Dye crystal morphology identification and differentiation by near infrared spectroscopy

S. Agyare Yeboah and Mark J. Robbins
Analytical Technology Division, Eastman Kodak Company, Rochester, NY 14650, USA.

Introduction

Dyes and couplers play an important role in photographic products. They are incorporated into sensitised photographic formulations to extend the spectral sensitivity range of silver halide image formers in the visible and infrared regions of the spectrum. These compounds can exist in different polymorphic states through different molecular packing rearrangements within the crystal lattice. If the lattice energies are small, the polymorphic forms may be subtle and insignificant, as in the case described by Sekulic *et al*.[1] However, for pharmaceutical and photographic applications, the changes may be significant enough to result in differences in properties and performance. In pharmaceutical applications, polymorphism is known to impact crystal stability, chemical reactivity, solubility, melting point and bioactivity.[2] In photographic and photolithographic applications, this phenomenon causes the crystals and other sensitisers to exhibit differences in hardness, solubility, visible absorption characteristics, hygroscopicity and stability.[3] Crystal growth in dispersions, precipitation, filterability and certain failures of sensitised formulations are all polymorph dependent. The exact morphology of a given dye crystal is, therefore, critical and must be carefully controlled during its production and subsequent keeping to ensure the preferred morphological state is obtained. The life cycle of a powder chemical may also include several stages. In many instances the crystals, after production, are milled to produce finer particles within a narrow distribution range of particle sizes. This process subjects the crystals to high impact pressures and shear forces. Interaction with moisture[4] and temperature[5] variations during storage can also lead to additional polymorphs being formed. In batch reactions, there is usually a desire to determine the end-point of crystallisation or the complete conversion of an intermediate polymorph to a final product. The use of analytical techniques and multivariate pattern recognition methods to follow these changes is important and has been well documented in the literature.[2,6–10]

A variety of analytical techniques have been used to identify and classify different crystal polymorphs. Among these, FT-IR, Raman, microscopy, differential scanning calorimetry and x-ray diffraction (XRD) techniques are most notable. XRD is usually more conclusive and lends itself to single crystal analysis. A more comprehensive review of the analytical techniques employed for the analysis of polymorhs has been compiled by Threlfall.[11] More recently, near infrared (NIR) diffuse reflectance spectroscopy has become the technique of choice, as manufacturers rely on its ability to be used off-line for identification and classification purposes[12,13] and on-line for process analysis and verification in batch reactors.[14] The use of NIR fibre optic probes for diffuse reflectance measurements and the minimal requirement for sample preparation minimises the likelihood of additional polymorphic forms being induced during measurements. The possibility to make measurements of the dry crystalline powder and its slurries with the same instrumental configuration allows chemists to follow the

Near Infrared Spectroscopy: Proceedings of the 9th International Conference

course of a polymorphic conversion during production and the stability of a crystalline material from storage to the time of use.

The identification and classification of different polymorphs is usually accomplished by the combination of an analytical technique and one or more multivariate pattern recognition methods. Mahalanobis distance methods and soft independent modelling of class analogy (SIMCA)[15–18] approach are both sensitive and easy to use. The primary goal of the project described in this paper was to provide a simple and reliable means of detecting the existence of different polymorphs in batches of dyes used in photographic formulations. It was also our intent to extend such capabilities to small-scale batch reactors to follow the crystallisation processes and determine the conversion end-points of one polymorph to another. In this paper the use of NIR diffuse reflectance measurement for the differentiation of various polymorphic forms of a dye, B1, during and after production is discussed. As will be discussed later, the dye can exist in various polymorhic forms. The real-time, in-line analysis of the conversion of polymorph I to polymorph V, the preferred form, in a laboratory reaction vessel is also presented. The Mahalanobis distance method is used to determine the end-point of the conversion.

