ELEMENTAL IMAGING FOR PHARMACEUTICAL TABLET FORMULATION ANALYSIS BY MICRO X-RAY FLUORESCENCE

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ABSTRACT

The analysis of the distribution of pharmaceutical materials in tablet formulations, such as drugs and matrix elements, is critical to product performance and is used in such areas as quality control, impurity testing, and process monitoring. Recently imaging techniques, such as Raman, near-IR, and fluorescence imaging, have become popular for “visualization” of pharmaceutical formulations, allowing for spatial and chemical composition information to be obtained simultaneously. These methods have been primarily focused on molecular imaging, or spatial analysis of the molecular characteristics of the tablet formulation. However, elemental species are also an important part of pharmaceuticals. Micro X-ray Fluorescence (MXRF) elemental imaging offers complementary information to molecular imaging techniques. In this study, MXRF was used for the elemental imaging of various commercial pharmaceutical drug and vitamin supplements. Specifically, elemental composition and heterogeneity were monitored for each different tablet.

INTRODUCTION

The distribution, particle size, and morphology of pharmaceutical materials within a tablet dosage form, such as drugs, lubricants, and excipient components, affect a number of properties of the tablet. These include physical attributes such as tablet hardness, robustness, and adhesion to the tablet punch, as well as more chemical characteristics such as tablet lifetime, component bioavailability and bodily uptake [1]. Many different types of analysis techniques have been used to study the characteristics of the components in pharmaceutical powders and oral dosage forms. For example infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy have been used to study pharmaceuticals at the molecular level [2]. Particle morphology and size distribution can be characterized by techniques such as scanning electron microscopy (SEM) and X-ray diffraction (XRD) [2]. Atomic force microscopy (AFM) has been used to study the effects of mechanical processing on surface stability of pharmaceutical powders [3]. Total reflection X-ray fluorescence (TXRF) has been used to study trace elements in drugs [4]. One of the major drawbacks of many of the techniques listed above, such as IR, SEM, XRD, and AFM, is that they only allow for either single point analyses or characterization of only a small area of a tablet. Techniques such as TXRF and NMR require that the pharmaceutical solid be dissolved prior to analysis, altering the original state of the material.

Imaging techniques are becoming popular as new methods for analyzing solid dosage forms. These techniques are often non-destructive and allow for the acquisition of component distribution information over a relatively large area of a tablet. They also require minimal sample preparation and manipulation, leaving the sample intact and in its original state during
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analysis. Specifically, molecular imaging techniques have been implemented for tablet and powder characterization. Fourier transform near infrared (FT-NIR) methods have been used to study the distribution of different organic ingredients in tablets with a spatial resolution of ~20-100 µm [5]. Attenuated total reflection infrared imaging has been used to look at the distribution of molecular species with a spatial resolution of ~4-15µm [6, 7]. Molecular Raman imaging, which is complementary to IR imaging, as also been successfully used for pharmaceutical tablet component characterization [5, 8].

Molecular imaging techniques such as IR and Raman are excellent for studying organic components and molecular species which have strong IR and Raman signals. However, pharmaceutical dosage forms also contain many inorganic constituents that are often very important to tablet function and may not be easily detected by molecular techniques. For example vitamin tablets, such as Vitamin B-12 or iron supplements, contain metals that are integral to their function. Inorganic species such as calcium phosphate and magnesium stearate are used as tablet binders and lubricants respectively. Similarly, monitoring of potential drug contamination by inorganic impurities, e.g. halogens, arsenic, and the heavy metals, is also important to pharmaceutical quality control. One alternative is to use elemental imaging for study of tablet inorganic components which would complement the information gathered by molecular imaging techniques. Laser induced breakdown spectroscopy (LIBS) has been used for elemental imaging of solid dosage forms[9]. However, the technique is destructive, as the laser ablates the surface of the sample during each analysis. In this study, a non destructive elemental imaging technique using Micro X-ray Fluorescence (MXRF) was used to study the elemental distributions of inorganic components in pharmaceutical oral dosage forms.

**EXPERIMENTAL**

MXRF imaging was performed in vacuum using an EDAX Eagle II XLP system equipped with a Rh target excitation source and a SiLi detector (EDAX, Mahwah, NJ). The X-ray source was equipped with a polycapillary focusing optic having a 60 µm nominal X-ray spot diameter (X-ray Optical Systems, Albany, NY). Figure 1 is a diagram of the MXRF experimental setup.

