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Some applications of near-infrared reflectance analysis in the pharmaceutical industry*

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Abstract: A non-comprehensive overview of near-infrared (NIR) reflectance analysis in the pharmaceutical industry is presented. This survey will include background information defining NIR (i.e. spectral region and spectral features), the spectroscopic measurement, and the merits of this technique. This presentation comprises selected pharmaceutical applications of NIR used within Wellcome. One use of NIR is for the identification of tablets in bulk and non-invasively inside individual blister pack cells using several sample presentations including a fibre-optic probe. NIR has been used to determine moisture in freeze-dried parenterals by non-invasively measuring spectra through the bases of product vials. The viability of transferring moisture measuring equations between different instruments within the same site and between two different sites is examined. Another application of NIR involves a rapid assay of the active in whole tablets without any sample preparation. Finally, the use of NIR to validate blending processes for solid formulations is briefly discussed. There are many other applications of NIR in the pharmaceutical industry which are beyond the scope of this presentation. The applications presented here are those being pursued within our company, and provide an indication of the capabilities of NIR.

Keywords: *Near-infrared; reflectance; spectrometry.*

Introduction

This paper presents a non-comprehensive overview of near-infrared (NIR) reflectance analysis in the pharmaceutical industry. The survey will include a brief introduction to the technique, a selection of applications being developed and used within Wellcome laboratories in the UK and USA, and assessments of the potential and merits of NIR.

NIR: The Spectral Region and Experimental Technique

Spectral region

The NIR spectral region is generally defined as the 800–2500 nm range, although commercial NIR spectrometers typically cover the range 1100–2500 nm, with extensions down to 400 nm in the visible region as an option.

Spectral features in the NIR region are overtones and combinations of vibrations observed in the mid-infrared (IR) region (see Fig. 1) and are therefore much less intense (10 to 1000-fold), broader and more overlapping than the parent absorptions, as shown in Fig. 2. The consequently greater difficulty of assigning NIR absorptions to structural features may be a disadvantage, but this is compensated by other aspects: unique combination bands offer information not available in the mid-IR, the reduced intensities allow direct measurements on undiluted samples, and measurements of, or in the presence of, water are readily performed. The offset between the spectra in Fig. 2 is caused by particle size differences in the solid sample. In reflectance spectrometry, the radiation is diffusely reflected from the surface layers of the sample: penetration has been estimated to range from 0 to 5 mm, depending on the particle size and degree of compaction. In order to focus on the chemical information, spectral derivatization can be used effectively to remove the physical effects, as illustrated in the second derivative plots of the same two spectra in Fig. 3.

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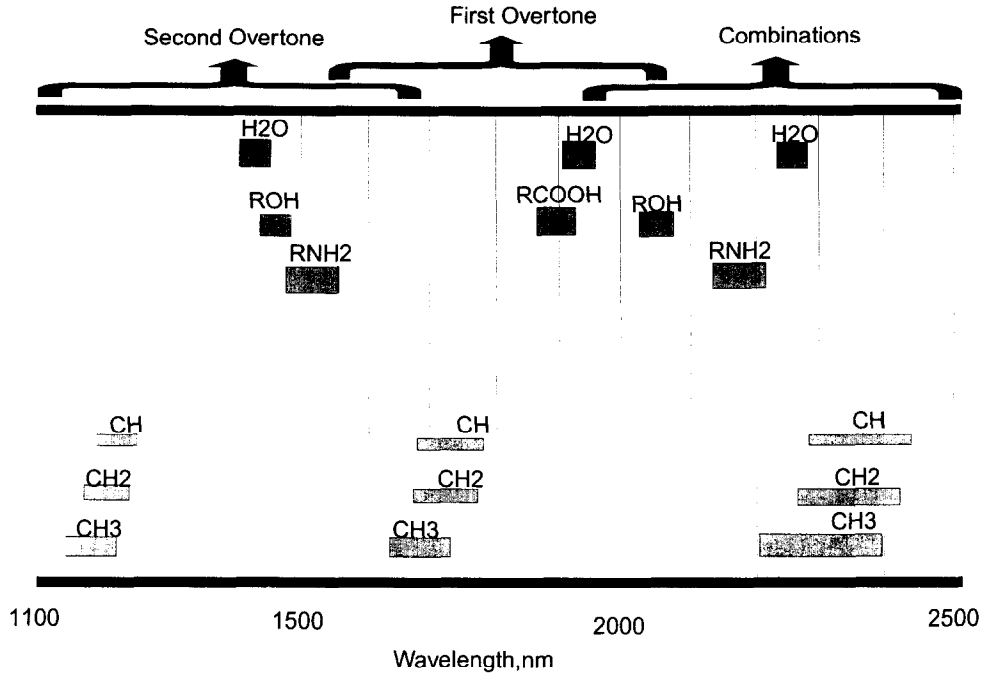

