] It offers a rapid, non-destructive method for qualitative and quantitative analysis. The NIR region of the electromagnetic spectrum ranges from 4000 – 12000 cm^{-1} and contains overtones and combination bands which are mainly due to CH, OH or NH vibrations with large mechanical anharmonicity. These overtones and combination bands are much weaker than the absorptions of the corresponding fundamental vibrations in the mid-infrared region. However, the smaller molar absorptivities allow the use of much larger sample thicknesses (up to cm) or undiluted samples. The radiation can penetrate for example into compacted materials like tablets and the diffusely reflected or transmitted radiation will provide a vast amount of spectral information about the sample.[2] The two most important measurement principles of NIR spectroscopy are the transmission and the diffuse-reflection mode. The principal difference between the two instrumental configurations is schematically outlined in Fig. 1.[3]

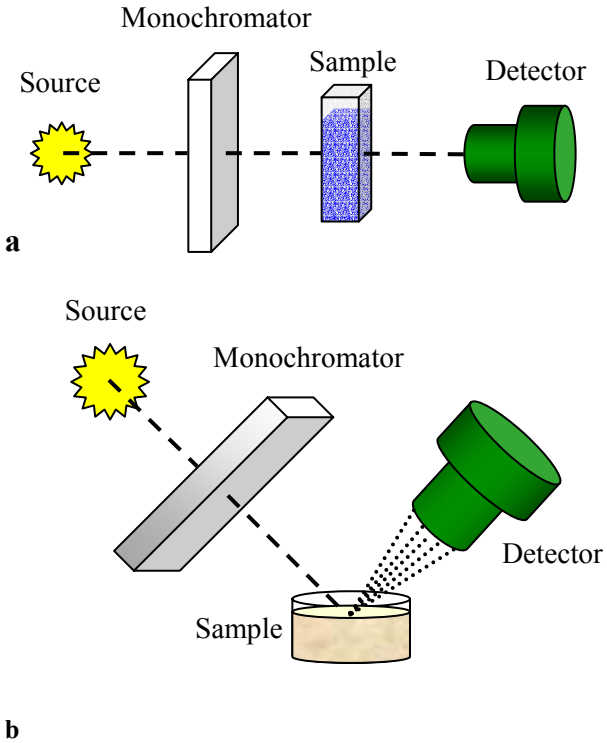

Fig. 1: Principal measurement set-up for the transmission (a) and diffuse-reflection (b) mode.

1.2 Chemometrics

Chemometrics involves mathematical and statistical methods to extract qualitative (principal component analysis (PCA)) and quantitative (partial least squares (PLS) regression) chemical and physical information from spectroscopic data of samples with varying complexity. The main idea of PCA is to reduce the dimensionality of a data set which consists of a large number of variables but to retain most of its variance. Each spectrum can be described as a point in the multidimensional wavenumber space. If we assume for simplicity that a spectrum consists of only three data points the spectrum x_1 of sample 1 should be a point in three-dimensional space (Fig. 2).

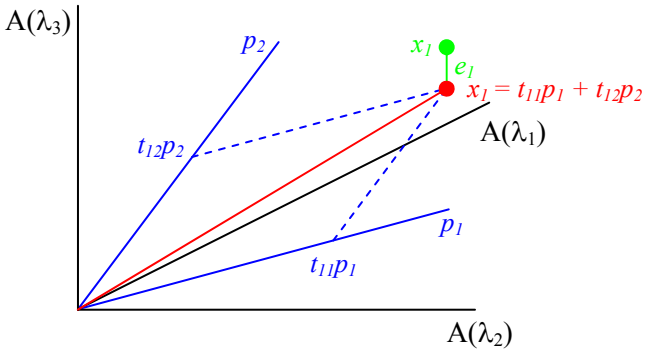

Fig. 2: Projection of a spectrum in a three dimensional space onto a plane.

