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Characterization of different laser irradiation methods for quantitative Raman tablet assessment

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Abstract

Quantitative Raman spectroscopy of conventional wet granulated pharmaceutical immediate release tablets and subsequent data evaluation was investigated. Different aspects of quantitative assessment of active pharmaceutical ingredient (API) in intact tablets with special focus on sub-sampling issues were addressed. Four different geometric laser irradiance patterns were examined to study the effect of sub-sampling within the tablets. The Raman data was evaluated using both univariate and multivariate techniques. UV absorbance spectroscopy was used as a reference method. The best result in terms of prediction error was attained by irradiating a large area of the tablets. Using multivariate calibration with multiplicative signal correction (MSC) the prediction error was 1.7%. In addition, the effect of tablet density on the Raman assessment was investigated. It was found that quantitative Raman assessment of chemical content can be made insensitive to variations in tablet density corresponding to a manufacturing compression interval of 5–20 kN provided that adequate data treatment is used. A short discussion about sample heating in the context of different irradiation patterns is included with reference to previous work. In conclusion, the present study provides a platform for developing an implementation strategy for quantitative Raman spectroscopy for both laboratory analysis and process analytical technology (PAT) applications.

Keywords: Raman; PLS; Quantitative; Pharmaceutical; Tablet; PAT

1. Introduction

In the last few years the need for fast and simple laboratory analyses as well as for at line/on line/in-line analyses for the pharmaceutical industry has increased dramatically [1,2]. With increased regulatory demands of control and increased number of assessments new tools have to be considered. The traditional analytical techniques often involving complicated sample pretreatment procedures are in this context too slow. Furthermore, all solid-state information about the active ingredient and matrix is lost when using wet-chemistry methods. Spectroscopic techniques, on the other hand, are both simple and fast [3]. Most spectroscopic techniques can be performed remotely via optical fibers or otherwise and can easily be multiplexed. So far the most utilized quantitative spectroscopic technique is near-infrared (NIR) spectroscopy. It is indeed both simple and fast but has some limitations. In NIR spectroscopy the broad spectral features overlap and often hiding the relevant chemical information. In addition, light scattering within solid pharmaceuticals is quite extreme and will act to distort the spectra even further. This makes spectral analysis complicated and multivariate evaluation is often required. Still problems exist with maintenance of multivariate models and transfer of methods to other laboratories.

There is a growing interest for implementation of Raman spectroscopy solutions for analysis of pharmaceuticals [2,4–6]. Still, up to date a surprisingly small number of quantitative Raman studies have been reported. Raman spectroscopy has been utilized for probing of blending and mixing of non-compacted powders. Deeley et al. compared mid-IR and Raman spectroscopy of mixtures of polymorphic cortisone acetate and found prediction errors of about 2% for both techniques [7]. Langkilde et al. reported on

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quantitative models for mixtures of polymorphic forms of an active substance [8]. Taylor and Zografi developed quantitative models of crystalline to amorphous content in dry mixtures of indomethacine [9].

Compacted solids present additional challenges for developing robust analytical procedures. The literature contains only a few examples where these problems are discussed. Kontoyannis reported on a Raman method for quantitative assessment of glycine/calcium carbonate tablets [10]. A relative standard deviation (R.S.D.) of about 2.5% for repeated measurements of single tablets was reported. Wang et al. reported on assay of aspirin as well as determination of its major degradation product salicylic acid in binary aspirin tablets [11]. Jedvert et al. used NIR and Raman spectroscopy to assay commercial isosorbide-5-mononitrate tablets [12]. Using multivariate evaluation a prediction error of lower than 2% was reported, both for Raman and NIR spectroscopy. Notably, in that paper a rotating sample holder was utilized in the Raman analysis in order to achieve high repeatability. The same principle was utilized by Szostak and Mazurek who also used a rotating sample holder and multivariate evaluation of the Raman spectra [13]. Powders of ground tablets containing acetylsalicylic acid and acetaminophen were used and they achieved prediction errors of less than 2%. The use of multivariate evaluation of Raman spectra was further reported by Dyrby et al. who showed the potential of variable selection to improve on the prediction errors [14]. Vergote et al. reported on determination of the content of diltiazem hydrochloride in diltiazem hydrochloride tablets using Raman spectroscopy [15].