Figure 1
Overtones and combinations in the NIR spectral region.

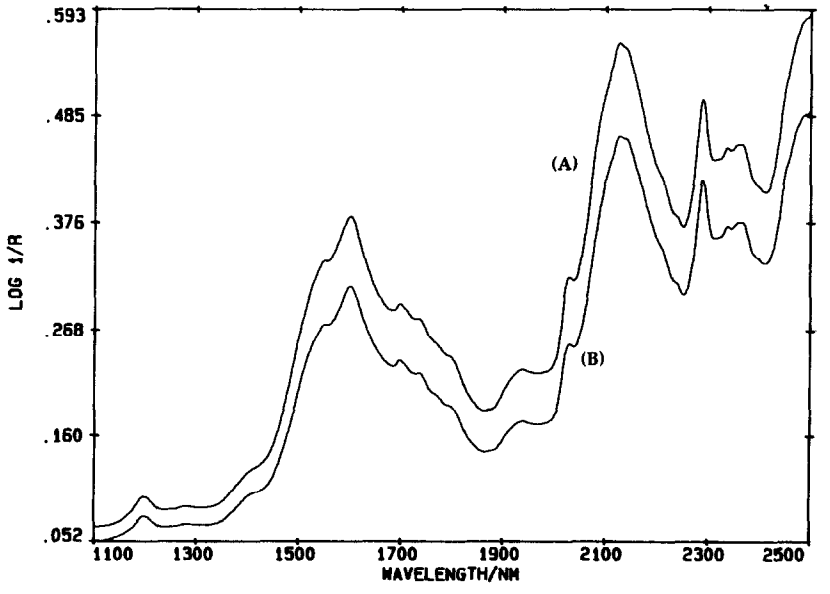

Figure 2
Zero-order NIR reflectance spectra: the low intensity bands are broad and overlapping. Note the offset on the absorbance (log $1/R$) scale caused by particle size differences. (A) Larger particles, (B) smaller particles.

Exploitation of the NIR region

The wealth of information residing in the broad overlapping NIR bands is not readily accessible: it must be extracted by sophisticated deconvolution techniques which have become available with the advent of powerful computer hardware and chemometric software, and which are largely responsible for releasing the full potential of NIR and prompting the explosive growth of the technique.

NIR is equally suited to reflectance, transmission or transflectance modes. There are many variations which provide extremely flexible options for handling all types of samples,