For each tablet, images were acquired with a pixel matrix of 128x100 and a 200 ms dwell time per pixel. Table 1 lists the element emission lines and energies that were monitored in this study. The commercially available pharmaceutical tablets used in this study were purchased at random at a local grocery store. A list of the different types of tablets used in this study as well a list of their major ingredients that could possibly be detected by MXRF is listed in Table 2. The tablets were prepared for imaging by slicing them in half with a stainless steel razor blade so that a flat, even surface was exposed to the instrument for analysis. Care must be taken that the razor blade is new (not rusty) and clean, and that it is cleaned between preparation of different specimens to avoid sample contamination.
Table 1. Element emission lines and energies monitored in this study.

<table>
<thead>
<tr>
<th>Element</th>
<th>Emission Line</th>
<th>Emission Line Energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>K-L</td>
<td>1.041</td>
</tr>
<tr>
<td>Mg</td>
<td>K-L</td>
<td>1.254</td>
</tr>
<tr>
<td>Si</td>
<td>K-L</td>
<td>1.740</td>
</tr>
<tr>
<td>P</td>
<td>K-L</td>
<td>2.015</td>
</tr>
<tr>
<td>S</td>
<td>K-L</td>
<td>2.308</td>
</tr>
<tr>
<td>Bi</td>
<td>M-N</td>
<td>2.423</td>
</tr>
<tr>
<td>Rh</td>
<td>L-M</td>
<td>2.696</td>
</tr>
<tr>
<td>Ca</td>
<td>K-L</td>
<td>3.691</td>
</tr>
<tr>
<td>Fe</td>
<td>K-L</td>
<td>6.403</td>
</tr>
<tr>
<td>C0</td>
<td>K-L</td>
<td>6.930</td>
</tr>
<tr>
<td>Zn</td>
<td>K-L</td>
<td>8.638</td>
</tr>
<tr>
<td>Bi</td>
<td>L-M</td>
<td>10.836</td>
</tr>
</tbody>
</table>

Table 2. Ingredients of pharmaceutical tablets investigated in this study that may be detected by MXRF.

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach Relief</td>
<td>Bismuth subsalicylate, calcium carbonate, magnesium stearate, mannitol, sodium saccharin, sodium</td>
</tr>
<tr>
<td>Iron Supplement</td>
<td>Calcium carbonate, ferrous sulfate, croscarmellose sodium, silicon dioxide, magnesium stearate, titanium dioxide sodium carboxymethylcellulose, sodium citrate</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>Dicalcium phosphate, magnesium stearate, cyanocobalamin</td>
</tr>
<tr>
<td>Multivitamin</td>
<td>Calcium carbonate, magnesium oxide, ferrous fumarate, croscarmellose sodium, zinc oxide, magnesium stearate, titanium dioxide, d-calcium pantothenate, calcium silicate, silicon dioxide, cyanocobalamin</td>
</tr>
</tbody>
</table>

MXRF elemental phase imaging was achieved with a multivariate statistical automated analysis program developed by Sandia National Laboratory (Albuquerque, NM). The program was originally developed for spectral imaging with scanning electron microscopy (SEM) and was adapted for use with MXRF. The program is described in detail in Kotula et al. [10].

ELEMENTAL MAPPING OF PHARMACEUTICAL TABLETS

Elemental X-ray mapping is a process by which a window about a range of X-ray energies is integrated and displayed as an image. From these mapped images, qualitative elemental distributions from a chosen area of a sample can be visualized and compared. Micro X-ray Fluorescence (MXRF) elemental mapping offers complementary information to molecular mapping techniques. MXRF can detect elemental composition for a given sample by measuring its characteristic X-ray emission wavelengths or energies. Mesoscale ($>10\,\mu m^2$) analysis is achieved through the use of a polycapillary focusing optic in conjunction with a Rh X-ray tube source, which focuses the source X-rays into a nominal spot size of $\sim60\,\mu m$ in diameter. MXRF allows for simultaneous elemental analysis with both quantitative and qualitative analysis of elements $Z = \text{Na}$. It is a nondestructive technique and requires minimal sample preparation. In this study, MXRF elemental mapping was used to study the distribution of inorganic components in pharmaceutical tablet formulations. The ingredients that could possibly be
detected by MXRF contained in each of the pharmaceutical tablets analyzed in the study are listed in Table 2.

Figure 2 shows the MXRF elemental maps for a stomach relief tablet. Bismuth and Ca could be detected in the tablet by MXRF. Both sodium and magnesium were present below the detection limit of the instrument and could not be mapped. The elemental maps show that the Bi and Ca are distributed very evenly throughout the tablet.

![BiM, CaK, BiL](image1)

**Figure 2.** MXRF elemental maps of a 3.9 mm x 3.7 mm area of a stomach relief tablet. Source setting: 30 kV, 0.160 mA.

Figure 3 shows the MXRF elemental maps for an iron supplement tablet. The elements that could be detected above background in the tablet include Si, S, Ca, and Fe. Again the distributions of the different elemental components can be observed in the MXRF maps and overlays. There is a strong correlation between the Fe and S signals due to the presence of Fe in the tablet as ferrous sulfate. The areas of high Fe and S intensity are complementary to those where Ca is present, indicating that the Ca is a separate component. Si is also present, with a signature that does not correlate with Fe, S, or Ca.