Analysis of compacted powders such as pharmaceutical tablets requires a great deal of considerations other than the more fundamental aspects of spectroscopy of solid materials. Due to spatial variations and inhomogeneities of the sample matrix spectroscopic assessment of samples is a delicate task that can yield different results depending on how the measurement is set up. This is often referred to as scale of scrutiny and has been thoroughly discussed for NIR spectroscopy of solids [16]. Compared with NIR, Raman spectroscopy is more critical because of the even smaller irradiated sample volume of pharmaceutical solids and only a small fraction of a cm-sized sample will be assessed by Raman measurements. Thus, in order to achieve a representative quantitative analysis of a single pharmaceutical unit such as a tablet, the sample has to be moved to multiple positions during Raman acquisition. This sub-sampling problem has been discussed before in the literature [12]. This has special implications for real-time process Raman measurements since obviously the assessment has to be rapid. One way to deal with the sub-sampling problem is to utilize rotating sample holders. In this way the collected signal will be acquired from a much larger area of the sample. This has also been used and reported earlier [11–13], however, the effect was never studied in greater detail.

In this paper, important aspects of quantitative Raman analysis of tablets are investigated. The sub-sampling problem is addressed by comparing different laser irradiance methods. The repeatability and prediction error of developed quantitative multivariate models will be compared for the different irradiance patterns and the results are discussed in view of the chemical homogeneity on the microlevel shown by NIR imaging of the tablets. Different spectral evaluation criteria are tested and compared with partial least squares (PLS) models. Lastly, the influence of tablet density on evaluated Raman spectra is studied and discussed in view of other spectroscopic techniques.

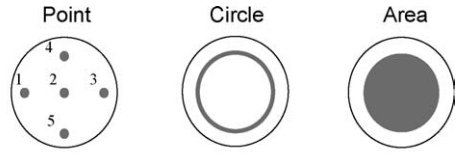
2. Materials and methods

2.1. Tablets

Tablets were manufactured according to an experimental design. The content of the active component (produced at AstraZeneca R&D Södertälje, Sweden) was varied between 45 and 55 mg. The tablets contained microcrystalline cellulose as a filler and five other excipients. The experimental design comprised varying content of active (five levels), lot active component (two batches) and lot microcrystalline cellulose (two batches). The amount of filler was adjusted for each level of content active to reach a tablet weight of 157 mg. In addition, another set of tablets were manufactured with variation in tablet compression force at four levels: 5, 10, 15 and 20 kN. The tablets were uncoated and had a diameter of 8 mm.

2.2. Raman spectroscopy

Raman spectra were acquired using a BioRad FTS 575C infrared/Raman spectrometer (Cambridge, MA, USA) equipped with a Nd:Yag laser operating at 1064 nm and a liquid nitrogen-cooled germanium detector. The laser power was set at 500 mW, the signal was an average of 64 scans, the recording time was ca. 1 min per spectrum and the resolution was 4 cm^{-1}. The diameter of the laser beam at focus was approximately 0.5 mm according to the instrument manufacturer. The tablets were placed in a rotating sample holder to irradiate various areas during spectral acquisition. The laser irradiation patterns used are shown in Fig. 1. For point irradiation five points on each side were measured while the tablets were stationary. For circle irradiation the tablets were rotated



	Point	Circle	Area
Irradiated area (mm^2):	0.2	3.9 (small) 7.9 (large)	19.6
% of tablet front surface:	0.4	8 (small) 16 (large)	39

Fig. 1. Different laser irradiance patterns for Raman assessment of tablets.

to yield a pattern of a circle with a diameter of 2.5 mm (small circle) and 5 mm (large circle), respectively. The area irradiation was achieved by simultaneously rotating and translating the tablets resulting in a spiral motion of the excitation beam on the tablet surface. The diameter of the sampled area was 5 mm. The Raman spectra were exported to Microsoft Excel where data evaluation was done. Multivariate evaluation was performed in Simca-P 8.1 (Umetrics AB, Umeå, Sweden).