If the vectors p_1 and p_2 define a plane, the combination $t_{11}\,p_1 + t_{12}\,p_2$ lies in the plane and e_1 joins x_1 to this point in the plane. The first step of the PCA is to determine the shortest e_1. This can be achieved, when e_1 is perpendicular to the plane (then $x_1 = t_{11}p_1 + t_{12}p_2$ is the projection of x_1 on the plane defined by p_1 and p_2). This step is equal to the determination of the direction through the cloud of points (spectra) along which the data show most of the information. This is the direction of the first two principal components (PC). Then x_1 is the point in the plane nearest to x_1. Similarly, but not shown in the Fig.3 x_2 and x_3 etc. are the points in the plane closest to the respective points x_2 and x_3. Figure 3 only displays the projection of a 3 three-dimensional point onto a plane.[4-5]

PLS is a quantitative spectral decomposition technique that is closely related to principal component regression (PCR). The PLS regression compares the information of the spectra with the physical or chemical properties of the investigated material. The central step of the PLS is to decompose the spectral matrix into eigenvectors which are arranged according to decreasing information content. So the first factor characterizes the main information of the spectra and the higher factors explain decreasing information in the data. The factorization of the spectra and the reference parameter values are performed simultaneously.

The aim of the present study was to develop qualitative PCA and quantitative PLS calibration models which permit high correlation between the spectra and the content of active ingredient, moisture and hardness of the investigated solid drug formulations.

2. Materials and Methods

Two different types of tablets with varying amount of active ingredient A and B, respectively, were investigated. The NIR-spectra were recorded in diffuse reflection and transmission on a Bruker Vector 22N FTNIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany). The spectrometer is equipped with a sample carousel for the automated measurement of 10 tablets, an InGaAs detector for transmission measurements and a PbS detector, which is integrated in an integrating sphere, for the reflection measurements. For spectra acquisition 32 scans with a spectral resolution of 8 cm^{-1} were accumulated.

The spectra were evaluated by „The Unscrambler"® (Camo, Norway).

The content of the active ingredient in the A-tablets was 0.1, 0.2, 0.3 and 0.8 mg. The B-tablets contained 2.5, 5, 10 and 20 mg of active ingredient.

The reference method for the active ingredient content of both types of tablets was the high-pessure liquid chromatography (HPLC); the tablet hardness was measured by a tablet hardness tester and the moisture content was determined by Karl Fischer (KF) titration.

3. Qualitative Tests

Generally, one issue of this study was to identify transmission or diffuse reflection spectroscopy as the preferential method to predict qualitative and quantitative parameters of the investigated tablets. In the first step it had to be determined whether it makes a difference when the NIR beam impinges first on the front or on the back side of the coated A-tablets in the two measurement configurations and whether the corresponding NIR transmission and diffuse-reflection spectra can be discriminated by PCA. Depending on the content of active ingredient the A-tablets had a stamp on the front side and a smooth reverse side (0.1 – 0.3 mg) or different stamps on both sides (0.8 mg). The transmission and diffuse-reflection spectra recorded from the front (blue) and reverse (red) side of the 0.8 mg A-tablets are shown in Figs. 3a and b, respectively.