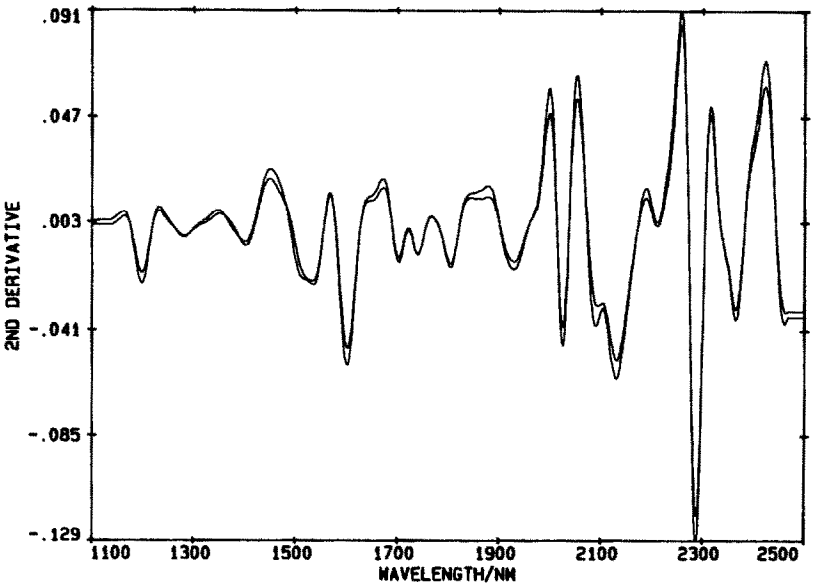

Figure 3
Second derivative plots of the two spectra shown in Fig. 2. Note the effective removal of the absorbance offset.

from solids through to liquids, thus making NIR an attractive technique for pharmaceutical analysis. The ability to work with undiluted samples is particularly exploitable for reflectance work on solid samples, since little or no sample preparation is needed and sample matrix information is retained.

The objective for multicomponent determinations, i.e. the determination of one or more analytes in a matrix, is to achieve a linear relationship between spectral intensity and analyte concentration as this offers the best potential for accurate concentration estimates. In practice, this is satisfied by expressing spectral intensities as log $1/R'$, where R' is the intensity of radiation reflected by the sample relative to that reflected by a non-absorbing standard (a white ceramic is normally used in NIR work).

Methodology

NIR instruments are rapid scanning devices. Spectral acquisition is typically completed in about 1 min, and a sample analysis, including the reference scan and filling the cell, takes less than 5 min. The same sample scan can be used for sequential qualitative and quantitative analysis, and simultaneous multicomponent determinations may be included in the quantitation.

Qualitative analysis involves the comparison of the sample spectrum with spectral libraries set up previously for known samples of known products. Sophisticated software assists in spectral matching, utilizing pre-set threshold values which provide stringent identification and discrimination criteria.

Quantitative analysis requires that the NIR response first be calibrated against reference analytical data generated by a standard technique (e.g. HPLC) on a calibration set of samples providing a necessary range of compositions. This calibration set must comprehensively cover all variations in analyte and matrix components likely to be encountered in real samples, in order that the calibration equation will be robust and reliable. The equation itself is generated by software application of linear regression or more complex chemometric approaches like Partial Least Squares (PLS) regression. The latter can offer advantages for complex sample matrices through making use of all the spectral data rather than just discrete wavelengths, because chemical interaction effects can in this way be modelled into the calibration. The calibration equation is stored electronically, and validated by generating results on known, independent test samples.

Applications

Identification

It is critical in clinical trials that patients be dosed with the correct product at the correct time. The complexity of many studies involving

tablet products poses the problem of correctly identifying and distinguishing between different tablets packed in blisters on the same blister card, where the position and order of the blisters relates to the dosing pattern required.

NIR reflectance methods have been developed in parallel in our USA and UK laboratories for discriminatory identification of tablets containing different strengths of the experimental active drug, a clinical comparator and a placebo, all manufactured to be of the same appearance, and packed into opaque white blisters. Three modes of sample presentation have been tried. In all cases NIR spectral scans were transformed to second-derivative spectra, and data analysis and results were generated using selected regions of the spectrum. Reference spectral libraries were first created by scanning at least 10 tablets of each tablet-type involved in the study, and confirming the identity of each of these tablets by a reference identification technique (typically TLC).

The most successful approach was by removing individual tablets from blisters and using a special template to position them on the optic window of the spectrometer. In this way, it proved possible to identify both active tablets containing 5, 10 and 20% (w/w) of the drug and clinical comparator tablets, and to distinguish these from each other and from 2% active tablets and placebo. Neither the 2% tablets nor the placebos could be positively identified, but in no case was a mis-identification made. The disadvantage of this sample presentation mode was the need to remove the tablets from the blisters: this extra operation lengthens the procedure and introduces potential for losing track of individual tablet positions on the blister card.