![Si K, S K, CaK, Fe K, Fe/S Overlay, Fe/Ca Overlay](image2)

**Figure 3.** MXRF elemental maps of a 3.9mm x 3.4mm area of an iron supplement tablet. Source setting: 35 kV, 0.1 mA.

Two different brands of vitamin B-12 supplements, each containing the same amount of Co containing cyanocobalamin (500 mcg), were chosen for MXRF imaging to show how MXRF can detect differences in the constituent makeup between different tablets of the same character. Figure 4 shows the MXRF elemental images for the two different vitamin B-12 tablets. The major elemental components are Ca and P from the dicalcium phosphate binder and Co from the
cyanocobalamin (also known as Vitamin B-12). Notice that for both tablets, the Ca and P signals seem to be correlated and that they are both complementary to areas showing significant Co intensity. Interestingly, the distribution of the three elemental components is very different between the two tablets. In tablet (a), the Ca and P are present as small particulates in the sample while Co is distributed evenly throughout the tablet. For tablet (b), the opposite is the case. Calcium and phosphorus are present throughout the tablet with Co filling the smaller gaps in between. So even though both tablets have approximately the same chemical make-up, the component distributions are very different.

Figure 4. Comparison of MXRF P, Ca, and Co elemental maps for two different brands, (a) and (b), of Vitamin B-12 supplement tablets. The area for each image is ~3mm x 2.5mm. Source setting: 35 kV, 0.15 mA.

Figure 5 shows the MXRF maps of the elemental species present in a multivitamin tablet. Notice that the tablet contains many different species that are detectable by MXRF. There seems to be some correlation between the Zn and Si species. Calcium is present in the highest abundance in the tablet and is present throughout. There are many more components in the multivitamin tablet than in the simpler tablets discussed above. Correlations by eye are much harder to make, and an elemental overlay was not shown for this example because the resulting image was too complicated to aid interpretation.

Figure 5. MXRF elemental maps of the major elemental components of a multivitamin tablet. The image area is approximately 3.4 mm x 3.4 mm. Source setting: 35 kV, 0.1 mA.

As shown in the examples above, MXRF offers powerful capabilities in elemental mapping. Areas, as small as several hundred micrometers and as large as tens of centimeters, can be
scanned to produce elemental images. These qualitative elemental images can clearly identify areas of heterogeneity, give relative distributions of the elements, and highlight areas of interest for higher resolution probes. However, the major drawback of all MXRF based mapping is the resulting elemental images can only be correlated to other elements visually or thought map overlays. In most cases this is simply achieved by visually inspecting the different elemental images and trying to correlate the elemental maps by eye. Generally this is not difficult when the composition consists of only a few elements. However, when the elemental composition increases to five or more elements, visual correlation of the individual elemental images can be difficult at best. The major concern is a critical elemental correlation can be overlooked if an element was not identified in the initial scan, or the elemental correlation is subtle or insufficient for distinct registration of the separate elemental images, effectively concealing any correlations.

**ELEMENTAL SPECTRAL IMAGING OF SELECT TABLETS**

As shown above, X-ray mapping involves the integration of X-ray energies in specific regions of interest (ROIs) over a given area of sample which are displayed as images. From these images one can visualize qualitative elemental distributions from an area of the sample. However, the mapping results are generally not quantitative without the use of standards. Mapping generally relies on the foreknowledge of elements in the sample so that specific ROIs can be designated for integration. Often with samples containing many different elemental components, elemental correlations are difficult. For example, Figure 4 shows a simple overlay of the P, Ca, and Co components in the Vitamin B-12 tablets. It can be seen that there is some correlation between the Ca and P components and that they are both complementary to the Co component. However, making elemental correlations with a more complex sample, like the multivitamin shown in Figure 5, is much more difficult. There seems to be some correlation of the Zn and Si components. Ca seems to be present throughout the tablet and complementary to all of the other elements. However, these conjectures are not definitive and elemental map overlays would be too convoluted to be of any interpretive help in this case.