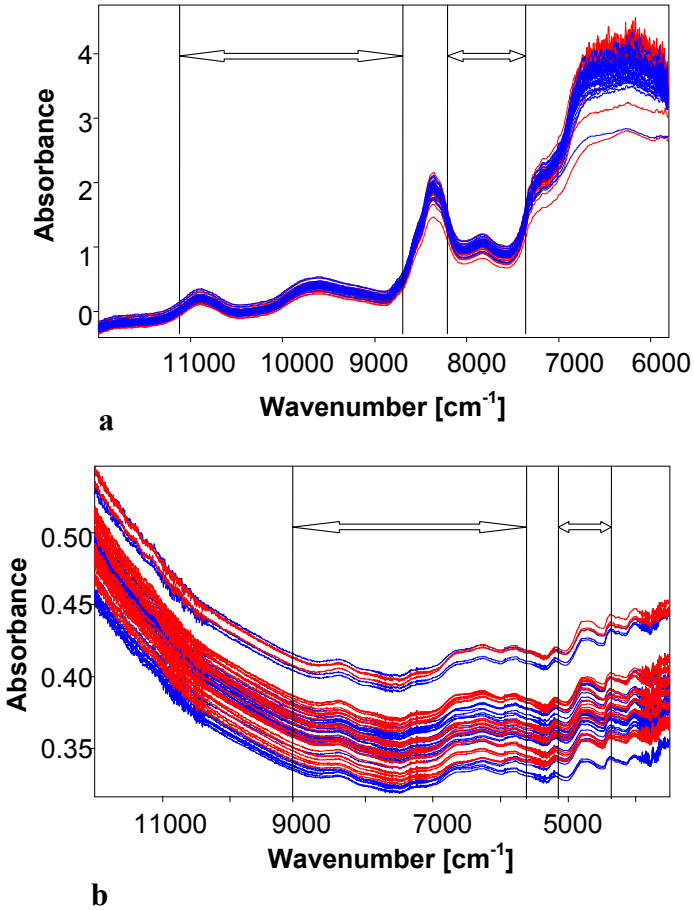

Fig. 3: Transmission (a) and diffuse-reflection (b) spectra of A-tablets (blue =front side, red = reverse side).

The score plot is the most important result of a PCA. It shows if a separation of the spectral variables is possible or not. Figure 4 shows a three-dimensional score plot derived from the PCA model of the recorded reflection spectra in terms of the tablet front/reverse side. The different colours demonstrate the different tablet sides (red: reverse side, blue/black: stamp side).

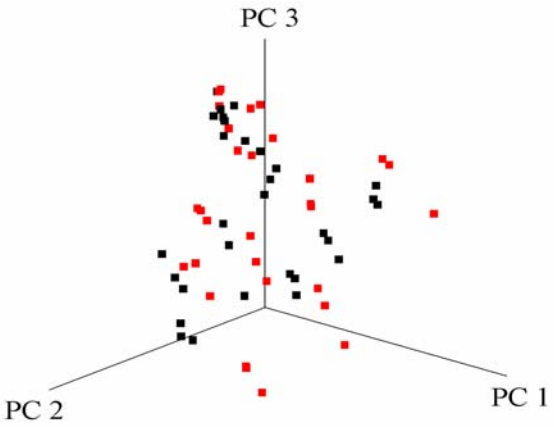

Fig. 4: Three-dimensional score-plot for the transmission spectra with illumination from the front (red) and reverse (black) side.

Thus, it can be derived that the spectra of the tablets recorded from different sides in diffuse reflection cannot be discriminated. The same result was obtained for the transmission spectra. Furthermore it was examined if a separation of the spectra measured in transmission or reflection mode of coated tablets was possible in terms of the active ingredient content.

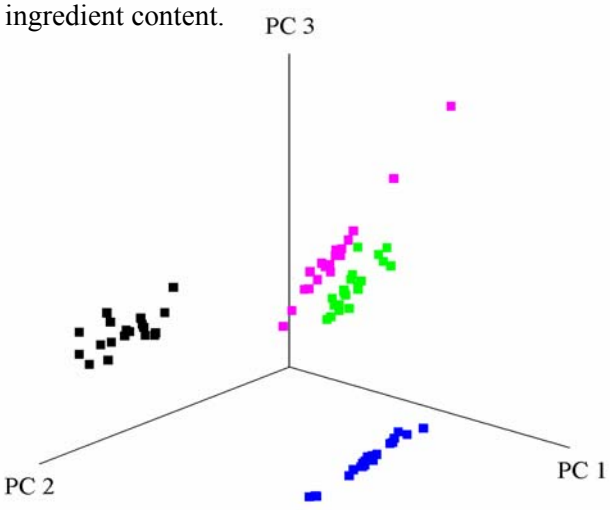

Fig. 5: Three-dimensional score-plot from transmission spectra for the different content of active ingredient.

