Anomalous Atomic Number Contrast in Compositional Backscattered Electron Images of Organic Compounds Due to Cathodoluminescence

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ABSTRACT

It is generally accepted that when multi-component materials are examined with a scanning electron microscope, the contrast differences observed using compositional backscattered electron imaging are due to variations in their average atomic numbers. With increasing atomic number, there is an approximate monotonic increase in backscatter coefficient, which is observed as an increase in the brightness of specimens. However, what seems to be less generally appreciated is that light emitted from cathodoluminescent compounds, especially organic compounds, will also contribute to the brightness in backscattered electron images, and this may result in anomalous compositional contrast. Backscattered electron detectors used with scanning electron microscopes are either a scintillator or a solid state semiconductor and, in addition to detecting high energy backscattered electrons, these detectors also happen to be very sensitive to light. Anomalous contrast had been observed with several organic pharmaceutically relevant compounds materials, where the images of some low average atomic number materials were unexpectedly brighter than those having higher average atomic numbers. This anomaly was found to be caused by cathodoluminescence. This discovery was a revelation because, if the published literature is an accurate guide, then organic compounds are rarely reported as being cathodoluminescent, whereas there is an abundance of information about the cathodoluminescence of numerous inorganic materials, especially minerals, ceramics and semiconductors. This paper describes several experiments using compositional backscattered electron imaging, elemental X-ray microanalysis and cathodoluminescence spectroscopy, which were performed to explore the cathodoluminescent behavior of several organic compounds that give rise to anomalous image contrast.

INTRODUCTION

When a multicomponent specimen is examined in a scanning electron microscope (SEM) using backscattered electron (BSE) compositional imaging mode, it is widely accepted that variations in the signal intensity are due to variations in the average atomic number of the individual components. This effect, known as atomic number, is a compositional or material contrast where bright regions in an image correspond to areas of high average atomic number, and darker areas are those having a lower relative average atomic number (1).

Consequently, BSE atomic number imaging is frequently exploited by electron microscopists to reveal the spatial distribution of components within specimens that can be distinguished by their different el-
 elemental compositions. BSE images do not, in themselves, show what elements are present, but they are valuable because they can be used to locate dissimilar components in specimens prior to analyzing their chemistry using electron probe microanalysis. Even though backscattered electron imaging is an established technique — as this paper demonstrates — it is not always variations in average atomic number that are responsible for differences in signal intensity. It has been discovered that the light emitted by cathodoluminescent organic materials can produce significant, sometimes extreme, contrast in backscattered electron images. This anomalous contrast can be misinterpreted as atomic number contrast, but is not necessarily a disadvantage because it provides image contrast that would otherwise be absent in low atomic number compounds.

This paper describes the results of an investigation to explore how the light emitted from cathodoluminescent organic substances can cause anomalous contrast in backscattered electron images. The effect can be so extreme that misleading or ambiguous interpretations may result when attempts are made to quantify the atomic number contrast of specimens to reveal compositional variations. Some inorganic substances, including many minerals and semiconductors, are cathodoluminescent and they, too, may exhibit anomalous contrast in backscattered electron images.

A BSE detector, either a scintillator type or solid state photodiode type, is usually fitted as standard to most scanning electron microscopes. It provides useful information about the compositional (chemical) and topographical (surface) variations of specimens and complements the signal derived from an Everhart-Thorlney (E-T) secondary electron detector (1). However, despite being used ubiquitously as electron detectors, their high sensitivity to visible light appears to be less appreciated. Indeed, in electron microscopy literature, any mention of this sensitivity to light is usually absent, or is so brief and understated that it is easily overlooked, as in Goldstein, et al., who declare on page 137 of their book, “Visible light can also affect the [solid state] detector, such as cathodoluminescence from the specimen” (1). The manufacturers of BSE detectors do not stress or warn users that they are sensitive to light, except where a cathodoluminescence accessory can be added. As a consequence, the contrast seen, in what might be considered to be a BSE image, may actually have a contribution from both backscattered electrons and the light that is emitted from cathodoluminescent materials as they are bombarded with high energy electrons. This sensitivity to light is the reason why BSE detectors are deactivated to protect them from overload as stray room light enters the SEM specimen chamber when specimens are changed or when an infrared chamber viewing system is operated.

In addition to atomic number contrast, other contrast effects are sometimes seen in BSE images of polycrystalline specimens due to channelling contrast, or orientation contrast (1, 2). Different shades of grey in monochrome images are derived from variations in the orientations of crystal grains as they interact with the incident electron beam. This effect can display extreme changes in contrast when individual grains are tilted by just a few degrees or the specimen consists of many randomly orientated crystals. When the electron channelling contrast for some materials is reduced as a result of orientation, the signal may be enhanced by exploiting the emission of light if they happen to be cathodoluminescent (3).

CALCULATION OF AVERAGE ATOMIC NUMBER

To establish if the contrast seen in a compositional BSE image is derived from backscattered electrons, from cathodoluminescence, or from both, the origin of the brightness variations due to backscattered electrons is appraised. Compositional differences across a specimen are seen as variations in the brightness that is related to the number of electrons it emits. The fraction of the incident electron beam that is backscattered from a single element is dependant on its atomic number (Z) and is expressed as its backscatter coefficient (η) (1). For multi-element specimens (such as organic compounds) that are homogeneous at the atomic level, the mean backscatter coefficient with η is used to estimate the contrast seen in images and is calculated by summing the mass fractions and individual coefficients of its constituent elements using Formula 1:

\[
\bar{\eta} = \sum_i C_i \eta_i
\]

where \(C_i\) is the mass fraction, and \(\eta_i\) is the backscattering coefficient for the pure element \(i\).

