RAMAN CHEMICAL IMAGING

Raman Chemical Imaging as a Tool for Measuring Layer Thickness in Sustained-Release Beads

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ABSTRACT

Site-specific delivery and controlled release of active pharmaceutical ingredients (APIs) have resulted in a high demand for modified-release products. Sustained-release beads can deliver stable levels of drugs, which result in less-frequent dosing. For consistent drug delivery, it is imperative for the bead coating to be uniformly applied. In this study, Raman Chemical Imaging (RCI) coupled with optical microscopy was applied to investigate API and polymer coating thicknesses in commercial sustained-release beads. This approach was compared to scanning electron microscopy (SEM) and dissolution data obtained for the same batch.

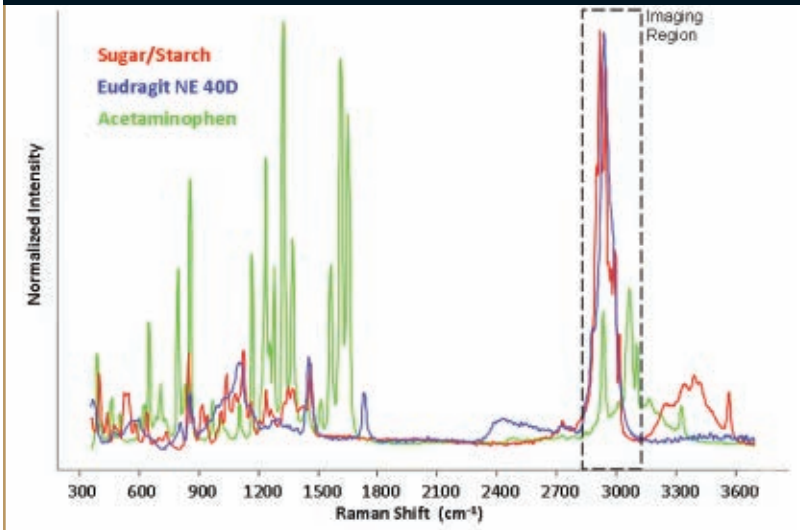
INTRODUCTION

Demand for modified-release products has grown in recent years due to favorable therapeutic qualities resulting from site-specific delivery and controlled release of an API. A modified-release mechanism ensures stable levels of the drug delivery and, therefore, lesser dosage frequency compared with instant-release formulations. These products include delayed-release and extended- (controlled, sustained) release products. Typical sustained-release systems use small beads encapsulating the drug in the core particle with a polymer coating or sandwiching the drug between an inert core and one or more polymer layers. In both cases, it is critical for consistent drug delivery that the bead coating is uniformly applied.

The validation of the coating process is generally achieved by surface analysis of coated bead cross-sections by SEM, which employs secondary or back-scattered electrons to produce nanometer-resolved surface images.[1] Because the analysis is carried out in vacuum, an SEM specimen should be dry and conductive in order to achieve high-quality imagery. Image artifacts may result from the charging of nonconductive specimens during electron beam scanning, therefore, most non-metal samples are coated with an ultra-thin layer of gold. One must distinguish coating layers by inherent morphology (or lack thereof), as the elemental composition of organic compounds is similar.

Matrix-assisted laser desorption/ionization with a time-of-flight mass analyzer (MALDI-TOF) is commonly used in tissue imaging and can also be used to analyze bead cross-sections.[2,3] It should be noted that image spatial resolution for MALDI-TOF is relatively low, about 20 micrometers, and is not always suitable for controlled-release beads. Both aforementioned analytical methods require special sample preparation and may not be sensitive to physico-chemical changes in the sample, such as hydration or polymorphism.

FIGURE 1



Raman dispersive spectra of pure components.

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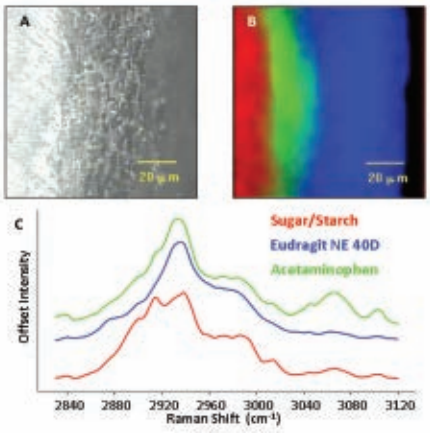
FIGURE 2



A typical optical and processed Raman chemical image of bead exterior at 20x magnification: (A) Optical microscopy image; (B) Processed Raman chemical image; and (C) Raman spectra of bead ingredients.

Raman spectroscopy is a laser-based vibrational spectroscopy technique that provides high specificity for determining the chemical composition and requires minimal or no sample preparation. Confocal Raman microscopy has been successfully applied to evaluate coating uniformity, thickness of the API layer, and drug-release mechanism.[4,5] Confocal Raman microscopy requires point-by-point mapping to construct an image and usually takes significant time to cover an appropriate area for layer thickness determination.