2.3. Reference analysis

The content of active ingredient in the tablets was determined using UV absorbance spectrophotometry. Each tablet was weighed and transferred into a 25 ml volumetric flask. Twenty milliliters of phosphate buffer pH 3.0 were added and the flask was shaken vigorously in a mechanical shaker for 30 min until the tablet was completely disintegrated. The samples were diluted to volume with phosphate buffer pH 3.0 and left to sedimentate for 3 h. Five milliliters of the clear solution were transferred to a 50 ml volumetric flask that was filled to volume with phosphate buffer pH 3.0. A diode array UV spectrophotometer (HP 8453, Hewlett Packard Sverige AB, Spånga, Sweden) was used. The spectral range was 190–1100 nm with a resolution of 1 nm. The light absorption was measured at 274 nm and the background at 550 nm.

2.4. NIR imaging

Intact and bisected tablets were imaged using a near-infrared imaging system (MatrixNIR, Spectral Dimensions Inc., Olney, MD, USA). Images were collected at 10 nm interval from 1100 to 1700 nm using a 1× or a 10× objective. The NIR data was processed using the evaluation program ISys (Spctral Dimensions Inc.).

3. Results

3.1. Irradiance pattern

A representative sample presentation is a key factor to attain reproducible and robust Raman assessment of tablets. The first aspect of sample presentation that was studied in this paper was the dependence of sample distance to focal plane on the Raman signal. A tablet was placed in the tablet holder, its position along the optical axis was varied and a Raman spectrum was collected for every 1 mm translated. In Fig. 2, the intensity of the Raman signal at 1612 cm^{-1} is plotted against the sample position along the optical axis. In addition, the evaluated Raman data expressed as the ratio of the intensity at 1612 cm^{-1} and the average intensity between 1000 and 1500 cm^{-1} is shown in Fig. 2. In spite of the significant effect on Raman intensity from different positions along the optical axis, the variation in evaluated signal is minimal after normalizing with peak ratioing. The calculated R.S.D. across 7 mm was only 2.5%. Thus, the Raman assessment

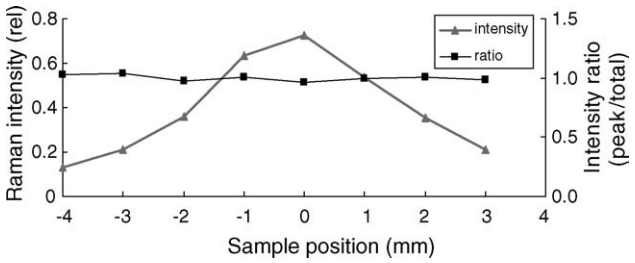


Fig. 2. Profiling of the effective focal depth of the Raman excitation laser. The Raman intensity at 1612 cm^{-1} as well as the intensity ratio at 1612 cm^{-1} and the average intensity between 1000 and 1500 cm^{-1} is displayed as a function of sample position along the optical axis.

can be considered to be robust against variations in sample position for most practical situations.

In order to visualize the effect of sub-sampling a few tablets were imaged using a NIR imaging system. Intact tablets were placed directly on the microscope sample table and images were recorded from both sides of the tablet. In addition, tablets were cut into half using a rotary cutter and cross sections both parallel and perpendicular to the tablet front face were collected. Also images of piles of pure active substance and excipients were collected. The spectral images were preprocessed using first order derivatives and spectral signatures were determined characteristic for each substance using spectra from the pure components. In Fig. 3a, 1st derivative images at 1620 nm are shown for four intact tablets displaying the distribution of the active substance at the front surface. The four images illustrate well the statistical nature of the distribution of the active component (white areas) with both smaller and larger regions within the tablets. In general, the active component was found to be present in larger domains (<1 mm), lactose was found at smaller domains on the limit of the spatial resolution and the microcrystalline cellulose appeared to be evenly distributed within the tablets. The cross sections of the bisected tablets were studied with respect to accumulation of any substance closer to the surface, however, no such spatial inhomogeneity could be found see (Fig. 3b).

To further characterize and to give a quantitative measure of the sub-sampling problem tablets were measured in the Raman system using the point irradiance pattern (Fig. 1, point irradiance). Tablets were placed in a stationary tablet holder and Raman spectra were acquired from five positions on each tablet side. The maximum intensity of the API peak at about 1612 cm^{-1} was found for each spectrum and a relative standard deviation of 10 recordings for each tablet was calculated. In Table 1, the calculated R.S.D. for five different tablets and the mean value of those are shown. As can be seen the mean value of R.S.D. values, that can be interpreted as the point to point measurement error, is as high as 5%.