Experimental

NIR spectra of the polymorphic forms of the powder and slurries of the dye were recorded using two different instruments. Powder and slurry measurements were conducted in sample cups using a Bruker IFS 28N FT-NIR spectrometer, interfaced to a fibre bundle diffuse reflectance probe and operated at a resolution of 8 cm^{-1}. An NIRSystems Model 5000 spectrometer (FOSS NIRSystems, Silver Spring, MD, USA) was employed in measurements of the crystallisation reactions and the polymorphic conversions. Two types of probes were used: a transflectance probe with a reflection tip forming a 2 mm sample gap was used for the dye formation and crystallisation experiments. A diffuse reflectance probe suited to slurry measurements was used for the polymorphic conversions following crystallisation. The latter spectrometer was operated at 120 scans, with a 120 seconds acquisition delay between spectra. The probe was inserted inside a 1 L temperature-controlled reaction vessel, with the measurement surfaceimmersed two inches below the surface of the reaction mixture. GRAMS/32 v.5.1 (Galactic Industries, Salem, NH, USA) was used for the multivariate analysis of the spectra. All spectra were converted to second derivative data to remove much of the variations caused by physical characteristics such as particle size and packing density differences. The derivative spectra were then inverted with respect to the intensity axis.

Results and discussion

Crystal powder spectra

To confirm the ability of NIR to distinguish among the various crystal forms of dye B1, a series of powder samples, previously established by x-ray diffraction techniques as having different polymorphic forms, were examined by NIR. XRD analysis of the dye samples produced distinctly different spectra,[19] thus prompting the XRD analysts to assign the nomenclature used to identify the samples in this report. As many as 17 forms of the dye, designated as Type I through Type XVII, were identified from two primary versions of the dye—triethylamine (TEA) and free acid (FA) forms. The TEA salt is produced in a 3-step process involving precipitation and protonation, followed by a digestion step in which the salt is converted to a free acid form. This polymorph, designated Type II, is less stable for the photographic formulation in which it is used and is, therefore, converted to a different polymorph, Type V, in a seeding process. The samples, without any prior treatment, were measured on the Bruker IFS-28N FT-NIR spectrometer, using a diffuse reflectance (DR) probe positioned about 0.5 cm above the sample cup under ambient conditions. No attempt was made to modify the original particle sizes,

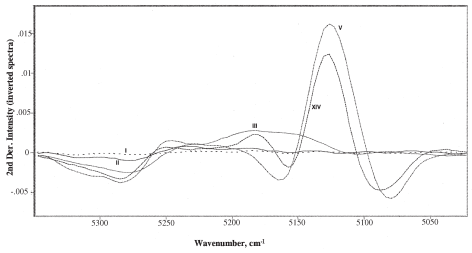

Figure 1. NIR spectra of Types I, II, IIIA, V and XIV measured on the Bruker IFS-28N FT-NIR spectrometer.

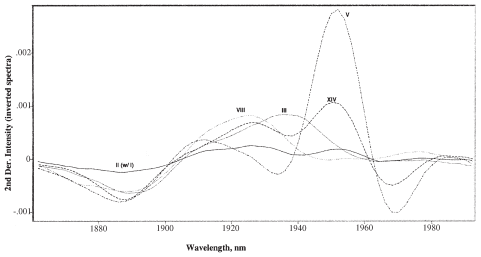

Figure 2. NIR spectra of Types II (with a contaminant amount of I), IIIA, V, XIV and XVIII.

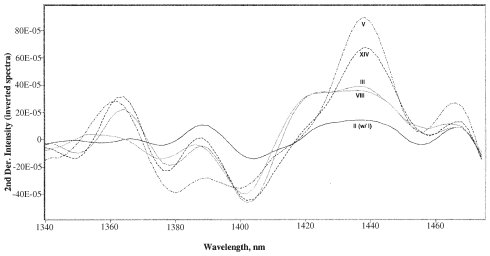

Figure 3. Spectra of Types II, III, V, XIV and XVIII.

