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A new role for cathodoluminescence spectroscopy and imaging as an analytical tool to support the development of pharmaceutical products

Applicaton Note

Cathodoluminescence (CL) analysis using a MonoCL™ system has recently been described as a technique for use with scanning electron microscopy to complement existing solid state spectroscopic and imaging tools that are used to support the understanding, development and manufacture of medicinal products.[1,2] This may seem unremarkable to the many existing users of CL spectroscopy and imaging, but it is a significant and overdue advance in pharmaceutical research and development, and as a way to support and defend intellectual property.

CL analysis is a well-established technique and many materials are now known to be cathodoluminescent (emit light when stimulated by an electron beam). Those that are studied most frequently are inorganic, predominantly as minerals, glasses, ceramics, gemstones, semiconductors, rare earths and optoelectronic materials. As a consequence, CL analysis is a routine technique that has become an invaluable analytical tool in many industries and in academia. CL provides unique insights into the chemical and crystallographic properties of these materials at the microscopic level and is used for the investigation of impurity induced defects, trace element analysis, and to map the spatial distribution of stress around defects and grain boundaries.

However, there is a dearth of published information about CL studies on organic compounds and none has been found that relates specifically to the analysis or characterization of pharmaceutical materials and medicinal products. This application note helps to redress the balance by demonstrating, with practical examples, that CL spectroscopy and spectral imaging have utility as investigative techniques to analyze and characterize many APIs (the biologically active drugs), excipients (the non-active organic and inorganic components), powder blends and formulated products.

Specimen preparation

CL spectra are acquired from powder specimens consisting of single components after they have been compacted into 4 mm diameter and 2 mm deep cavities drilled into the centers of 12.5 mm diameter aluminium pin-type stubs. Multicomponent powder samples (such as blends containing at least one API and excipients) to be imaged are sprinkled onto conductive, self-adhesive, carbon-based Spectrotabs stuck onto SEM stubs. Solid dosage forms, (such as tablets and multi-particulate beads) are examined either intact or as cross-sections that are prepared by fracturing them at room temperature. To maximize the collection efficiency of emitted light, specimens were not coated with any conductive metal.

CL spectroscopy and imaging

Cathodoluminescence spectra and monochromatic (single wavelength) CL images were acquired from uncoated specimens of APIs, excipients and products at room temperature using a MonoCL3™ system fitted to a Carl Zeiss SUPRA 40 VP variable pressure scanning electron microscope (SEM). The SEM was operated at 10 kV with the specimen chamber back-filled with nitrogen gas at a pressure of 15 Pa. This low vacuum enabled the imaging of specimens without causing beam charging artefacts; however this created an unexpected artefact with two low intensity peaks observed at about 390 nm and 425 nm that dominated the CL spectra of some weakly luminescent materials and was attributed to luminescence of the variable pressure mode gas. Typical acquisition times for the CL spectra shown in this report (acquired using a CCD and 150 l/mm diffraction grating) were a few seconds and for CL images approximately one minute.

Results and discussion

Exploratory work indicates that around 80% of drugs and other drug-like compounds are cathodoluminescent.[1] It has also been discovered that at least 80% of commonly used excipients are either non-cathodoluminescent or are very weakly luminescent. This has practical advantages because it means that there are likely to be very few issues with interference when solid dosage forms (such as tablets and blends) are imaged for the presence of APIs.

Results and discussion (continued)

Examples of CL spectra derived from seven commercially available APIs and seven commonly used excipients are given in Tables 1 and 2, respectively.

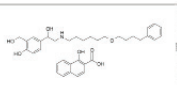
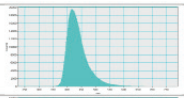
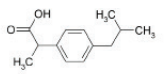
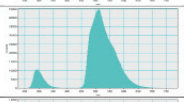
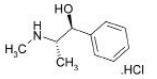
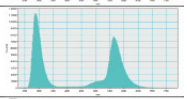
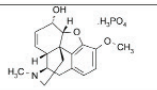
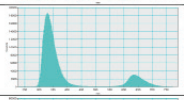
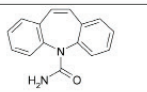
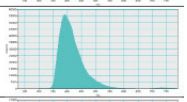
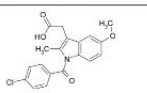
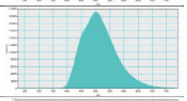
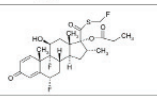
Compound name Chemical formula Molecular weight	Molecular structure	Cathodoluminescence emission spectrum
salmeterol xinafoate $C_{25}H_{37}NO_4.C_{11}H_8O_3$ 603.75		
ibuprofen $C_{13}H_{18}O_2$ 206.28		
pseudoephedrine HCl $C_{10}H_{15}NO.ClH$ 201.70		
codeine phosphate $C_{18}H_{21}NO_3.H_3PO_4$ 397.36		
carbamazepine $C_{15}H_{12}N_2O$ 236.27		
indomethacin $C_{19}H_{16}ClNO_4$ 357.79		
fluticasone propionate $C_{25}H_{31}F_3O_5S$ 500.57		

Table 1. CL spectra of some APIs.