The second approach was similar to the first except that the blister card itself was presented to the spectrometer such that individual blisters were exposed one at a time through the special template: the spectra of the tablets inside the blisters were scanned through the blister plastic. Spectral sub-regions of 440–1060 and 1140–1650 nm were selected in order to omit the range above 1650 nm in which there was significant spectral contribution from the blister plastic. There was still a small blister contribution to the spectra in the regions retained for analysis, and this decreased the capability of the technique compared to the exposed tablet approach, such that identification of the 5% tablet was less reliable. The 10 and 20% tablets and comparator tablets were still positively identified in all cases, and no mis-identifications were made.

The third approach utilized a fibre optic extension to the spectrometer, which allowed remote measurement of the tablet spectra. This procedure was optimized by clamping the fibre optic probe in a rig which allowed reproducible placing of the probe on successive blisters on the card, and by selecting a narrow spectral range (1466–1480 nm) for analysis. Identification and discrimination proved entirely reliable for 10 and 20% active tablets, the comparator and placebo, but only in the absence of 2 and 5% tablets. The latter could not be discriminated from each other or the placebo when using the fibre optic, and this represented a trade-off against much better speed and convenience when using this accessory.

Results from such a work can be obtained either by visual examination of spectral plots, in which the different tablets appear as families of curves, as indicated in Fig. 4, or by use of the NIR spectral matching software to indicate acceptable correlation between test and library spectra in terms of correlation coefficient and conformity within a pre-set number of standard deviations from the library mean, as indicated in Fig. 5. In practice, a threshold distance value of five or six standard deviations has been typically applied.

Moisture determination

Many injectable products are currently presented in capped vials as freeze-dried solids, the moisture content of which is an important control parameter in the product specification. Specification limits of up to about 5% (w/w) are typically applied, depending on the product. Karl Fischer (KF) titration methods are commonly used to determine the water contents, but can be prone to errors in all but experienced hands, and risk compromising the sample when the vial is opened to atmosphere in order to dissolve and titrate the contents: the procedure also destroys the sample.

NIR reflectance methods have been developed for determination of moisture by placing the unopened product vials on the optic window of the spectrometer and scanning the spectra through the bases of the vials. Since water has pronounced NIR absorption bands,

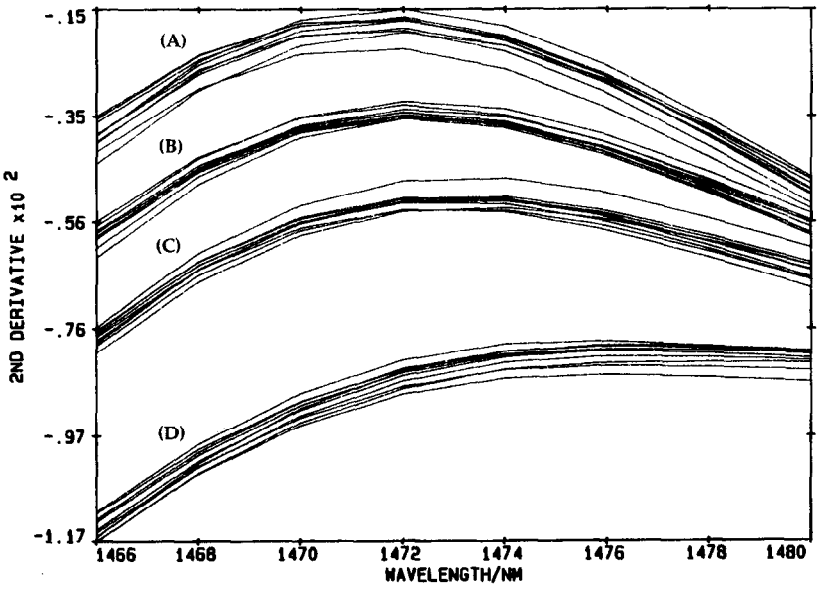

Figure 4
Visual comparision of spectra of different tablet strengths, demonstrating grouping and the basis for discrimination. (A) Placebo; (B) 50 mg active; (C) 100 mg active; (D) 200 mg active.