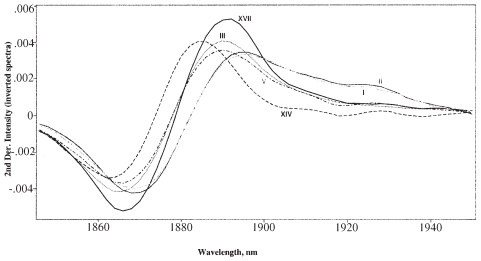

Figure 4. The 1890 nm band profiles of slurries of Types I, II, III, V, XIV and XVII polymorphs.

nor was any effort made to subject the samples to any uniform packing density. Thus in multiple measurements involving reloading of the samples into cups, the spectral intensity for a given sample could vary depending on the final packing density. Band locations, on the other hand, remained unaffected by sample handling.

Figures 1 to 3 present spectra of Types I, II, III, V and XIV. The spectral regions around 1908 nm and1420 nm (5240 and 7040 cm^{-1}, respectively) are found to be the most sensitive to the morphological changes in the crystals. The sensitivity found in these regions suggests that the polymorphic differences arise from interaction of the host molecules with water, leading to variations either in the level of hydration or the strength of hydrogen bonding formed with the hydroxyl bonds.[20,21] XRD analysis conducted on these samples revealed that the interconversion of the polymorphs could occur in some cases simply by exposure to different humidity or temperature.[19] The types identified and measured by NIR were found to be stable after formation. Figure 1 shows spectra of different polymorphs originally measured on the Bruker FT-NIR spectrometer. Figure 2 shows spectra of the crystal types measured several weeks later from a different set of samples, using the NIRSystems Model 5000 spectrometer. Comparison of Figures 1 and 2 shows spectra of the same crystal type to be consistent. Once formed, a polymorph remains stable so long as its environment and storage conditions remain unchanged. Figure 3 shows spectra of the same samples in the spectral region from 1340 nm to 1470 nm, demonstrating similar sensitivity to the different crystal forms. The NIR spectral regions selected for the differentiation of the various crystal forms also correspond to the combination and first overtone bands, respectively, of hydroxyl vibrations. Since the ultimate objective was to monitor the real-time conversion of the crystal types during the digestion phase, an instrument possessing on-line monitor-

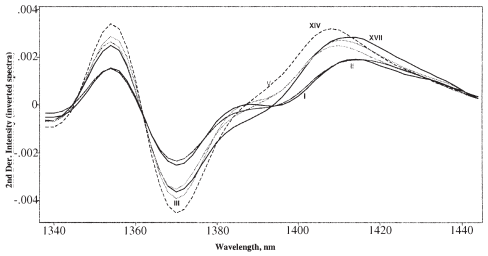

Figure 5. The 1400 nm band profiles of slurries of Types I, II, III, V and XVII polymorphs.

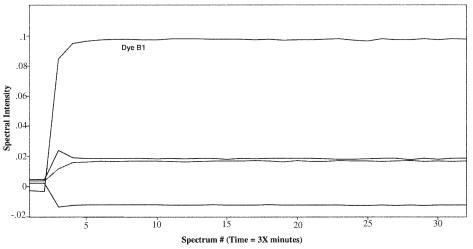

Figure 6. Component profiles from dye B1 reaction monitoring.

ing capabilities was required for any further analysis. The Bruker instrument was, therefore, replaced with an NIRSystems Model 5000 spectrometer for all subsequent measurements.

Since the dye is hygroscopic, its moisture absorption bands should appear in the same spectral regions as water bands. That the band profiles in these regions vary significantly and consistently from one crystal type to the next, is most likely an indication of the different manner in which water molecules are bound to the different crystal lattices. Further work is being done to determine whether all of the water associated with a particular crystal form exits within the lattice or is mostly held at the surface of the crystal and what effect the water distribution will have on the spectral profile.