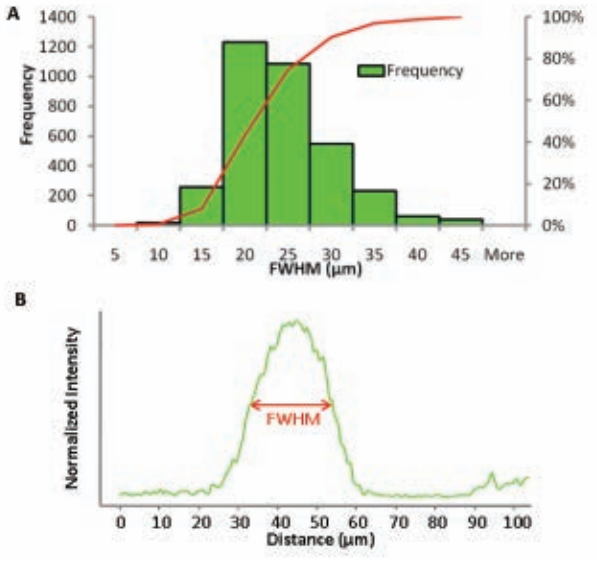
Wide-field RCI is a hyperspectral imaging method based on liquid crystal tunable filter technology that transmits spatially resolved wavelength frames to a Charge Coupled Device (CCD) detector. Wide-field RCI determines the chemical identity of individual components of a heterogeneous sample by combining the objectivity of Raman spectroscopy with the visual perception of digital imaging. It provides exceptional value for a variety of applications, including pharmaceutical research and development.[6] A full or partial Raman spectrum is captured for each pixel in the chemical image corresponding to a spatial location on a sample. Thus, a resulting data set, or hypercube, contains two spatial dimensions as well as a wavelength dimension. Each chemical entity in the field of view (FOV) can be identified by its distinctive spectral profile and correlated with an associated optical image. Specific Raman spectral planes are used for identification, placement, and sizing purposes. Advanced chemometric techniques may be used to isolate unique Raman signatures and separate multiple ingredients in complex systems or matrices.

In this study, RCI coupled with optical microscopy was applied to investigate API layer thickness in sustained-release beads. This method was compared to both SEM and dissolution data obtained for the same batch.

MATERIALS & METHODS

Sustained-release beads with acetaminophen (APAP) as an API were prepared by Vector Corporation. Sugar spheres (3 kg, 30-35 mesh) were loaded into a Vector Granurex GXR-35 Conical Rotor Processor (Vector Corporation) equipped with a K-Tron KT-20 precision powder feeder. Micronized Acetaminophen (Mallincrodt) was fed via the KT-20 into the GXR-35 dry and layered onto the spheres to a level of 15% w/w, using a 5% aqueous solution of PVP K-30 in water as a binder. The drug-layered beads were then functionally coated in the

FIGURE 3



C

Category	Value
Average (μm)	21.8
Std. Dev. (μm)	5.8
Measurements	3475
D10 (μm)	15.5
D50 (μm)	20.7
D90 (μm)	29.9

Distribution of measured API layer thickness based on 29 individual beads and SNR ≥ 10σ: (A) Acetaminophen layer thickness distribution based on FWHM; (B) Example calculation of FWHM of the API layer; (C) Acetaminophen layer thickness summary statistics.

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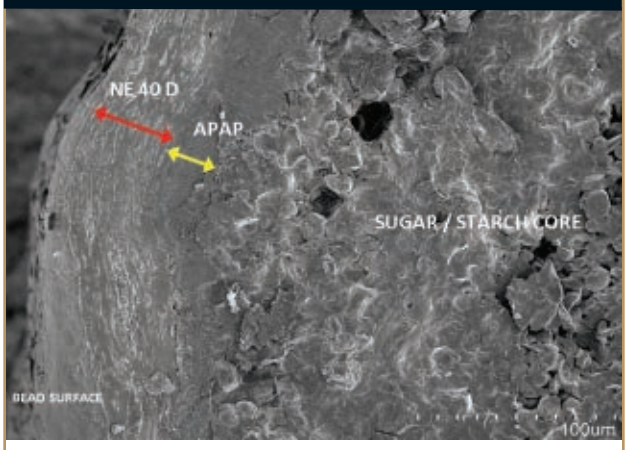
FIGURE 4



Representative SEM image of a cross-sectioned NE 40 D-coated APAP bead.

GX-35 rotor processor with the sustained-release polymer Eudragit® NE 40D, an aqueous dispersion of ethyl acrylate and methyl methacrylate copolymer (Evonik Industries) using the precision powder feeder to add minute amounts of talc to prevent blocking of the NE 40D-coated beads. The beads were coated to a target polymer content of 25% w/w at 300 rpm and 40°C air temperature. Produced beads looked smooth and uniform with better than 98% coating efficiency.

Dissolution testing was carried out using a Hewlett Packard 8452AUV-VIS photo-diode spectrophotometer using Method 06029 (University of Iowa, College of Pharmacy) for sustained-release APAP beads.