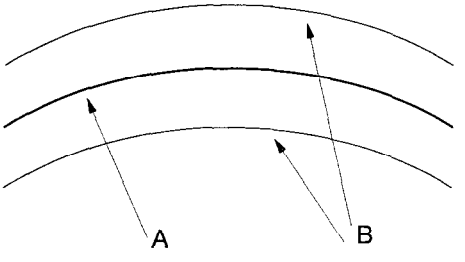

Figure 5
Representation of mean library spectrum and the threshold envelope based on a pre-set number of standard deviations of the library group, within which a test spectrum must lie for positive identification. (A) Mean of library spectra; (B) limits of standard deviation envelope for library spectra.

single or dual wavelength calibrations have often proved viable based primarily on the 1940 nm combination band. One example of this application is the collaborative work carried out in our laboratories in the UK and USA on one product manufactured at both sites to the same formula but filled in different dose amounts into vials sourced locally. Excellent calibrations, using selected production batches were achieved in each case, using as reference data the KF results generated on the same vials scanned in the NIR, and an example of such a calibration is shown graphically in Fig. 6. Independent test sets were then used to successfully validate the calibrations at each site, prior to electronically transferring calibrations between two instruments at the USA site and between instruments at the USA and UK sites. The water contents of all the sample sets used in the calibration and subsequent test sets are shown in Table 1. Excellent results were obtained with these transferred calibrations on the local test sets, demonstrating that methods/calibrations generated on a particular instrument at a particular site for locally manufactured product are robust enough to generate accurate data on different instruments at different sites for product manufactured at the second site. Examples of these results are shown in Tables 2 and 3 and in Fig. 7. In 10 of the 11 test set predictions summarized in Table 3, paired *t*-tests demonstrated that there was no significant difference between KF and NIR results at the 95% level, and in the remaining set there was no significant difference at the 98% level.

Whole tablet assay

Traditional analytical procedures like HPLC or UV spectrophotometry necessarily involve time consuming sample extraction/dissolution/dilution stages when applied to solid products. An NIR method has been developed to provide rapid assay of bulk whole tablets with no sample preparation. A large rectangular cell was filled with tablets in random orientations and, using a motorized spectrometer accessory, was longitudinally transported past the