With increasing atomic number, there is an approximate monotonic increase in backscatter coefficient. This relationship, although not universally followed, is fortunate because it is the basis for compositional backscattered electron imaging and is utilized for the examination and analysis of a wide range of inorganic and organic materials. For the accurate
quantitative analysis of BSE images of multi-element specimens, methods have been proposed to predict backscattering coefficients and average atomic number ($\bar{Z}$) values that are based upon electron fractions rather than mass fractions (4). However, because of the close relationship between atomic mass and atomic number across the periodic table, the method that is generally accepted and is used most frequently to calculate the approximate ($\bar{Z}$) is the simple summation of the mass fraction (w) and atomic number (Z) for each of the atoms in a compound using Formula 2:

$$\bar{Z} = w_1Z_1 + w_2Z_2 \ldots$$

In addition to calculating average backscatter coefficients and average atomic numbers that enable quantitation of compositional backscattered electron images, relative values can also be determined from the images themselves. By measuring and quantifying the brightness and contrast levels in images, it is possible to perform rapid quantitative image analysis of multiphase specimens, such as the identification of minerals in rocks (5), the analysis of cement clinkers (6) and the degree of mineralization in human bone (7). This is achieved by measuring the brightness and contrast levels for each phase in a specimen so that relative $\bar{Z}$ values can be assigned. This procedure works well because quantitation is possible from observed gray level intensities by measuring the response of the backscattered detector using pure specimens of the phases sought. However, there are materials for which the observed gray level intensities do not correspond with expected values of $\bar{Z}$ and, as a consequence, quantitative backscattered electron imaging is unreliable or impossible. Howell et al. (8) encountered inconsistencies when they discovered that the correlation between the predicted backscattered electron coefficients and experimental values for many low atomic number materials did not agree. The possibility that these inconsistencies may be the result of anomalous contrast caused by cathodoluminescence should not be discounted.

BACKGROUND AND REASON FOR THIS STUDY

The observations which suggested that average atomic number differences may not be the only cause of contrast in compositional BSE images of several powdered organic materials were made several years ago in our laboratory. Uncoated powders were examined at low vacuum in a variable pressure SEM equipped with a scintillator-type backscattered electron detector and the images produced showed anomalous contrast. These powders were experimental pharmaceutical blends prepared to support the development of medicinal products and consisted of excipients (i.e., the non-active ingredients in pharmaceutical preparations, such as $\alpha$-lactose monohydrate, starch and microcrystalline cellulose) that are intimately mixed with an active pharmaceutical ingredient (API).

The APIs used in these blends were small molecule organic compounds having medium molecular weights of 100 to 500, and many contained a relatively heavy atom, such as sodium, sulphur, chlorine or bromine. In backscattered electron imaging mode, many of these APIs were readily visible as bright objects, and it was assumed, not unreasonably, that they were bright because they contained heavy atoms. This assumption was reinforced by the observation that in some blends, particles of the inorganic excipient, dibasic calcium phosphate (which contains two heavy atoms, Ca and P), were also bright. These observations made technical sense, and analytical judgements, such as the evaluation of blend homogeneity and the visual assessment of the sizes of individual API particles, were based upon them. However, when a binary blend consisting of $\alpha$-lactose monohydrate (containing C, H and O; $\bar{Z} = 6.73$) and a development API having no heavy atom (it contained C, H, N and O; $\bar{Z} = 6.14$) was examined, the particles of API were unexpectedly much brighter than the $\alpha$-lactose monohydrate. This observation suggested that the image contrast was anomalous and was being controlled not by the difference in average atomic number, but by another mechanism resulting from the API’s interaction with the electron beam.

This anomalous behavior made sense when it was realized that, in addition to being sensitive to high energy electrons, the scintillator-type BSE detector used was also very sensitive to visible light. This suggested that the unexpectedly bright compound may be emitting light when bombarded by electrons; in other words, it was cathodoluminescent. As a consequence, bright objects seen in BSE images do not necessarily have a high $\bar{Z}$; they may actually be low $\bar{Z}$ compounds that happen to be cathodoluminescent. In these instances, it is the visible light, rather than backscattered electrons, that produces the contrast in BSE images. For some compounds that contain a heavy atom, the intensity of the expected compositional BSE signal may be exceeded by the cathodoluminescence signal. It was also observed that for many compounds, the intensity of the cathodoluminescence signal de-
increased with increasing exposure time to the electron beam. This observation is discussed later.

Cathodoluminescence is a property exhibited by many non-metallic materials, including semiconductors, minerals, ceramics and some organic compounds (3). As with ultraviolet stimulated fluorescence, cathodoluminescence of organic materials is an intrinsic property resulting from the interaction of high-energy electrons with conjugated and aromatic molecules, many of which are found in pharmaceutical compounds. The incident radiation raises delocalized π-electrons to an excited state, and upon relaxation to the ground state, excess energy is emitted as luminescence, very frequently as visible light. Previous studies have shown that there is a good correspondence between the cathodoluminescence spectra and the fluorescence spectra of organic solids (9). In addition, the relationship between the molecular structures of several organic compounds and their cathodoluminescence yields has been discussed (10).