Figure 5 demonstrates this effect for the transmission spectra of the tablets where each cluster corresponds to spectra of tablets with a certain content of active ingredient. A similar result (not shown here) was obtained for the diffuse-reflection spectra suggesting that the difference in active ingredient is significant enough to discriminate the tablets also in the diffuse-reflection mode. However, it should be pointed out, that the tablet coating contained varying amounts of Fe_2O_3 depending on the content of active ingredient. In order to verify the influence of the coating further investigations were performed with the spectra of uncoated A-tablets recorded in the transmission and reflection modes. The spectra were subjected to a PCA to see if a separation of uncoated tablets with reference to the content of active ingredient was possible. The result of this test was, that it was possible to separate only the transmission spectra according to the content of active ingredient whereas the PCA was not able to separate the spectra recorded in the diffuse-reflection mode. In view of the fact that in the reflection mode the NIR-beam does not penetrate into the core of the tablet the separation obtained in the score plot of the diffuse-reflection spectra was obviously caused by the different coating for the different assays.

From these introductory qualitative investigations of the A-tablets is has to be assumed that only transmission measurements provide the necessary reliability for the NIR spectroscopic determination of the active ingredient. To further support this conclusion PLS models were developed with the specification values (0.1, 0.2, 0.3 and 0.8mg) of the A-tablets. While the predicted values for transmission spectra were in good agreement with the actual values, in the diffuse-reflection mode only the 0.8 mg tablets yielded acceptable prediction accuracy. Thus, the minimum limit to predict the concentration of the active ingredient in the reflection mode is reached by the 0.8 mg tablets.

4. Quantitative Tests

Based on this preliminary work, further quantitative evaluations where only based on transmission spectra of the two different drug formulations. For the A-tablets PLS-1 models were developed with the HPLC and hardness values, respectively, of 120 calibration samples. In analogy, for the B-tablets PLS-1 models were developed for the active ingredient, hardness and water content (with Karl Fischer reference values) (30 tablets per concentration for the active ingredient content, 40 tablets for the hardness and 20 tablets for the moisture content). The Tables 1a and 1b summarize the best results of these PLS models for the different parameters of the two drug formulations.

Table 1a: Results of the PLS models of transmission spectra of the active substance A tablets

	Active ingredient content	**Hardness**
Pre-treatment	Vector normalization	Straight line substraction
R^2 [%]	99.58	70.89
RMSEP [µg o. N]	16.9	5.41
Factors	4	5
Wavenumber-Range [cm-1]	7500 – 8100 8870 - 11100	7498 – 9751

Table 1b: Results of the PLS models of transmission spectra for the active substance B tablets

	Active ingredient content	**Hardness**	**Moisture content**
Pre-treatment	No Pre-treatment	MSC	Offset correction
R^2 [%]	99.70	89.10	98.41
RMSEP [mg, N, %]	0.366	5.73	0.224
Factors	6	5	6
Wavenumber Range [cm-1]	8790 – 12000	8652 – 11224	8625 – 11329

The most meaningful results of the PLS are the "Actual vs. Predicted-Plots" and the "R^2/RMSEP vs. Factor-Plots". The "Actual vs. Predicted-Plot" permits the determination of the R^2-value which indicates the calibration model quality to predict the actual values. Figure 6 shows an Actual vs. Predicted-Plot for four different concentrations of the active ingredient A tablets for the PLS model with vector normalization as spectral pretreatment.

The R^2/RMSEP vs. factor plot shows the contribution of the separate factors. The R^2-value represents the correlation coefficient and

the RMSEP means Root Mean Square Error of Prediction. Each factor explains its contribution of information to the model. In Fig.7 the R^2/RMSEP vs. factor plot of active ingredient A-tablets for the PLS-1 model with vector normalization is given.