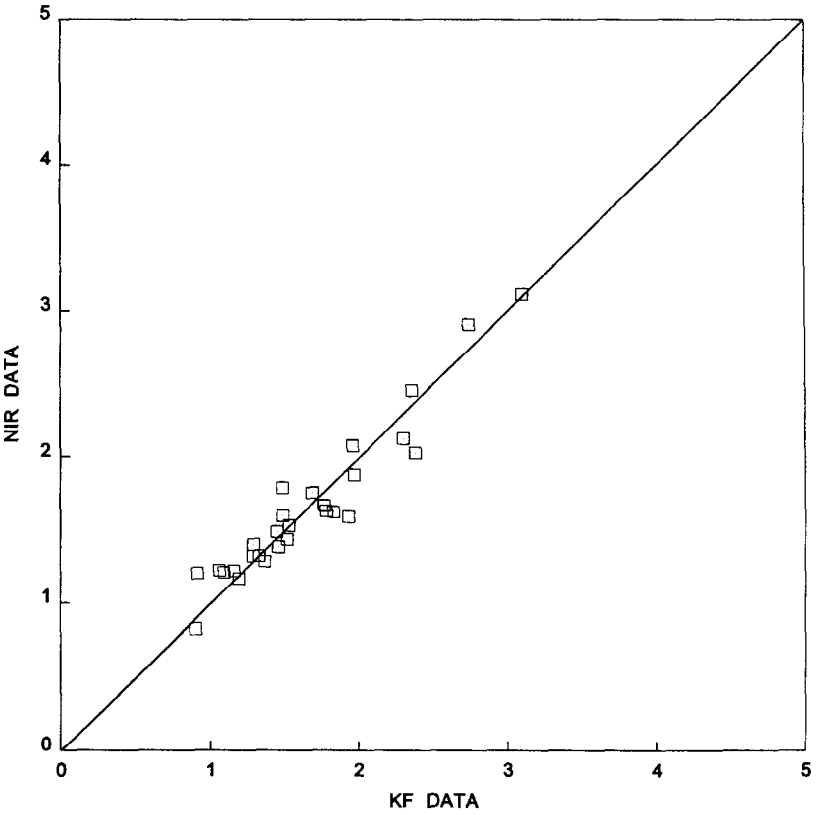

Figure 6
Example of an NIR moisture calibration (dual wavelength, 1914 and 1340 nm).

Table 1
Water contents of calibration and test sets

		Water content (% w/w) by KF		
Sample set	Number in set	Mean	Range	RSD
Calibration sets				
UK	28	1.66	0.90–3.10	33
USA	32	2.11	1.34–4.90	33
Test sets				
UK	12	1.59	1.16–2.13	20
USA1	25	1.84	1.27–2.70	20
USA2	27	1.35	0.84–1.71	16
USA3	19	1.39	1.12–1.71	12

NIR beam during spectral scanning. In this way, replicate rapid scans were subsequently averaged by the instrument to provide a mean spectrum which incorporated variations due to different tablet orientations as well as spectral scan variations.

The calibration set was comprised of tablet compositions specially designed to incorporate variations of approximately ±10% of the formula concentrations of all ingredients, including the active, with minimized intercorrelations, together with authentic production batches in which the composition was always close to the formula levels. Reference assay data were generated by a well established UV procedure, and a PLS regression calibration was developed, and is shown graphically in Fig. 8. This calibration was tested on a range of independent tablet samples including specially prepared batches with high and low assay values and production batches, and the results are shown in Table 4. Excellent agreement between the NIR and UV results was obtained, and a paired *t*-test revealed no significant

Table 2
Moisture (% w/w) in UK test samples predicted using USA single wavelength NIR calibration (1920 nm)

	Reference data (KF)	NIR prediction	Residual
	1.450	1.571	0.121
	1.830	1.616	−0.214
	1.600	1.608	0.008
	1.470	1.377	−0.093
	1.320	1.412	0.092
	1.160	1.277	0.117
	1.170	1.309	0.139
	2.120	2.238	0.118
	1.610	1.577	−0.033
	1.650	1.659	0.009
	2.130	2.064	−0.066
	1.530	1.557	0.027
Mean	1.59	1.61	+0.02 Bias 0.09 Accuracy
SD	0.32	0.29	0.11 Bias 0.06 Accuracy

Table 3
Summary statistics of NIR test set data

	Residuals, NIR − KF			
	Bias		Accuracy	
Test set	Mean	SD	Mean	SD
(A) Using UK2-wavelength calibration				
UK	−0.04	0.11	0.09	0.07
USA1	−0.01	0.22	0.19	0.10
USA2	−0.06	0.20	0.16	0.14
USA3	−0.01	0.23	0.16	0.16
USA3Q	−0.06	0.20	0.16	0.13
(B) Using USA1-wavelength calibration				
UK	+0.02	0.11	0.09	0.06
USA1	0.00	0.24	0.20	0.13
USA2	−0.05	0.25	0.19	0.17
USA3	−0.04	0.24	0.16	0.17
USA3Q	−0.09	0.20	0.17	0.13
(C) Using USA2-wavelength calibration				
UK	−0.03	0.17	0.14	0.08

USA1, 2 and 3 are three independent USA test sets scanned on the same instrument at that site: US3Q is test set USA3 scanned on a second USA instrument: UK is a UK test set scanned on the UK instrument.