Following this revelation that some organic compounds are cathodoluminescent, it was discovered that many of the small molecule organic compounds used in marketed pharmaceutical products, and those encountered during the development of new medicinal products, will emit light when bombarded with electrons. Cathodoluminescence imaging, as a way to analyse rapidly the spatial distribution of single components in formulated medicinal products, has the potential to complement established spectroscopic imaging methods, such as infrared, Raman and elemental X-ray analysis, which are already applied as routine techniques in pharmaceutical research and development. So, in addition to this investigation, the cathodoluminescent behaviors of many drug compounds, drug-like compounds and pharmaceutically important excipients have been explored (11).

### MATERIALS AND SPECIMEN PREPARATION

Six organic compounds, available from Sigma-Aldrich (Gillingham, Dorset, U.K.), were used for the preparation of test specimens for imaging and cathodoluminescence spectroscopy (Table I). These organic compounds comprised four commercial APIs (carbamazepine, fluticasone propionate, furosemide and verapamil hydrochloride), a commonly used pharmaceutical excipient (α-lactose monohydrate) and a non-pharmaceutical compound (BBOT). The reason for selecting carbamazepine, fluticasone propionate and BBOT as model compounds is that they display contrast in compositional BSE images that appears to be unrelated to their average atomic numbers or the presence of a heavy atom.

For each compound, the average atomic number was calculated using formula 2. Additional materials used were copper metal (\(Z = 29\)), aluminium metal (\(Z = 13\)), and carbon-based adhesive tabs (\(Z \approx 6\)).

The cathodoluminescence emissions from three different types of commercially available self-adhesive tabs were also determined to select the one that has negligible light emission for use with the test specimens. The tabs tested (supplied by Agar Scientific Ltd., Stansted, Essex, U.K.) were Carbon Tabs, Sticky Tabs and Spectrotabs (having product codes G3347N, G3109 and G3358, respectively).

For imaging using a combination of backscattered electrons, secondary electrons and X-rays, three types of test specimen were prepared: 1) two non-mixed powder specimens, 2) a composite specimen, and 3) a mixed powder specimen. Each was mounted onto a 12.5 mm diameter aluminium pin-type stub (supplied by Agar Scientific Ltd.) using a self-adhesive Spectrotab.
A fourth type of test specimen was prepared to explore the cathodoluminescence emission of the organic compounds and gain a greater understanding of how the intensity of the emitted light is affected by prolonged beam exposure (the so-called “beam effect,” which is discussed later). These specimens consisted of bulk powders filled into cavities drilled into 12.5 mm diameter pin-type stubs.

To ensure that even the weakest cathodoluminescence emission could be detected, the test specimens were not metal-coated. Then, having established that some compounds did emit light, an experiment was conducted using the mixed powders specimen to find out if the presence of a thin metal coat would suppress the emission of light. The mixed powder specimen was re-examined after it had been sputter coated with about 20 nm of platinum using a Polaron SC500 sputter coater (supplied by Fisons Instruments, U.K.) that was operated with an argon gas pressure of 0.08 mBar, a sputter current of 20 mA and a total coating time of four minutes (the specimen was tilted during coating and was rotated through 180° after two minutes to ensure a uniform coating of the uneven surfaces). After the two non-mixed powders and the composite specimen had been examined in variable pressure mode, they were also sputter coated with platinum under these same conditions so that secondary electron images could be recorded to show their components in greater detail.

The four types of test specimen were as follows:

1. Non-mixed powders specimens

Two stubs were prepared using pairs of powders mounted side-by-side on Spectrotabs so that they were in close contact but not mixed. The pairs were a) fluticasone propionate and carbamazepine, and b) fluticasone propionate and BBOT. Fluticasone propionate and carbamazepine were chosen as a pair because they have dissimilar \( Z \) values (6.990 and 5.998, respectively) and the former also has a heavy atom, sulphur and so would be expected to be the brighter of the two compounds when viewed using BSE compositional mode. Fluticasone propionate and BBOT were chosen as a pair because they have similar \( Z \) values (6.990 and 6.654, respectively) and similar sulphur contents (6.4% and 7.4% by weight, respectively) and should have similar compositional BSE brightness. To prevent intermixing, a razor blade was held vertically on the stubs as the powders were placed on either side before the blade was removed. This allowed the powders in each pair to be imaged simultaneously to ensure that the contrast observed was due to variations inherent in the powders and not variations due to exposure conditions. The three powders used to prepare these two specimens were carefully selected because they demonstrate significant and unexpected contrast differences that appear to be unrelated to their average atomic numbers or to the presence or absence of a relatively heavy atom (sulphur).

2. Composite specimen

The composite test specimen comprised a mixture of five different organic and inorganic materials. Crystals of carbamazepine and \( \alpha \)-lactose monohydrate, copper metal and a narrow strip of aluminium foil were stuck onto a low atomic number, non-cathodoluminescent, carbon-based Spectrotab. The copper was an Athene Old 400 mesh TEM grid having 45 \( \mu \)m square apertures (supplied by Agar Scientific Ltd.). As metals are non-cathodoluminescent, the two different metals, Cu (\( Z = 29 \)) and Al (\( Z = 13 \)), were selected to produce compositional BSE contrast without any contribution from emitted light.

3. Mixed powder specimen

A single specimen comprising a 50:50 mixture of carbamazepine and \( \alpha \)-lactose monohydrate powder particles was prepared to simulate a simple pharmaceutical blend. Each powder had been sieved (\( \geq 89 \mu \)m and \( \leq 211 \mu \)m) prior to mixing to remove the fine and coarse particles. The powder mix was mounted onto a Spectrotab.