Raman measurements were performed using the different laser irradiance patterns shown in Fig. 1. The circle irradiance pattern was done both for 2.5 and 5 mm diameter. Two recordings were made on each tablet, one from each side, and the difference of the two was calculated as a measure of the

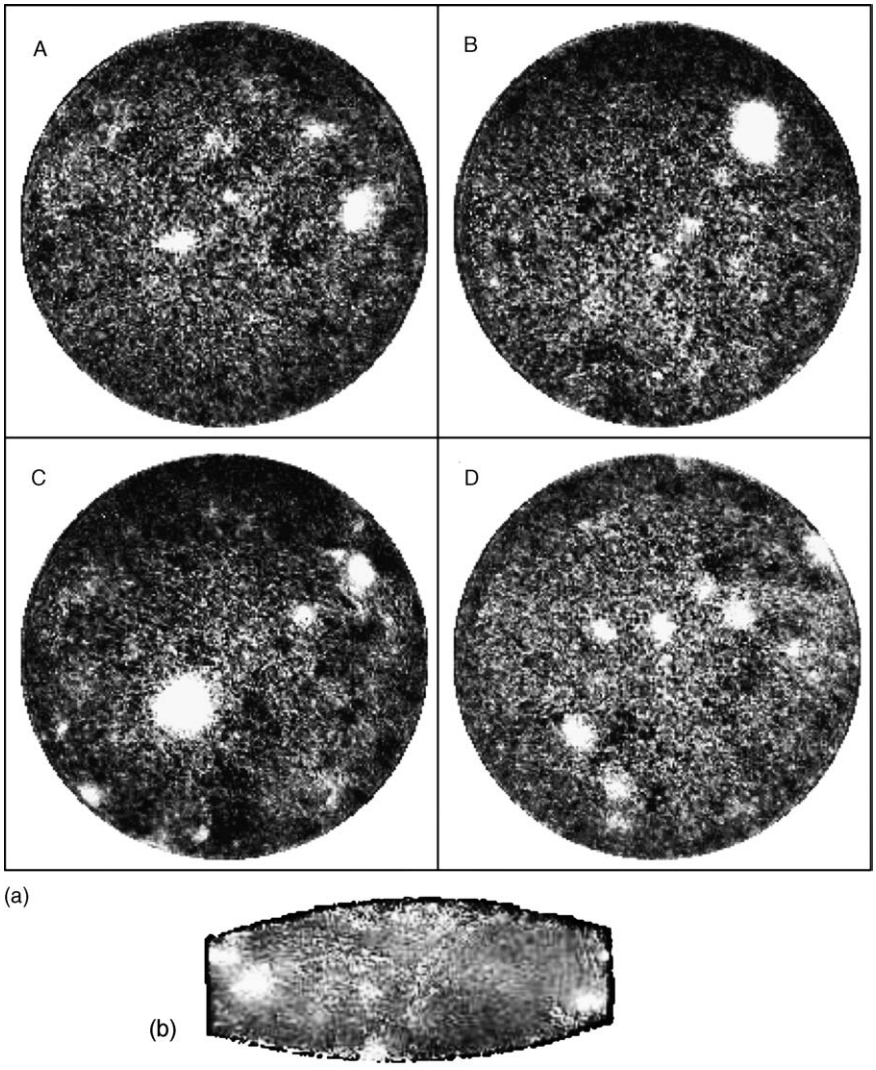


Fig. 3. NIR images of the surface of four different intact tablets (a) and one bisected tablet (b). The images were preprocessed using first derivative and show the corresponding values at 1620 nm. White areas correspond to areas of a high concentration of API. The size of the tablets was 8 mm.

sub-sampling error. The average values of recordings from 20 different tablets are shown in Table 2. The Raman data was evaluated as the ratio of the peak at 1612 cm^{-1} and the average intensity between 250 and 1500 cm^{-1}. Table 2 shows the benefit from increasing the sampled area as the progressive lowering of the sub-sampling error. It is also shown that no improvement is attained going from a 5 mm circle pattern to a 5 mm area pattern.