Table 4
NIR assay of whole tablets: results on independent test set

	Assay, % label strength	
UV data	NIR prediction	Residual
103.6	105.0	1.4
99.6	98.8	−0.8
100.7	100.8	0.1
94.8	94.9	0.1
102.0	101.1	−0.9
99.7	99.7	0.0
108.0	107.7	−0.3
99.6	99.6	0.0
102.6	101.5	−1.1
88.0	90.2	2.2
	Mean	+0.1 Bias (0.7 Accuracy)
	SD	1.0 Bias (0.7 Accuracy)

difference at the 95% level. In addition, the agreement between NIR and UV data was shown to be as good as the agreement observed between two sets of UV data generated in different laboratories.

Blending validation

It is necessary to validate the process of blending the component solids prior to compression into tablet products. This validation may include various blending equipment parameters as well as time of blending, and the major means of assessment is to check homogeneity by analysing samples taken from different points in the bulk. The large numbers of samples necessarily involved are time consuming to analyse by conventional procedures, and turnaround time is long.

NIR procedures are currently being developed in Wellcome's UK laboratories in order to speed up the procedure and facilitate faster and possibly more extensive blending validation studies. In order to avoid the necessity of manufacturing a wide range of blend compositions for use as an NIR calibration set, which would itself place heavy demands on both pharmaceutical and analytical resources, it was decided to develop an alternative approach. Rather than generating assay values for each sample, NIR spectral matching is being investigated as a means of demonstrating

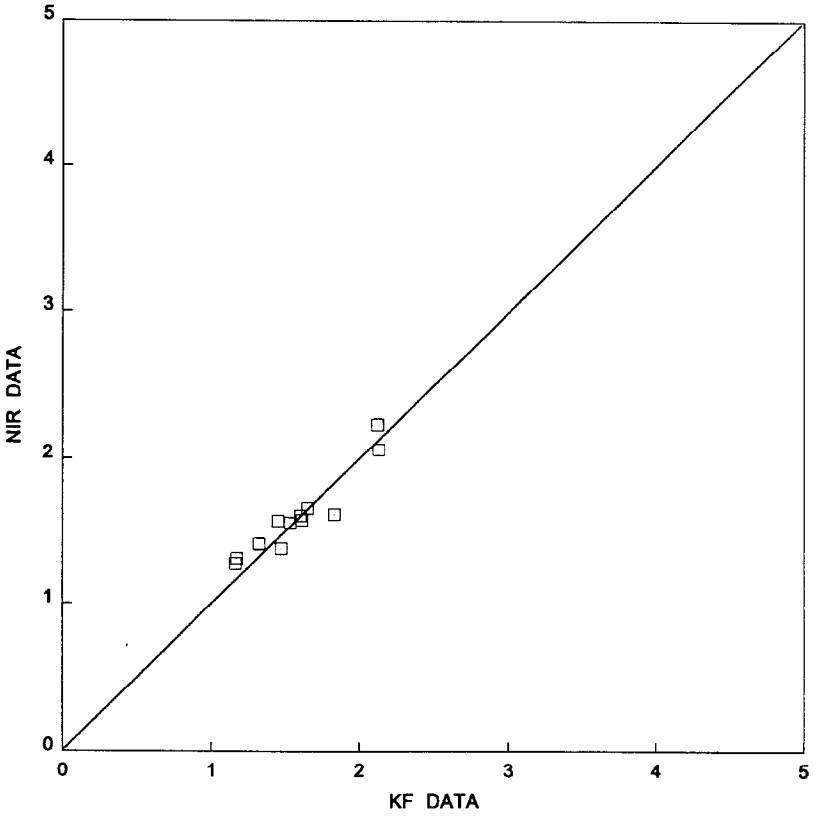

Figure 7
Example of NIR moisture test set prediction (UK test set predicted by USA single wavelength calibration, 1920 nm).