4. Bulk powder specimens

Bulk specimens were prepared for the acquisition of cathodoluminescence reference spectra from the six organic compounds. These were prepared by hand-pressing (using the end of a metal rod) about 20 mg of each powder into 4 mm-diameter by 2 mm-deep cavities that had been drilled into the centers of 12.5 mm-diameter aluminium pin stubs. Each powder was well-packed into the cavities to exclude pockets of air that could expand explosively as specimens are evacuated in the SEM chamber. In addition to spectroscopic analysis, bulk specimens of the APIs, furosemide and verapamil hydrochloride, were also used to investigate the way that the cathodoluminescence signal changes during exposure to the electron beam.

EXPERIMENTAL METHODS

The specimens were examined using two scanning electron microscopes that were equipped with different detectors to distinguish between anomalous and real compositional backscattered electron imag-
The first was a Topcon SM-300 variable pressure SEM (Tokyo) with an E-T secondary electron detector, a Centaurus BSE detector (KE Developments, Cambridge, U.K.) and a PGT Spirit energy dispersive X-ray microanalyser (Princeton, N.J.) with a detector takeoff angle of 21° from the horizontal. The second was a Carl Zeiss SUPRA 40VP variable pressure field emission SEM (Carl Zeiss NTS Ltd., Cambridge, U.K.) with a variable pressure secondary electron (VPSE) detector.

Fitted to the SUPRA 40VP was a Gatan MonoCL3 dispersive spectrometer (Gatan U.K., Abingdon, Oxford, U.K.) configured for the rapid acquisition of cathodoluminescence spectra. The light emitted from specimens was dispersed using a 150 lines/mm diffraction grating and directed to a Pixis 100 Peltier-cooled CCD camera (Princeton Instruments, N.J.). The calibration of the cathodoluminescence spectrometer was checked using the mercury emission spectrum from an in-line lamp (supplied by Gatan), which produces sharp peaks at precisely known positions within the visible spectrum without the need to vent the SEM specimen chamber.

**Specimen tabs**

Cathodoluminescence emission spectra across a wavelength range of 220 nm to 790 nm were acquired from the three non-coated, self-adhesive tabs and the empty pin stub (which acted as a noncathodoluminescent control) using the Gatan MonoCL3. The weak intensity light signal emitted across the selected spectral range from each specimen was captured with a paraboloidal mirror positioned directly above each specimen using the fast spectroscopy ParaCL parallel detection mode. Spectra were acquired at room temperature for 20 seconds and the dark noise (i.e., the quiescent signal generated by the CCD camera when no light is being detected) was subtracted automatically from each spectrum. The SUPRA 40VP SEM was operated in variable pressure mode at a chamber pressure of 15 Pa (with nitrogen gas), a beam accelerating voltage of 10 kV and a 60 μm beam aperture. The specimen working distance of 12 mm was optimized for cathodoluminescence detection. Specimens were scanned rapidly at a magnification of about 200x with a scanned area measuring approximately 700 μm by 500 μm (0.35 square mm).

**Non-mixed powders specimens**

The interfaces between the pairs of uncoated organic compounds on the two nonmixed powder test specimens were examined at a magnification of 50x using the Topcon SM-300. The SEM was operated with a chamber pressure (air) of 130 Pa, a beam accelerating voltage of 20 kV and the non-tilted specimens had a working distance of 12 mm. Images of these specimens were produced using the Centaurus BSE detector.

Qualitative elemental energy dispersive X-ray microanalysis (EDX) spectra from the fluticasone propionate, carbamazepine and the BBOT were acquired using the PGT Spirit system to show the elements they contain. For the compositional BSE images, the compounds containing a relatively heavy atom (in this case, sulphur) would be expected to show the greatest contrast. So, to complement the BSE images, digital dot maps were produced to show the spatial distributions of sulphur across both of the non-mixed test specimens.

Secondary electron images of the non-mixed powder specimens were also recorded after they had been sputter coated with platinum to show clearly the spatial distribution of the particles.

**Composite specimen**

The composite specimen was examined with both the Topcon SM-300 SEM and the Zeiss SUPRA 40VP SEM using complementary imaging modes. Before being examined by electron microscopy, a reflected light photomicrograph was taken of the composite specimen to show the distribution of the five components.

The Topcon SM-300 SEM was used to generate a backscattered electron image and a cathodoluminescence image of the same field of view of the composite specimen using the Centaurus BSE detector at a magnification of 100x in variable pressure mode at 20 kV. This detector incorporates a retractable tip that is fitted with a curved scintillator surrounding the primary electron beam entry hole, as shown in Figure 1. Light generated, when backscattered electrons strike the scintillator, is detected by a photomultiplier tube, which has a spectral response of 300 nm to 650 nm. Although a cathodoluminescence tip is available for use with the Centaurus detector, this accessory was not used. So for the panchromatic detection of cathodoluminescence with the Centaurus detector, a temporary modification was made. The backscattered electron signal was decoupled from the cathodoluminescence signal by covering the curved scintillator with a close-fitting mask made of aluminium foil having its shiny side facing the specimen to reflect light into the photomultiplier tube (Figure 1).