Table 1
R.S.D. of evaluated Raman data expressed as the intensity at 1612 cm^{-1}

Tablet	R.S.D. (%), $n = 10$
1	7.3
2	4.8
3	4.2
4	4.0
5	4.9
Mean	5.1

The R.S.D. values are based on 10 recordings from each tablet.

3.2. Data evaluation

A number of different data evaluation criteria are compared here utilising the area irradiance pattern. Traditionally, sophisticated data evaluation are not utilised due to the fact that Raman spectra in most cases include sharp features that are clearly separated from each other. Thus, in many cases the peak height or peak area of one significant Raman feature

Table 2
Comparison of different laser irradiance patterns and effect on quantitative assessment

Pattern	Difference side A – side B, $n = 20$
Point	4.0
Small circle	2.1
Large circle	1.4
Area	1.4

The difference between two evaluations from the same tablet is calculated. Reported values are mean values of the differences from 20 tablets.

Table 3
Different data evaluation criteria

Evaluation	RMSE (%)
Peak height	2.7
Peak area	2.7
Peak area-background	2.8
Peak area/total	1.9
Peak area/excipient 1	2.3
Peak area/excipient 2	2.2
PLS[a]	1.7

Peak height: maximum intensity close to 1612 cm^{-1}, peak area: integrated intensity of 1612 peak, background: spectral background close to 1612 cm^{-1}, total: integrated intensity 1000–2000 cm^{-1}, excipient 1: maximum intensity close to 360 cm^{-1}, excipient 2: maximum intensity close to 1097 cm^{-1}.
[a] RMSEP value.

is used. In our case the peak height or peak area gave similar results, as shown in Table 3. The root mean square error (RMSE) was calculated as the relative standard deviation of residuals from linear regression models of Raman and reference data. Since the background intensity may originate from both sample fluorescence and hidden non-resolved Raman features it should be beneficial to subtract the background from the peak area. However, no improvements were noticed from this procedure. The background procedure was repeated for other data from the same tablets and again without any noticeable improvement. A further improvement was found to be peak normalization. This was done using the integrated intensity over a large portion of the relevant Raman spectrum as well as using selected excipient peaks. The most successful normalization was found to be normalization against the total area rather than selected excipient peaks (Table 3).

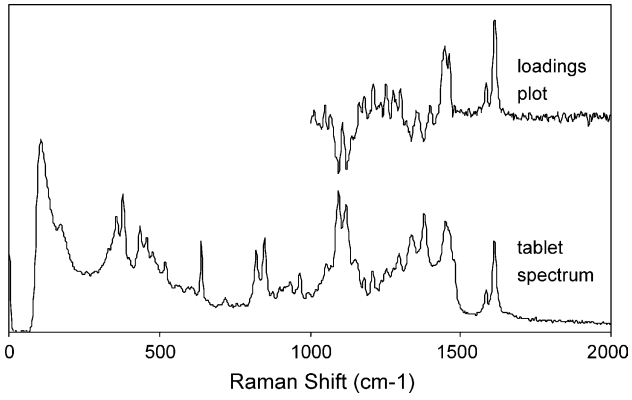


Fig. 4. Raman spectrum of tablet together with loadings plot from PLS calibration. Raman data between 1000 and 2000 cm^{-1} were used in the PLS calibration.

A more powerful data evaluation scheme is the use of multivariate analysis. Multivariate analysis is frequently used in combination with NIR spectroscopy where the spectral features are less distinct. However, the combination of Raman spectroscopy and multivariate evaluation is quite unusual. Here, PLS models were developed as a comparison to the other evaluation criteria reported in Table 3. Different data pretreatment protocols were utilized: second derivative, multiplicative signal correction, standard normal variate transform (SNV) as well as combinations of those. It was found that an MSC correction resulted in the lowest prediction error. In Fig. 4 a typical Raman spectrum is shown together with a loadings plot. Using MSC pretreatment a single PLS component was sufficient to accurately model the API content. From the tablet spectrum and loading plot of Fig. 4 it is clear that the