Slurry measurements

To test the capability of NIR to differentiate among slurries of the various polymorphs, the dye B1 samples were dispersed in 25% water/acetone mixtures at a concentration of 8.95 wt.% dye. The samples were measured using the NIRSystems 5000 spectrometer with a fibre bundle DR probe. Spectra of the slurries of Types I, II, III, V, XIV, XVII are shown in Figure 4. Two observations are made. First, the average band centre of the spectral region found to change with respect to crystal type has shifted to around 1890 nm. Second, the variation among the spectra of the different polymorphs is less striking for the slurries than it is for the powders. This large shift is a result of the presence of liquid water. But it is also possible that the degree of hydration of the crystals may be extensive enough that this spectral region alone lacks sufficient differentiation power. As Figure 5 points out, the region around 1400 nm also shows spectral shifts due to the different polymorphs. Each spectral region may not exhibit strong enough eveidence to stand on its own. However, the combination of the two regions should be sufficient to distinguish among the polymorphs. The ability to clearly distinguish among slurries of the polymorphs can be further enhanced by the application of discriminant analysis techniques based on the Mahalanobis Distance (MD) algorithms.

Reaction monitoring

The reaction monitoring process attempted to determine the composition and reaction end-point in a new prototype design that involved precipitation, protonation and digestion steps after the initial production of TEA salt of dye B1. Figure 6 shows the component profiles in the reaction step leading to the formation of the TEA salt. The TEA salt and other component variations can be clearly followed. However, as indicated by the time axis, the entire reaction is complete within ten minutes of initiation. Hence the need to invest in an on-line spectroscopic monitoring system was not deemed a valuable enhancement to the reaction process. Interestingly, however, analysis of the NIR data from the digestion

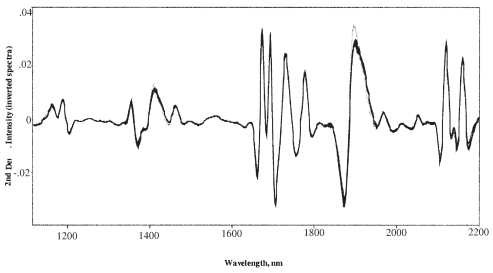

Figure 7. Digestion step.

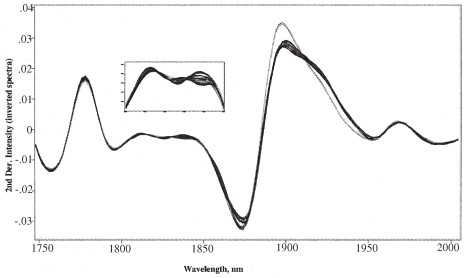

Figure 8. Digestion step (expanded view).

step of the reaction process demonstrated the possibility that the conversion of the crystal morphology could be followed by NIR and, therefore, the possibility to determine the point of complete conversion to the morphological form of interest. Figure 7 shows spectra of the digestion step of the TEA conversion to FA form of the dye. Prior to these experiments, changes in the spectra of the dye powder as a function of crystal type had been observed earlier in the region between 1800 and 2000 nm. The same spectral region in the digestion step demonstrates similar changes as shown in the expanded view in Figure 8.

Discriminant analysis

In order to use spectra to verify the identity or quality of a chemical, mathematical procedures have been established that can "learn" and recognise spectral patterns as a means of classifying the chemical. The spectrum of an unknown is compared against a set of previously collected spectra belonging to samples of the chemical known to be of acceptable quality within defined tolerances. A statistical measure is then calculated to represent the quality of the match. Several such algorithms exist and are generally classified as pattern recognition or discriminant analysis techniques. A technique that has been used successfully to measure spectral similarity in chemical analysis is Mahalanobis distance.[4] These techniques are able to be used because spectra from different materials are never exactly alike. A detailed discussion of the technique is beyond the scope of this report.