To confirm that it is light and not backscattered electrons that give rise to the anomalous contrast shown by the carbamazepine, the composite test speci-
men was also examined with the Carl Zeiss SUPRA 40VP SEM at 100x in variable pressure mode. The SEM was operated at 10 kV with a chamber gas (nitrogen) pressure of 25 Pa, and a secondary electron image was collected using a variable pressure secondary electron (VPSE) detector having a bias voltage of +300 V. The VPSE detector does not detect electrons, but it responds to the visible light that is generated when secondary electrons emitted from specimens interact with gas molecules in the specimen chamber. This process is known as gas luminescence or scintillation (12). The process is enhanced by applying a positive bias voltage to the detector to draw and accelerate electrons towards it and by optimizing the gas pressure. By virtue of its design, where light is collected and directed into a photomultiplier tube along a transparent glass light guide, the VPSE detector is very sensitive to low levels of visible light and, as a consequence, will function as a panchromatic CL detector. Its CL collection efficiency is not optimum, because it is positioned to one side of the specimen, rather than being positioned directly above the specimen, as is the case with the purpose-designed Gatan MonoCL3 collection mirror. So when a zero or a negative bias voltage is applied to the VPSE detector at a low gas pressure (e.g., 5 Pa), secondary electrons are not attracted to it and the light emitted from cathodoluminescent materials is detected. A second image of the specimen was acquired using the VPSE detector to capture the light that was being emitted.

**Mixed powder specimen**

The uncoated mixed powder specimen was imaged in variable pressure mode (with a chamber pressure of 39 Pa) using the Zeiss SUPRA 40VP SEM at a magnification of 50x and a beam accelerating voltage of 12 kV. It was observed in secondary electron mode using the VPSE detector (with a bias voltage of +200 V) to view both components in the mixture and then with a bias voltage of 0 V to detect emitted light only.

To establish if the presence of a thin coating of metal would inhibit the emission of light from a cathodoluminescent material, the mixed powder specimen was sputter coated with platinum. The same field of view that was observed previously was re-examined using the VPSE detector with a bias of 0 V, a chamber pressure of 10 Pa, but with a reduced beam accelerating voltage of 10 kV. The slightly lower beam voltage was selected to optimize the image and also to establish if light would still be emitted.

**Bulk powder specimens**

Bulk powder specimens were prepared for two purposes: to acquire cathodoluminescence reference spectra, and to explore electron beam induced changes to the cathodoluminescent yield from organic compounds.

1) Cathodoluminescence emission reference spectra for fluticasone propionate, carbamazepine, BBOT, and α-lactose monohydrate were acquired from uncoated specimens with the Gatan MonoCL3 under the same experimental conditions as used for the specimen tabs (see the “Specimen tabs” section on page 152).

2) For many organic compounds, it had been observed that the intensity of the emitted cathodoluminescence signal decreased as specimens were exposed to the electron beam, especially when it was concen-
treated into a smaller area at higher magnifications or with a reduced area scan. Sometimes, the cathodoluminescence intensity reduced after just a few seconds of irradiation. This phenomenon was explored to determine how cathodoluminescence emission is affected by exposure to the electron beam using uncoated bulk powder specimens of furosemide and verapamil hydrochloride. These were examined using the same acquisition conditions as described for the specimen tabs. For the furosemide, a low magnification (25x) image was recorded with the Carl Zeiss SUPRA 40VP SEM at 10 kV in variable pressure mode (15 Pa), using the VPSE detector to show how it appeared before a 20 second duration reduced-area scan was made at a magnification of 200x near to the center of the specimen. A second image was then recorded at 25x to show how the rastered area had become darker as a result of exposure to the beam. Then, to establish if the darkened area would revert to its original state seen prior to the reduced-area scan, the specimen was left inside the SEM specimen chamber for 14 days (under vacuum with no exposure to an electron beam or to light), and a third image was recorded. For the purpose of this investigation, the assessment of specimen darkening was qualitative rather than quantitative. Quantitative measurements would require an accurate measurement of the primary electron beam current, which is straightforward to do in a high vacuum using a Faraday cage and a picoammeter. When examining specimens in the gaseous environment of the variable pressure SEM, accurate current measurements are complicated due to ionization of the gas, beam spreading and also by the influence of the bias voltage applied to the VPSE detector (13).

During this investigation, furosemide was found to be an example of a compound with a single cathodoluminescence emission peak that decreased in intensity with electron beam exposure. So a second compacted powder specimen of furosemide was prepared to allow the intensity of the peak to be monitored as six cathodoluminescence spectra were recorded from the same irradiated area at 20 second intervals over a two-minute period.

In contrast to the furosemide, verapamil hydrochloride is an example of a compound that has a more complex spectrum with multiple peaks. It was also observed that during prolonged exposure to an electron beam, the relative intensities of the peaks in the multi-peak spectrum will vary. To illustrate this effect, cathodoluminescence spectra were acquired after 20 seconds duration and again after 60 seconds from the same area on a bulk specimen of verapamil hydrochloride.

RESULTS AND DISCUSSION

The results of the observations made during the examinations of the adhesive tabs and each of the four types of test specimens are discussed.

Cathodoluminescence of the specimen tabs

The cathodoluminescence emission spectra for the three adhesive specimen tabs and the empty pin stub are shown in Figure 2. The spectrum for the empty pin stub has a signal intensity that does not exceed 80 counts and has no distinctive emission peak above background; it is, essentially, electronic noise. This confirms that the aluminium pin stub is non-cathodoluminescent within the wavelength range measured. In comparison, the Carbon Tab has a com-
Figure 3. SEM images of the non-mixed specimen each showing fluticasone propionate on the left and carbamazepine on the right. The left micrograph was acquired using backscattered electron imaging mode to show the anomalous contrast displayed by the carbamazepine (which has the lower average atomic number). The middle image reveals the spatial distribution of sulphur X-rays, and the right secondary electron image was recorded after sputter coating with platinum.