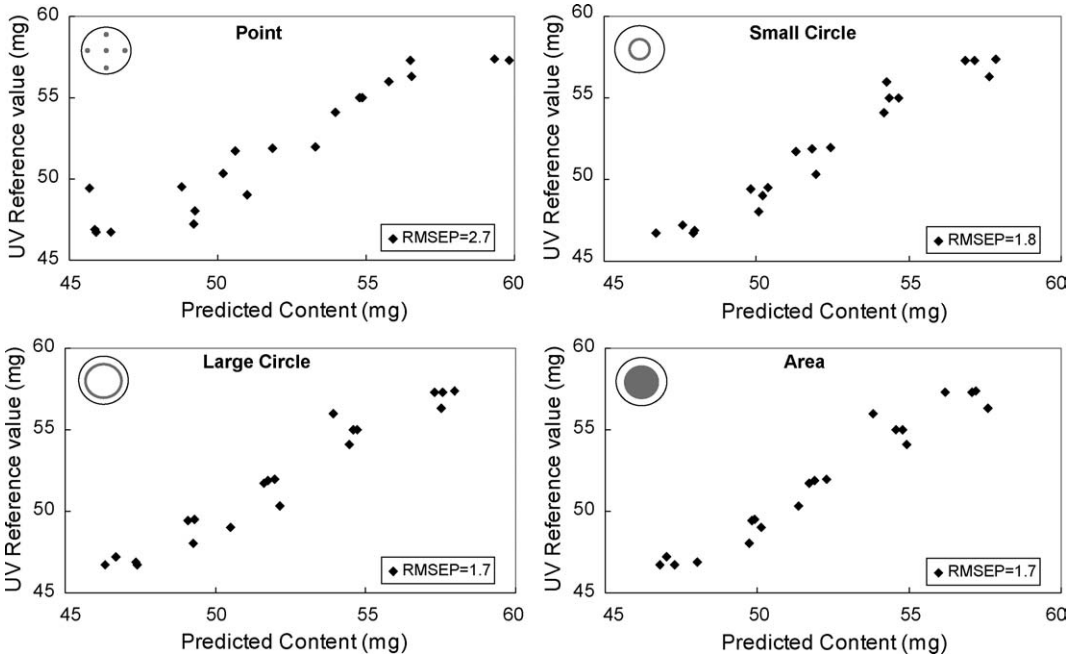


Fig. 5. Prediction of content API vs. UV reference values for four different laser irradiance patterns. The calibrations include one PLS component and MSC pretreatment.

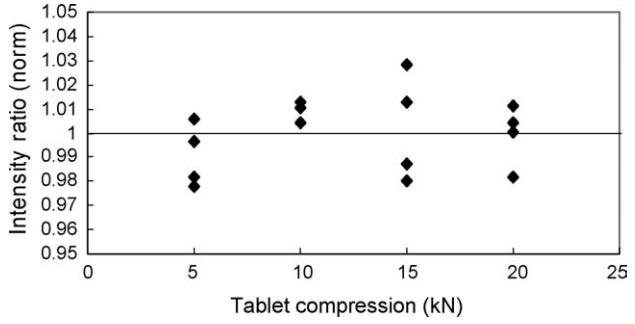


Fig. 6. Influence of tablet compression force during manufacturing on Raman signal. The evaluated Raman signal is expressed as the ratio of the Raman peak at 1612 cm^{-1} and the integrated intensity from 100 to 1600 cm^{-1} and normalized against reference analysis data.

API gives a positive contribution and the excipients a negative contribution to the loading vector. Comparing the different models root mean square error of prediction (RMSEP) values were used, where the error is predicted from an independent data set not included in the PLS model. In Fig. 5, prediction plots for the different laser irradiance patterns are shown. Again, irradiating a larger tablet surface seems to result in lower prediction errors. Comparing the values of Table 3 the multivariate evaluation seems to be slightly better than a manual data evaluation at discrete frequencies.