The Mahalanobis distance technique was employed to test our ability to identify the Type V crystals generated from the Type I form of the free acid dye. Six experiments of the free acid polymorph conversions were performed. The NIR spectra from these reactions are shown in Figure 9 in the lower pattern, B. Set A in Figure 9, resulting from the TEA-FA (Type I), is included for comparison. The initial and final collection of six spectra in each of sets A and B represent a pure component. The spectra at the upper and lower boundaries of set A correspond to TEA and FA (Type I), respectively. FA (Type I) and FA (Type V) are similarly represented in set B. XRD analysis of the final products from each experiment was used to confirm the complete conversion of the polymorphs from Type I to Type V. This means that intermediate spectra should correspond to a mixture of the two types, varying in relative amounts as the conversion progresses. To develop the Mahalanobis distance method for the prediction of the conversion end-point, two collections of spectra were employed. Twenty spectra comprising the last five spectra from four of the conversion experiments were collected into one set. These are spectra of Type V crystals. A second set consisted of a total of 18 spectra from the beginning of four of the conversion experiments, representing Type I crystals. The four experiments were selected so that only two of them were common to both sets of spectra. Both sets were then combined into a single training set. It is important to remember that these spectra are measured from slurries. All pattern recognition calculations were performed using the training set of 38 spectra after submitting the data to second deriva-

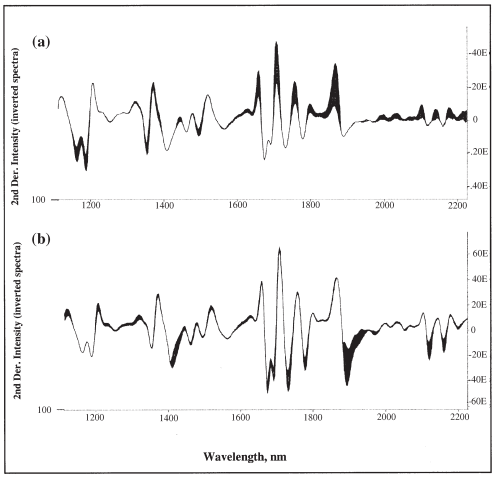

Figure 9. Spectral patterns of DYE B1 dyes; (a) TEA salt to free acid form and (b) Types I, II to Type V free acid form.

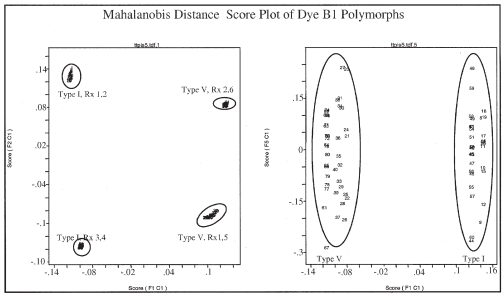

Figure 10. Discriminant analysis score plots of the Types I and V free acid from DYE B1.

tive treatment. A combined principal component and Mahalanobis distance model was then developed. Five principal components, with a cumulative variance of 99.99%, were required to sufficiently classify spectra of the two polymorphs as two different types. For the test set, 12 spectra were included from the two remaining conversion experiments, selecting spectra from various points along the reaction time axis. Spectra from the TEA-FA (Type I) experiments were also included to allow a comparison of the predicted Mahalanobis distances for the Type I polymorpths from the two types of reactions. The comparison of the Type I polymorphs only serves to identify any changes imposed on the crystals as the final products from the TEA-FA reactions are dried and prepared for the subsequent conversion experiments.

As Figure 10 illustrates, the Mahalanobis distance score plot with two principal components distinctly separates four clusters of spectra. Two of the clusters represent Type I crystals, with the Type V crystals belonging to the other two. That four clusters are formed is not surprising. The series of reactions were conducted in two different time blocks separated by several weeks. Thus, it is possible that unintended differences in reaction conditions resulted in producing crystals of slightly different particle sizes. When five principal components are used, the Type I crystal spectra are forced together into a single cluster with distinct boudaries, as are spectra of the Type V crystals. These are also shown in Figure 10. This result not only demonstrates the sensitivity of the Mahalanobis distance method in detecting differences within the

Table 1. Mahalanobis distances results for test samples.

Samples	M. Distances	Limit Test
1	378.76	Fail
2	301.65	Fail
3	276.14	Fail
4	183.98	Fail
5	127.99	Fail
6	89.85	Fail
7	30.45	Fail
8	3.237	Fail
9	0.995	Pass
10	0.491	Pass
11	0.166	Pass
12	0.224	Pass

same crystal polymorph, even though such differences arise from slight variations of the reaction conditions. More importantly, it permits differentiaion of the final product from the starting polymorph, thereby serving as the means for the conversion end-point detection.