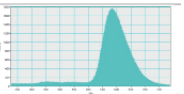
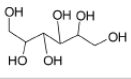
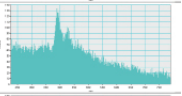
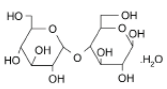
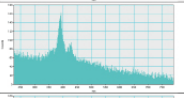
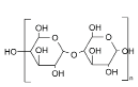
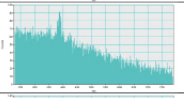
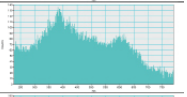
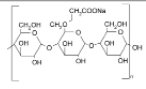
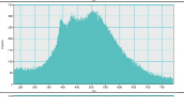
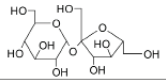
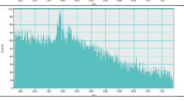
Compound name Chemical formula Molecular weight	Molecular structure	Cathodoluminescence emission spectrum
dibasic calcium phosphate $CaHPO_4$ 136.06		
mannitol $C_6H_{14}O_6$ 182.20		
lactose monohydrate $C_{12}H_{22}O_{11}.H_2O$ 360.31		
microcrystalline cellulose $(C_6H_{10}O_5)_n$ > 5,000		
calcium carbonate $CaCO_3$ 100.09		
sodium starch glycollate $(C_{20}H_{16}O_{17}Na)_n$ > 10,000		
Sucrose $C_{12}H_{22}O_{11}$ 342.31		

Table 2. CL spectra of some commonly used excipients.

As part of this exploration of the utility of CL for the analysis of pharmaceutical materials, it was discovered that for some compounds, CL could distinguish between different solid forms of some drug compounds. The four known polymorphs of carbamazepine have different crystal structures, but although their CL spectra are similar with a single broad peak (Figure 1), there are measurable differences between them. The CL spectra from crystalline and amorphous forms of a compound can also be very different, and this is exemplified by indomethacin (Figure 2). In addition, different salt forms of many compounds also have distinct CL spectra. These observations are exciting because they enable CL analysis to be used to study any changes to the solid form of an API after it has been formulated into a tablet, pellet or blend.

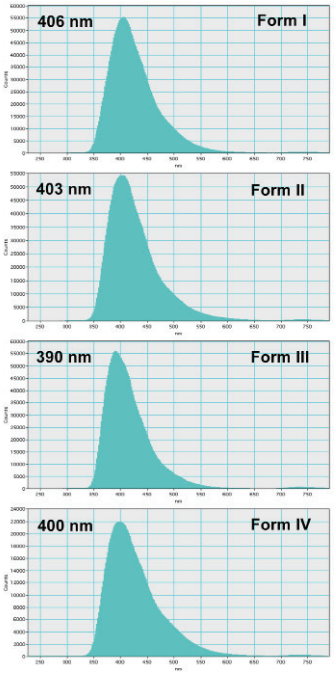


Figure 1. CL spectra for polymorphs I, II, III and IV of carbamazepine with the peak wavelength values indicated.

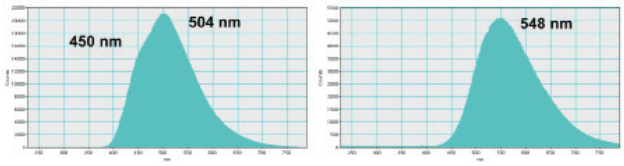


Figure 2. CL spectra for crystalline indomethacin (left) and amorphous (melt quenched) indomethacin (right).

Results and discussion (continued)

In general, adjacent peaks in a CL spectrum of an organic compound tend to be poorly resolved and those that are separated by less than about 150 nm are severely overlapped. These overlapping peaks in a spectrum can be de-convoluted to reveal its constituent peaks using spectrum analysis software supplied with the MonoCL3 system. Alternatively, the peak resolution could be enhanced by cooling specimens whilst they are being analyzed to reduce peak broadening, but the possibility that sub-ambient solid state phase transitions may occur needs to be considered because different polymorphic forms may have different CL spectra.