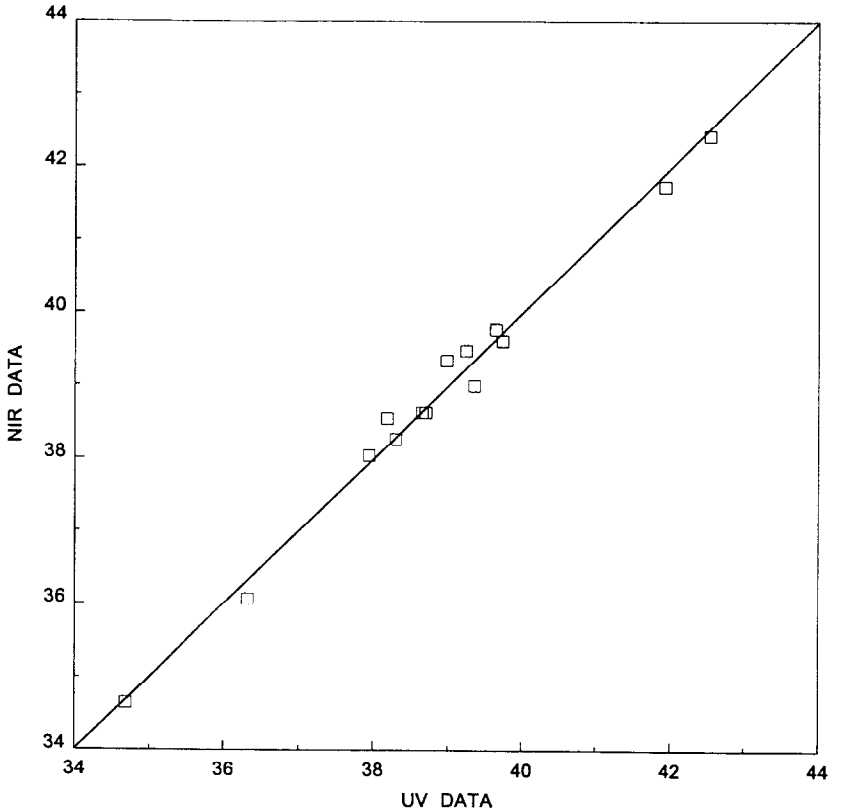

Figure 8
PLS NIR calibration plot for whole tablet assay.

conformity or otherwise of each sample with other samples, and ultimately with a correct, fully blended composition. The NIR software approaches used here are similar to those described earlier for tablet identification, and are based on correlations of sample spectra with the mean and standard deviation envelope of a reference spectral library. Once homogeneity has been demonstrated by spectral conformity of samples from all points in the bulk, duplicate conventional assays (e.g. by HPLC) on a composite sample can be carried out to confirm that the homogeneous mix is actually of the correct assay. It is recommended that this method find acceptance as a rapid alternative to multiple assays: it should offer advantages in facilitating more extensive blending studies and in confirming conformity of the entire blend composition rather than just correct active ingredient content.

Conclusions

NIR is gaining acceptance in the pharmaceutical industry, although regulatory approvals are still relatively few in number. There are many other applications beyond those described in this paper, but the work carried out so far within Wellcome has demonstrated the success of the technique for product identification, moisture determination and active ingredient assay.

NIR methods are very fast in routine application, although the necessity of carefully setting up and validating spectral libraries or quantitative calibrations during method development must be emphasized. Routine analyses take less than 5 min per sample and can simultaneously provide identification and assay with no sample preparation. The technique is non-destructive and non-invasive, thus allowing the same samples to be used for other tests and ensuring that sensitive samples are not compromised during analysis, e.g. by exposure to atmospheric moisture.

NIR is applied directly to samples without the need to disrupt the sample matrix by extraction or dissolution as required by conventional analysis. This allows NIR analysis to take full account of matrix effects, particularly in solid samples, and so to provide information relating either to a particular ingredient as it exists in that matrix or to the whole sample matrix, e.g. NIR identity confirmation of a solid product like a tablet demonstrates that the entire product composition is correct, not merely that the correct active ingredient is present.

Finally, it is worthwhile reiterating that the necessary effort to establish accurate, robust and validated NIR spectral libraries or calibrations which incorporate all variations to be encountered in real samples is the pre-requisite to reaping enormous benefits in rapid, convenient and efficient routine or quality control testing. NIR methods established in this way should then have every probability of gaining early regulatory acceptance.

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