Figure 4. SEM images of the non-mixed specimen each showing fluticasone propionate on the left and BBOT on the right. The left micrograph was acquired using backscattered electron imaging mode to show the extreme anomalous contrast displayed by the BBOT. The middle image reveals the spatial distribution of sulphur X-rays, and the right secondary electron image was acquired after sputter coating with platinum.

plex emission spectrum having three relatively intense peaks at about 290 nm, 335 nm and 565 nm and a weak peak at about 500 nm. For this reason, a Carbon Tab is unsuitable as a support for cathodoluminescent powders, especially if the powder is a weak emitter of light, because it would cause interference in both emission spectra and spectral images.

Inspection of the spectrum for the Sticky Tab reveals that it does have two low intensity peaks at about 290 nm and 560 nm that are just above background. These peaks correspond approximately to the two most intense peaks for the Carbon Tab and are most likely derived from the adhesive. A Sticky Tab would certainly be more suitable than a Carbon Tab for cathodoluminescence studies.

Finally, the spectrum for a Spectrotab is practically devoid of cathodoluminescence emission phenomena with just two very weak, broad peaks evident at about 280 nm and 550 nm. When the spectrum for the Spectrotab is compared with that for the empty pin stub in Figure 2, they are seen to be very similar. For this reason, Spectrotabs are recommended for use with powdered specimens when a low background support is required for cathodoluminescence spectroscopy and imaging.

Non-mixed powder specimens

Figure 3 shows images of the non-mixed specimen comprising fluticasone propionate and carbamazepine; Figure 4 shows images of the non-mixed specimen with fluticasone propionate and BBOT.

In Figure 3, the left micrograph was acquired in backscattered electron compositional imaging mode, and it is evident that the carbamazepine (right) is brighter than the fluticasone propionate (left). This observation suggests that the carbamazepine has an average atomic number that is considerably greater than that of the fluticasone propionate. However, carbamazepine has the lower $Z$ (5.998), while fluticasone propionate has the greater $Z$ (6.990).
Carbamazepine is clearly displaying anomalous compositional BSE contrast. In addition, unlike carbamazepine, fluticasone propionate contains a relatively heavy atom, sulphur, as shown when their EDX spectra are compared (Figure 5). Using the sulphur as a marker element, an X-ray elemental distribution map (Figure 3, middle image) confirms that sulphur is confined to the fluticasone propionate. The secondary electron image (Figure 3, right image) of this specimen, after being platinum coated, shows the particles of the two compounds in greater detail and the cuts in the Spectrotab caused by the razor blade when the specimen was being prepared.

The non-mixed specimen prepared using fluticasone propionate and BBOT also shows anomalous compositional BSE contrast, as shown in Figure 4. However, they were expected to have very similar BSE contrast, because these two compounds have similar average atomic numbers (6.990 and 6.654, respectively) and, as shown by their EDX spectra (Figure 5), they have similar sulphur contents (6.4% and 7.4% by weight, respectively). The left image in Figure 4 was acquired using backscattered electron compositional imaging mode. The BBOT (right) is seen to be considerably brighter than the fluticasone propionate and is also much brighter than the carbamazepine shown in the left image in Figure 3. The BBOT used was “scintillator grade,” so it is no surprise that it is cathodoluminescent. The surprise is that it is excessively brighter than the fluticasone propionate, because both compounds have similar $Z$ values. The middle image in Figure 4 shows the spatial distribution of sulphur across the non-mixed specimen, and the two components cannot be distinguished because they contain similar amounts of sulphur. The right image in Figure 4 shows the specimen viewed using secondary electrons after it had been sputter coated with platinum.

The evidence collected from the two non-mixed powder specimens indicates that the anomalous compositional contrast exhibited by carbamazepine and BBOT is not caused by atomic number contrast. Cathodoluminescence spectra for fluticasone propionate, carbamazepine and BBOT that were acquired from the bulk powder specimens are shown in Figure 6. The carbamazepine and BBOT are intensely strong emitters of light with peak maxima at about 390 nm and 475 nm, respectively. The spectrum for fluticasone propionate, on the other hand, suggests that it is a poor emitter of light with just three very weak peaks at about 390 nm, 425 nm and 470 nm, which are just above the background (compare the spectrum with that obtained from the empty, non-cathodoluminescent, aluminium pin stub in Figure 2). The source of these three weak peaks is discussed later in the section “Mixed powder specimen” in relation to the cathodoluminescence spectrum derived from $\alpha$-lactose monohydrate. It can be concluded, therefore, that the anomalous contrast observed in these two specimens is caused by the light being emitted and collected by the Centaurus BSE detector.
Composite specimen

Figure 7 shows the composite specimen viewed as a reflected white light photomicrograph and as a secondary electronmicrograph (having been sputter coated after all of the SEM imaging experiments had been completed). These images show the spatial distribution of the four components stuck to the black Spectrotab with the copper TEM grid overlain with a vertical strip of aluminium foil, particles of \(\alpha\)-lactose monohydrate at lower left and particles of carbamazepine at lower right.