Luminescence is a phenomenon exhibited by many organic compounds which possess aromatic molecules or molecules having conjugated double bonds when they are irradiated, with an electron beam for example. Delocalized electrons are excited by the incident electron beam and when they relax to the ground state, excess energy is emitted as photons of light that range from the ultraviolet, through the visible and into the infrared with wavelengths from about 160 nm to about 2000 nm. The observation that different crystal polymorphs of the same compound can have different CL spectra indicates that the CL signal is not just derived from the molecule, but also from intermolecular interactions resulting from the way molules are packed in crystals.

Having established that many APIs emit light and produce characteristic spectra and that most commonly used excipients are not cathodoluminescent, CL imaging has been exploited to visualize APIs in formulated solid dosage forms. The spatial distributions of API in tablets, multi-particulate beads (from capsules), and dry powder inhalation blends have been studied using monochromatic spectral imaging. These experiments are used to assess the homogeneity of products to support the development and optimization of manufacturing processes.

Examples showing how CL analysis is being used to image APIs in products are illustrated using commercially available medicines. Figure 3 is a fractured cross-section through a Nurofen® Plus pain relief tablet and in the secondary electron image, it is not possible to distinguish between the two APIs, ibuprofen and codeine phosphate. The spatial distributions of ibuprofen (at 510 nm) and codeine phosphate (at 350 nm) are clearly seen when monochromatic imaging is used.

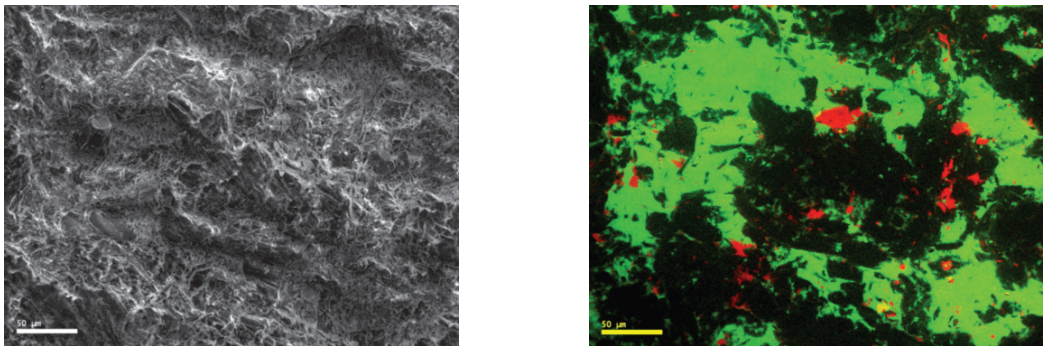


Figure 3. Cross-section taken through a Nurofen® Plus tablet showing its secondary electron image and a composite colour CL image that reveals the spatial distribution of ibuprofen at 510 nm (green) and the distribution of codeine phosphate at 350 nm (red). Scale bar = 50 µm.

Some capsule products are formulated with multi-particulate beads and these beads consist of sugar and starch-based cores with one or more APIs deposited as concentric layers separated with controlled-release polymer layers. Figure 4 shows a cross-section through a single bead, which measures about 750 µm across, from the prolonged release decongestant, Contac® which contains the API, pseudoephedrine hydrochloride. By using CL imaging to detect light emitted at 560 nm from the API, the drug-rich layer (colored yellow) is revealed. The sugar-starch core and the external control release layer (both shown as brown) are not cathodoluminescent.

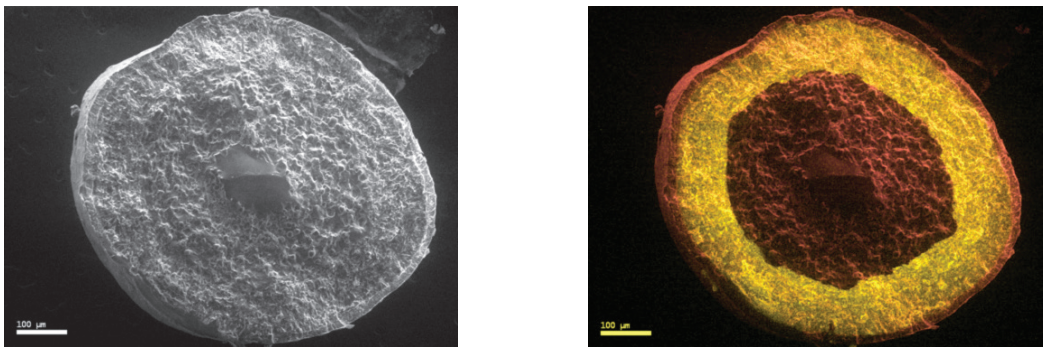


Figure 4. Electronmicrograph showing the internal structure of a Contac bead in a fractured cross-section and a composite colour CL image that reveals the layer of pseudoephedrine hydrochloride (yellow) collected at 560 nm. Note that the sugar-starch core and the external polymer control release layer (both shown as brown) do not emit light. Scale bar = 100 µm.