An X-ray spectral image is a two-dimensional array of pixels taken from an area of sample that contains a complete X-ray spectrum from each point. X-ray spectral imaging overcomes the disadvantages of X-ray mapping because a complete spectrum is collected from each pixel; no spectral information (elements) is left out or lost. However, spectral imaging often involves the time-consuming collection of very large data sets, much of the information contained in these data sets is redundant, and it is usually difficult to extract chemically relevant data from it. To overcome the problems of conventional X-ray spectral imaging, a new multivariate statistical analysis technique was developed by Sandia National Laboratory (SNL) to automatically analyze X-ray spectral data sets from electron excitation on an SEM [10] and was adapted for application to X-ray tube excited spectra. The method returns physically accurate component spectra and images with only a few minutes of data processing time. The result is not only elemental images, but also elemental phases or chemical phases based on elemental composition. This then removes the subjective nature of the visual correlation and enables the visualization of both major and subtle elemental correlations. No assumptions about the absence or presence of any constituent are made prior to analysis. The method has the ability to handle extremely noisy data and significant spectral overlaps, so data acquisition times can be drastically reduced to obtain
experimentally relevant data. Image processing times are very short (minutes) and quantitative agreement is maintained between raw and reconstructed data.

Figure 6 shows the 4 principal components as well as the component overlay returned by SNL’s automated image analysis program for a Vitamin B-12 tablet. Notice that the image area for the automated analysis is the same as that shown for the X-ray elemental mapping for tablet (a) in Figure 4. The initial X-ray mapping experiments took approximately 1 h with a 200 ms dwell time per pixel. Conversely, data acquisitions for automated analysis only took 18 min with 50 ms per pixel with no distinct loss in resolution. The automated analysis of the spectral data took on the order of 2 min to complete, for a total acquisition and analysis time of 20 min; a factor of three times less than for X-ray mapping. For the Vitamin B-12 tablet analysis, phase 1 is primarily made up of the Bremsstrahlung background. Phase 2 consists of both Ca and P, and is indicative of the calcium phosphate binder present in the tablet. Phase 3 is a separate Ca phase (possibly due to Ca present in lactose or corn starch). Notice that this component is not visually observable in the elemental maps present in Figure 4. Phase 4 is Rh scatter from the Rh X-ray source off of the sample surface. Also notice that the overlay of 4 automated analysis components is much easier to visualize and interpret than those shown for elemental mapping.

![Figure 6. Elemental phase images of Vitamin B-12 tablet 1 (same area as shown in Figure 4). The yellow circle identifies the Co peak from cyanocobalamin.](image)

Interestingly, a separate phase for the Co from the main cyanocobalamin component in Vitamin B-12 is absent from the principal components returned by the spectral imaging program. If one looks carefully, one can see that the small Co X-ray peak is present in Phase 1 as part of the Bremsstrahlung background (peak is circled in yellow). This is because the small Co signal could not be separated from the very large Bremsstrahlung background. A background
subtraction routine is currently being integrated into the program to allow for trace components to be more easily identified.

Figure 7 shows the 5 principal components as well as the component overlay for the more complex multivitamin matrix. Phase 1 is primarily made up of calcium. The major component of phase 2 is Fe. Phases 3 and 4 have Zn and Mg in highest abundance respectively. Phase 3 also shows traces of S and Si, while phase 4 contains traces of Ca, Ti, Fe, and Co. Phase 5 is composed of both Rh scatter from the X-ray source as well as Ca. The distribution of these different phases is very easy to see in the component overlay.

Figure 7. Elemental phase images of a multivitamin tablet (same area as shown in Figure 5).

In short, multivariate statistical analysis allows for much easier viewing and interpretation of elemental correlations within a sample. Furthermore, components are often identified, like the extra Ca component in the Vitamin B-12 tablet, which would easily be missed otherwise.

CONCLUSIONS

In this study, MXRF was used as a new tool for elemental imaging of pharmaceutical oral dosage forms. The distribution of inorganic species within a tablet can easily be visualized and correlations can be made between different components. Multivariate statistical analysis techniques can enhance elemental imaging capabilities by allowing for the extraction of elemental “phases” from raw spectral imaging data, eliminating bias errors and elemental omissions from human visual interpretation and comparison of elemental images. However, not all components in a tablet dosage form are inorganic in nature. Pharmaceutical materials are often made from a variety of both organic and inorganic components. Therefore, both elemental as well as molecular imaging techniques are needed to properly make a full characterization of
the distribution of all tablet constituents. The next logical step of this work is to implement “integrated chemical imaging”, where both elemental and molecular imaging techniques are used to characterize pharmaceutical materials.

To achieve successful chemical imaging, tablets must be prepared so that they can be imaged by a variety of techniques in a routine manner. Specifically, very precise methods of sample preparation will be needed so that the exposed tablet areas are very smooth and flat for optimal analysis by different imaging methods. Also, stage registration techniques will have to be implemented so that the exact same area on a given sample can be imaged by many different techniques in different instruments so that direct comparisons can be made between inorganic and organic component distributions. These efforts are currently being investigated.

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REFERENCES