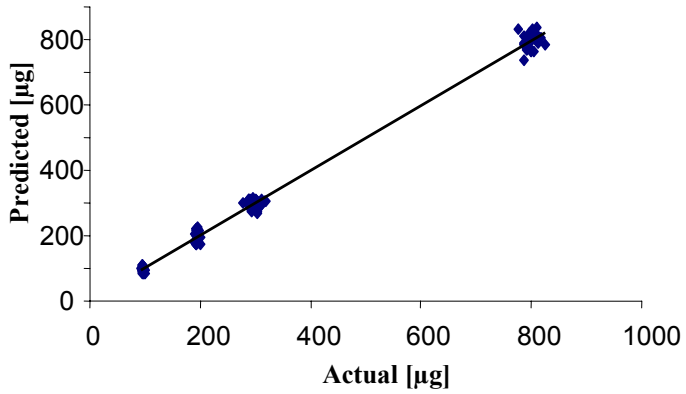

Fig. 6: Actual vs. Predicted plot for active ingredient of A-tablets.

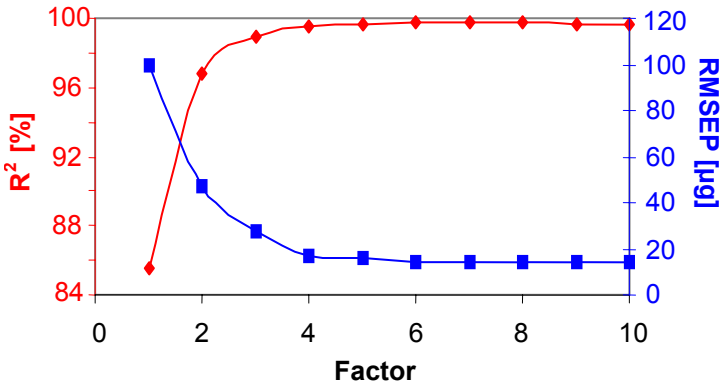

Fig. 7: R^2/RMSEP vs. Factor-Plot for active ingredient A-tablets.

The models were improved by outlier detection (F-test) and/or variation of the wavenumber range. The spectral range was chosen to allow for the inclusion of maximum information for the model. The improved spectra were tested versus new samples which were not included in the calibration model. In the prediction of concentration for the active substance A-tablets the deviation between the actual value and the prediction value must not exceed 15% (for the 0.1 – 0.8 mg active ingredient content). For the active substance B-tablets the deviation for the concentration and moisture content may be 5% (for the 2.5 – 20.0 mg active ingredient content). The hardness deviation varied within 10 N (for hardness values between 110 – 170 N).

The differences between the actual values and the predicted values of the 0.1 mg active ingredient content of the active substance A tablets are unacceptable. A quantitative prediction of the active ingredient content for active substance A-tablets is only possible for concentrations > 0.2 mg. The models for the determination of the hardness were improved by the use of tablets which had the same size (0.1 mg – 0.3 mg). The prediction of the concentration of the active substance B tablets yielded numerous outliers in the 2.5 mg and 5 mg range. A new model was generated which only included tablets of two concentration types (2.5 + 5 mg, 5 + 10 mg, 10 + 20 mg). The results of these models were better than the models which contain all concentrations (2.5 – 20 mg). The calibration should contain tablets with consistent sizes and different concentrations of active ingredient. The results of the hardness of the active substance B-tablets were acceptable. Presumably the model could be improved by the use of the same tablet size in analogy to the calibration of the concentration. The modeling of the moisture content yielded acceptable results but the deviation between the actual value and the predicted values of the 2.5 and 10 mg tablets was very high. The results derived from models of reflection spectra were not acceptable. The coating of the tablets has a large influence on the spectra.

5. References

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