3.3. Tablet density

A set of tablets was measured with variation in density as an effect of manufacturing compression force ranging from 5 to 20 kN. Raman spectra were acquired using the same measurement conditions as with the other tablets and using 2.5 mm rotation diameter of the sample holder. The spectra were evaluated by calculating the ratio of the active substance carbonyl peak at 1612 cm^{-1} and the integrated Raman intensity from 100 to 1600 $cm^{-1}$and normalized against the reference value as determined by UV absorbance analysis. The evaluated data are shown in Fig. 6 as a function of tablet compression force. As can be seen from Fig. 6 no significant effect of tablet density on the evaluated Raman signal is observed. The R.S.D. of all values was calculated to be 1.5% which is comparable with values reported above for tablets of a single compression force.

4. Discussion

The use of spectroscopic techniques for quality control of pharmaceutical materials has increased rapidly in the past few years. Raman spectroscopy has the potential to be developed into the first choice for assessment of API in solid products. In particular, Raman solutions for process analytical technologies (PAT) require both rapid and reliable technologies. The main challenge for process control is the continuously changing process conditions that are difficult to predict or simulate. This will put the focus on the robustness of a PAT tool and not only on a high precision. The robustness of an analytical procedure is a product of fundamental properties of the technique, instrumental realisation and the utilised sampling technique. Here, a number of important issues have been addressed that have to be thoroughly investigated before reliable Raman assessment can be realised.

Representative measurements is of particular importance in Raman spectroscopy due to the limited irradiated volume per scan. From the NIR images in Fig. 3 it is clear that the size of the API region in the utilised tablets is comparable to the laser focus diameter used here, about 0.5 mm. Thus, with point irradiance very large errors may be introduced unless the sample (or excitation beam) is moved during data acquisition. On the other hand, already a limited sample movement is sufficient to lower the assessment error below 2% and with a further increased irradiated area the precision is not much improved. A similar conclusion can be made from the multivariate evaluation as shown in Fig. 5. In the latter case the benefit from moving the sample seems to be less important. This may be a result of multivariate evaluation being capable of sorting out the relevant information, thus resulting in more robust models. Furthermore, multivariate evaluation results in lower prediction errors than manual data evaluation. The best manual evaluation technique was found to be normalization against the total intensity, which is not surprising since such measurements are largely insensitive to both sample movements and excipient inhomogeneities. It is important to point out that the conclusions made here are based on one type of tablet only and may not be generalized. However, the tablets were manufactured according to a widely used wet granulation procedure and should at least to some extent be representative for other immediate release tablets.

Another aspect of different irradiation patterns to be considered is potential heating of the sample. It has been shown that for some materials already low laser powers are sufficient to raise the temperature locally during acquisition and that spectral shapes might be altered [17]. However, sample heating can be avoided again by moving the sample during acquisition in order to distribute the excess heat to a larger volume although for non-compacted powders the heat dissipation is very poor. Hence, a slight movement of samples during Raman assessment takes care of both the sub-sampling problem and potential sample heating. An in-depth discussion can be found elsewhere [17].

The Raman measurements here on tablets manufactured at different compression forces clearly show that Raman assessment is not much influenced by tablet density. The calculated R.S.D. for all measurements was less than 1.5% and still the design covers a wide range of compression forces. These results are interesting in view of the current status of NIR spectroscopy. Near-infrared spectra and associated calibrations are strongly influenced by changes in the tablet matrix due to changes in light scattering properties. These effects can be limited by data pretreatment but still results in offsets in existing calibrations. Possible reasons why Raman

spectroscopy does not seem to be influenced by tablet compression may be that sharp spectral Raman features are less influenced by light scattering than broad NIR features and that the sampled volume and thus mean optical path length is smaller in Raman spectroscopy. Again, these results are based on measurements on one single type of tablet.

5. Conclusions

A number of practical issues of Raman assessment of tablets were addressed. Different laser irradiance patterns were investigated and compared in terms of the assessment error. It was shown that extending the irradiated area lowered the assessment error substantially. This was attributed to scale of scrutiny effects. However, the entire surface does not have to be scanned. Errors below 2% were attained both for circle and area irradiance patterns. Different data evaluation schemes were compared, both direct peak height calculations and multivariate, and here MSC pretreatment followed by PLS calibration gave the best results. In addition, a set of tablets manufactured at different tablet compression forces were measured. No effect of compression force on the evaluated data were found. In conclusion, Raman spectroscopy appears to be a competitive candidate for the future analytical toolbox both for laboratory and process applications.

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