Results and discussion (continued)

In addition to the analysis of solid dosage forms, CL imaging may also be used to examine powder blends. Most powder blends are compressed into tablets, but some are formulated and used as loose powders in capsules or as dry powder inhalation (DPI) products. For DPI formulations, which usually comprise a mixture of micronized API and lactose monohydrate (acting as the drug carrier), the blend homogeneity and product performance, when aerosolized, depends upon how the API binds to, and is released from, the carrier. The distribution of a cathodoluminescent API throughout a DPI blend can be visualized rapidly and at high resolution using spectral imaging in the SEM. The example used here to illustrate the technique is Serevent® which contains the API, salmeterol xinafoate, and lactose monohydrate. Figure 5 shows an area of the blend measuring about 0.2 mm^2 viewed using secondary electron imaging (to show both the API and the lactose particles) and cathodoluminescence spectral imaging at a wavelength of 415 nm to reveal the locations of API particles. The distribution of the API is very clear in the CL image and it appears to be uniformly dispersed, non-agglomerated and adhering to both the fine and the coarse lactose particles; this is crucial information for product formulators.

Other spectroscopic techniques can also be used to achieve this, but CL analysis benefits from rapid data acquisition (typically spectra <1 s and images <60 s) and the high spatial resolution afforded by using a scanning electron microscope.

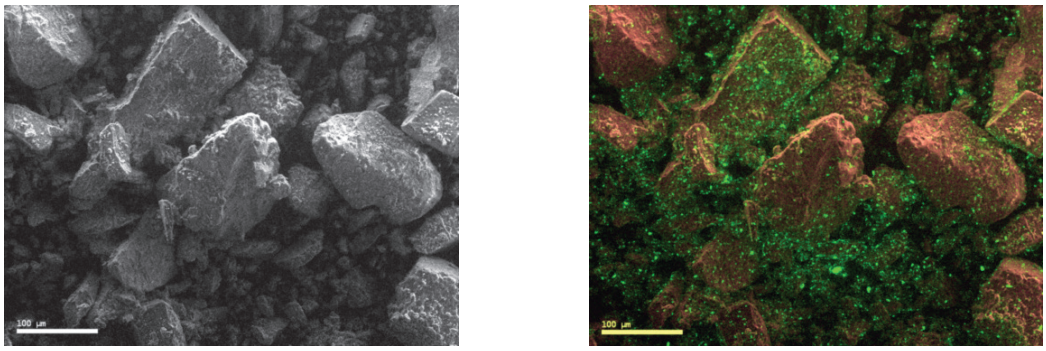


Figure 5. Secondary electron image of a binary dry powder inhaler blend (Serevent™) and a composite color CL image (collected at 415 nm) showing the spatial distribution of the micronized drug, salmeterol xinafoate (green), mixed with coarse and fine particles of non-cathodoluminescent lactose monohydrate (brown). Scale bar = 100 µm.

Conclusion

The discovery that many APIs and API-like compounds are cathodoluminescent has resulted in several exploratory spectroscopy and imaging experiments to show that this technique has potential for the analysis and investigation of pharmaceutical materials, different solid forms and as an alternative way to visualize the spatial distribution of APIs in formulated products.

The results of this investigation indicate that up to 80% of APIs and API-like compounds are moderately to strongly cathodoluminescent and this contrasts with up to about 80% of excipients that are either weakly- or non-cathodoluminescent.

CL analysis provides rapid spectral imaging at high resolution and this capability complements existing spectroscopic and chemical imaging techniques used in pharmaceutical R&D, such as elemental X-ray microanalysis, Raman spectroscopy, mid-infrared spectroscopy and near-infrared spectroscopy.

References

1. Nichols, G., 2012. Applications of Cathodoluminescence Spectroscopy and Imaging in the Characterisation of Pharmaceutical Materials. European Journal of Pharmaceutical Sciences, 45, 19-42.

2. Nichols, G., 2011. Anomalous atomic number contrast in compositional backscattered electron images of organic compounds due to cathodoluminescence. The Microscope, 59(4), 147-163.

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