When examined in the Topcon SM-300 SEM with compositional BSE mode, using the Centaurus detector with the scintillator uncovered (normal operation), the copper TEM grid and aluminium foil were visible, as shown in the left image of Figure 8. As expected, the copper was much brighter than the aluminium and the \(\alpha\)-lactose monohydrate particles were invisible. However, the carbamazepine was unexpectedly visible and had brightness similar to that of the aluminium. This observation suggested that their average atomic numbers were similar, but carbamazepine has an average atomic number of 5.998, which is less than half that of the aluminium (\(\bar{Z} = 13\)). Additionally, the \(\alpha\)-lactose monohydrate has an \(\bar{Z}\) of 6.730, so it should be brighter when viewed using compositional BSE imaging than the carbamazepine, but it is not. To establish if the anomalous contrast displayed by the carbamazepine was due to cathodoluminescence, the same field of view was imaged using the BSE detector after it had been reconfigured to detect light rather than electrons. The right image in Figure 8 shows just the light being emitted from the composite specimen, and it is only the carbamazepine that is visible. Note that each image in Figure 8 was adjusted to give the optimum brightness range for the visible objects, and this has resulted in the carbamazepine crystals appearing to be darker in the left image compared to those in the right image.

Figure 9 shows the uncoated composite specimen viewed in variable pressure secondary electron mode (left image) and in cathodoluminescence mode (right image) using the Zeiss SUPRA SEM. In the VPSE image, all of the materials are visible (including the Spectrotab), but the carbamazepine is excessively bright. As described earlier (see the “Composite Specimen” section on page 152), the VPSE detector responds to the light generated as secondary electrons interact with gas molecules in the chamber as they are accelerated under the influence of a positive detector bias. As the carbamazepine is extremely bright when compared with the copper, aluminium and \(\alpha\)-lactose monohydrate (which all have average atomic numbers that are greater than carbamazepine), the VPSE detector is most likely responding to both secondary electrons and light emitted from the composite specimen. To confirm this, the VPSE detector bias was set to 0 V (to prevent the attraction of secondary electrons towards it and to allow the detection of light only) and, as shown in Figure 9 (right image), only the cathodoluminescent carbamazepine is visible. Close inspection of this image reveals further convincing evidence to verify that the carbamazepine is cathodoluminescent, because the edges of the TEM grid and the aluminium foil adjacent to the particles of carbamazepine are illuminated by the glare of the light being emitted.

Mixed powder specimen

The left micrograph in Figure 10 is the VPSE image of the uncoated mixed powder specimen of \(\alpha\)-lactose monohydrate and carbamazepine that was...
produced with a positive bias voltage on the detector. This image resembles a BSE compositional image, and both compounds are clearly visible. The bright particles of carbamazepine are easily distinguished from α-lactose monohydrate because they are much brighter. It is even possible to see several small particles of carbamazepine (those that were produced by attrition during the gentle mixing of the two sieved components) on the surfaces of the α-lactose monohydrate particles. The high contrast shown by the carbamazepine relative to that of the α-lactose monohydrate was also observed in the composite specimen (Figure 9) and is due to the cathodoluminescence of the carbamazepine. The cathodoluminescence spectra for carbamazepine and α-lactose monohydrate acquired at low vacuum are shown in Figure 6.

As mentioned earlier, carbamazepine has a very intense peak emission at about 390 nm, while α-lactose monohydrate appears to be very weakly cathodoluminescent with at least three low-intensity peaks that occur in the ultraviolet and near-ultraviolet at about 350 nm, 390 nm and 425 nm. These peaks
coincide with the low-intensity peaks for fluticasone propionate (see the “Non-mixed powder specimens” section on page 155). As these two compounds have very different chemical compositions (Table 1), it is unlikely that these shared peaks are due to a chemical effect. These cathodoluminescence peaks result from electron-induced luminescence of the nitrogen gas as the specimens were being examined and analyzed using the variable pressure mode. This phenomenon in a SEM operated with a gaseous environment is known as gaseous scintillation, which is well-documented (12, 14).

As described earlier (see the “Composite specimen” section on page 152), gaseous luminescence is also the process used by the VPSE detector in the Zeiss SUPRA 40VP SEM to detect secondary electrons for the imaging of specimens in variable pressure mode. By operating the SEM in high vacuum mode, it has been demonstrated (11) that the weak gas emission peaks are absent. This observation means that both fluticasone propionate and α-lactose monohydrate are actually non-cathodoluminescent and the low-intensity gas emission peaks are an artifact of the analysis conditions. In addition, it has been discovered that many other non-cathodoluminescent organic compounds also display the weak gas emission peaks when they are analyzed for cathodoluminescence in a variable pressure SEM (11).

The middle micrograph in Figure 10 is the cathodoluminescence image produced when the VPSE detector bias voltage was zero. The signal from the particles of α-lactose monohydrate has been suppressed so that only the bright particles of carbamazepine are visible; even the smallest particles of carbamazepine are easily seen. This image is effectively a binary image (black and white) and would be suitable for image analysis without the need for complex image preprocessing to quantify the number and the sizes and spatial distribution of the carbamazepine in the mixture.

Even when the specimen has been sputter coated with platinum (as shown in the right micrograph in Figure 10), the light emitted from the carbamazepine is so intense that some of it leaks through the coating.

This experiment has highlighted the ability to distinguish between different components visually in a mixture based upon differences in their cathodoluminescence behaviors. As a consequence, it provides the opportunity to rapidly examine a wide variety of blended powders, manufactured products and many other types of specimens with the benefit of imaging using the high resolving power of the SEM.

**Bulk powder specimens**

The reference cathodoluminescence spectra from the bulk powder specimens of fluticasone propionate, carbamazepine, BBOT and α-lactose monohydrate are shown in Figure 6.