As part of the Mahalanobis distance tests, we were interested in assessing the ability of the method to correctly identify samples based on their polymorph types. Hence, a second model was developed using only the first 20 spectra in the original training set. These spectra belong to the Type V crystals. The test set of 12 spectra were then predicted. Table 1 reports the predictions. As expected the Mahalanobis distances for the samples (corresponding to different times in the conversion reaction) range from as large as 378.76 to 0.224. The limit test correctly identifies those samples that belong to the Type V crystals and rejects those that are either Type I (at the beginning of the conversion reaction) or mixtures of the two types. The trend in the distances also suggests the possibility that the Mahalanobis distance method can be related to the amount of the undesired polymorph in the final product.

Conclusion

The results presented in this paper successfully demonstrate that near infrared spectrosocpy, in combination with Mahalanobis distance methods, can provide a means to differentiate polymorphic forms of the B1 dye. The ability to follow the conversion reaction and determine its end-point without removing samples is particularly valuable in process monitoring applications. The techniques are simple, sensitive and easy to use. It should provide benefits in terms of time-saving in the transition from chemical process development to production.

References

1. T. Norris, P.K. Aldridge and S.S. Sekulic, *Analyst* **122,** 549 (1997).
2. J. Halebin and W.J. McCrone, *Pharm. Sci*. **58,** 911 (1969).
3. M.C. Etter, R.B. Kress, J. Bernstein and D.J. Cash, *J. Am. Chem. Soc*. **106,** 6921 (1984).
4. A. Burger and K. Schulte, *Arch. Pharm*. **314,** 398 (1981).
5. E. Smakula, A. Gari and H.H. Wotiz, *Spectrochim. Acta*. **9,** 346 (1957).
6. S. Kitamura, L.-C. Chang and K. Guillory, *J. Int. J. Pharm*. **101,** 127 (1984).
7. K.J. Hartauer, E.S. Miller and K.J. Guillory, *Int. J. Pharm.* **85,** 163 (1992).
8. J. Anwar, S.E. Tarling and P. Barnes, *J. Pharm. Sci* **78,** 337 (1989).
9. M. Lagas and C.F. Lerk, *Int. J. Pharm*. **8,** 11 (1981).
10. F. Forni, G. Coppi, V. Iannuccelli and R. Cameroni, *J. Therm. Anal*. **36,** 35 (1990).
11. T.L. Threlfall, *Analyst*, **120,** 2435 (1995).
12. P. Corti, E. Dreassi, L. Savini, S. Petriconi, R. Genga, L. Montecch and S. Lonardi, *Process Control Qual*. **2,** 131 (1992).
13. P.K. Aldridge, C.L. Evans, H.W. Ward, II, S.T. Colgan, N. Boyer and P.J. Gemperline, *Anal. Chem*. **68,** 997 (1996).
14. K. De Braekeleer, R. De Maesschalck, P.A. Hailey, D.C.A. Sharp and D.L. Massart, *Chemometrics and Intelligent Laboratory Systems* **46,** 103 (1999).
15. M. Thompson and B. Martens, *Analyst* **119,** 2777 1994.
16. N.K. Shah and P.J. Gemperline, *Anal. Chem*. **62,** 465 (1990).
17. P.J. Gemperline and N.R. Boyer, *Anal Chem*. **67,** 160 (1995).
18. J.B.M. Droge, W.J. Rinsma and H.A. Van't Klooster, *J. Chemometrics* **1,** 231 (1987).
19. T. Blanton, private communications.
20. J.A. McCauley, R.J. Varsolona and D.A. Levorse, *J. Phys. D: Appl. Phys*. **23**, B85 (1993).
21. P.C. Buxton, I.R. Lynch and J.M. Roe, *Int. J. Pharm.* **42**, 135 (1988).