Figure 11 shows three low-magnification electronmicrographs recorded using the Zeiss VPSE
Micrographs of the mixed powder specimen imaged with a Zeiss VPSE detector. The secondary electron image of the uncoated specimen (left) shows both the $\alpha$-lactose monohydrate and carbamazepine. The middle micrograph reveals just the cathodoluminescent carbamazepine when the VPSE detector bias voltage was zero. The carbamazepine still emits light after the specimen has been sputter coated with platinum (right).

Electron micrographs showing the effect of electron beam exposure on a bulk specimen of furosemide powder: before a reduced area scan (left), after a 20-second reduced area scan (middle) and after 14 days with no beam exposure (right).

detector configured for cathodoluminescence detection of a single bulk specimen of furosemide. The left image shows the specimen before a reduced area scan (at higher magnification) was acquired. After scanning the specimen at 200x for just 20 seconds, the smaller scanned area is clearly visible in the middle image as the darker rectangle. The right image is of the same area after 14 days without any beam exposure (except to record this image) and the darkened area remains present with no appreciable change. This visible darkening is a phenomenon that has been recognized by other researchers when examining cathodoluminescent organic materials and has a significant affect on the luminescent yield, which deserves discussion here.

De Mets and Lagasse (15) observed this drop in cathodoluminescence yield and described it as the “beam effect.” It has a measurable reduction of the intensity of emitted light, as noted by Niitsuma, et al. (16). The images shown in Figure 11 are typical of the beam effect. Figure 12 is the cathodoluminescence spectrum for furosemide with a single emission peak centred at 417 nm. Figure 13 shows a succession of six cathodoluminescence spectra (an expanded wavelength scale is used for clarity) for furosemide recorded at intervals of 20 seconds over a two-minute period to illustrate how the intensity of the peak at 417 nm reduces in intensity over time without a shift in the peak wavelength.

Although the rectangular area shown in Figure 11 has the appearance of classic electron beam damage that can reduce the yield of secondary electrons due to the deposition of carbon and other breakdown products after prolonged beam exposure (17), the beam effect discussed here seems to result from a completely different, less-destructive process. The beam effect shown in Figure 11 was caused after just 20 seconds of beam exposure in a SEM benefiting from having a completely oil-free vacuum system. The interaction between the specimen and the electron beam causes the visible darkening. This is probably analogous to luminescence quenching where the electronic environments...
in and around molecules are disrupted and the ability to emit light is reduced or even stopped. In addition, the area displaying the beam effect remains dark (i.e., it emits less light than a non-irradiated area) even after being given the chance to recover for 14 days inside the SEM specimen chamber. This suggests that the beam effect is non-reversible; this is in accord with previous observations (15).

Studies of aromatic organic compounds in the SEM by Egerton, et al. (18) have shown that a measure of the radiation damage to specimens is given by the variation in CL yield with incident beam energy, and that the CL signal decays exponentially during irradiation. Also, when incident beam energies are above 1 keV, it is suggested that each molecule of a cathodoluminescent compound will emit just one photon of light before the emission is suppressed because of radiation damage. This is the most likely cause of the beam effect.

When the cathodoluminescence emission spectrum from a compound comprises several peaks, the reduction in luminescence intensity may not be uniform for each peak. To illustrate this, Figure 14 shows the spectrum for verapamil hydrochloride with its three emission peaks at 315 nm, 530 nm and 611 nm. After just 60 seconds of continuous exposure to electrons, the relative peak intensities change considerably, and the 315 nm peak red shifts to 337 nm and its intensity is reduced by about half. In addition, the intensity of the 530 nm peak increases more than fourfold (to about 31,000 counts from about 7,000 counts), and the peak at 611 nm reduces to a weak shoulder on the high wavelength side of the 530 nm peak.

The beam effect clearly has a profound influence on the emission spectra of compounds, and this could preclude the use cathodoluminescence for quantitative analysis and some imaging. If qualitative analysis or imaging is adequate to investigate a material, then a weak cathodoluminescence signal may still be detected using the highly sensitive MonoCL3.
CONCLUSION

The observation that some organic compounds with low average atomic numbers produced unexpectedly high contrast in compositional backscattered electron images has been explored. Experiments using four types of test specimens resulted in the discovery that many drug and drug-like compounds are cathodoluminescent and emit considerable amounts of light when exposed to an electron beam. The anomalous contrast was understandable when it was realized that backscattered electron detectors are not only sensitive to high energy electrons, but are also very sensitive to visible light. So, when a cathodoluminescent material is examined in a scanning electron microscope, the contrast in a BSE image is boosted. This is because the signal collected is a combination derived from both light and electrons. Only by confirming the actual chemical compositions of materials that appear bright, using a technique such as elemental X-ray microanalysis, can cathodoluminescent materials be distinguished from those that happen to have a relatively high \( Z \).

The cathodoluminescence signal emitted by some of the organic compounds tested, especially carbamazepine, furosemide, verapamil hydrochloride and BBOT, was so intense that cathodoluminescence spectra and images could be acquired in just a few seconds. Although cathodoluminescent inorganic compounds (such as minerals, ceramics and semiconductor materials) were not examined as part of this investigation, the intensity of the light emitted from them is often much less than that emitted from many organic compounds and so data acquisition usually takes much longer. The compositional BSE signal from inorganic compounds, which tend to contain heavier atoms (such as silicon, sulphur, calcium, iron, etc.) than those found in organic compounds, is likely to exceed the cathodoluminescence signal. Therefore, anomalous compositional BSE contrast may only be encountered when low average atomic number organic compounds, such as pharmaceuticals or agrochemicals, are studied using scanning electron microscopy.

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REFERENCES

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