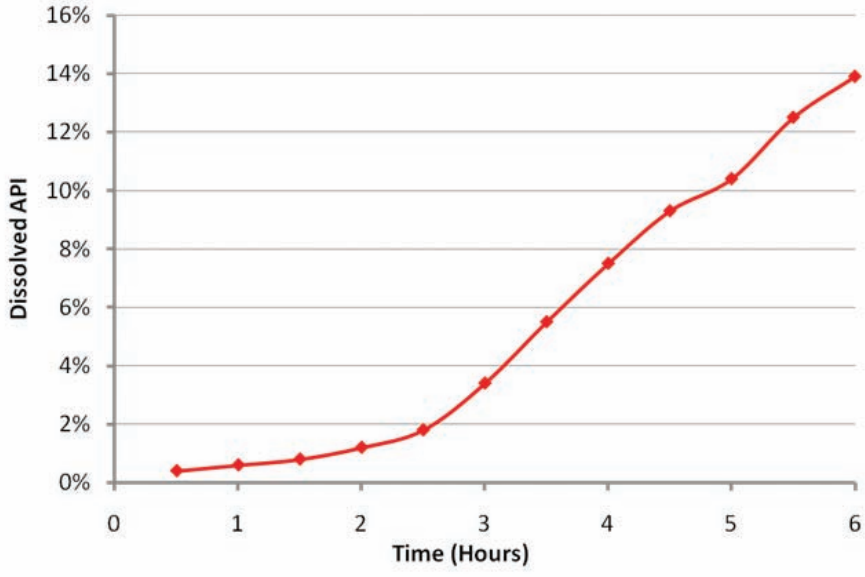
The surface and cross-sectional morphology of the 20 coated beads were observed via SEM images collected using a Hitachi S-4800 SEM. Beads were cross-sectioned to expose the core and inner layers for the RCI study. A total of 29 beads were investigated. All data was collected using a FALCON II™ Wide-Field Raman Chemical Imaging System (ChemImage Corporation) with 532-nm laser excitation. Brightfield reflectance and Raman chemical images were collected in an automated mode at 20x magnification across the right side for 29 individual beads. At 20x magnification, an FOV of 100 x 100 micrometer is interrogated. The following experimental conditions were employed. The laser power was set to 250 mW at the laser head. Each FOV was photobleached for 20 seconds before starting the acquisition. The C-H region was scanned from 2830 to 3120 cm^{-1} at a 5 cm^{-1} interval. Each frame was integrated for 1.5 seconds and 3 averages. All imaging data was processed and analyzed using the ChemImage Xpert™ software package.

RESULTS & DISCUSSION

Raman spectra of the pure component materials were acquired to construct a Raman spectral signature library as shown in Figure 1. Based on the Raman library of pure components, the imaging spectral range for efficient discrimination of each constituent was selected in the C-H spectral region (2700-3200 cm^{-1}).

The FALCON II microscope was focused on the edge of each bead to include all three layers. The RCI processing steps included cosmic ray removal, baseline correction, and vector normalization. For final ingredient discrimination, a spectral unmixing algorithm called Spectral Mixture Resolution (SMR) was applied to the RCI data. SMR evaluates each pixel spectrum using a linear combination of the pure component spectra to achieve an overall spectral contribution. The result is presented as a spatial distribution of each

FIGURE 5



Dissolution profile for NE 40 D-coated APAP beads.

ingredient. A representative data set comparing optical microscopy and RCI is shown in Figure 2. The RCI-derived ingredient images can be used to objectively measure the thickness of individual coating layers. The frame corresponding to the API was isolated and smoothed using a convolution filter with three averages, and an intensity profile for each row of pixels (128 in each frame) was generated. Intensity profiles (IP) with signal-to-noise ratio below 10 standard deviations were discarded. The thickness value was measured as the Full Width at Half Maximum (FWHM) of the IP trace. The average thickness value then was calculated based on 3475 measurements for 29 samples. A representative IP trace and API layer thickness distribution is shown in Figure 3. The APAP layer is 21.8 ± 5.8 micrometers thick (n = 29), D10 = 15.5 micrometers, D50 = 20.7 micrometers, and D90 = 29.9 micrometers. A consistent distribution of the drug layer within the sampled bead population was observed.

SEM results from 20 beads from the same batch revealed the API thickness was 15-20 micrometers. A representative SEM image of the cross-sectioned bead is shown in Figure 4. SEM data correlates well with the RCI results. Differences in the RCI-measured thicknesses can result from several factors such as the larger sample population, nonlinear dependence of Raman signal on sample volume, and FWHM metric for layer thickness determination.

Dissolution testing was carried out to evaluate sustained-release properties of the polymer-coated bead. The dissolution results showed a fairly linear zero-order release after 2 hours in 0.1N HCl media as shown in Figure 5. Due to the relatively large polymer coat, the dissolution was retarded to a greater extent than a typical formulation but was suitable for this investigation. At 6 hours, just under 14% of the drug had been released with a standard deviation of 0.7 (N = 6).

CONCLUSIONS

API layer coating thickness has been measured using wide-field RCI to characterize multilayered beads used for sustained-release drug delivery. Rich spectral and spatial information contained within the RCI data cube can provide valuable feedback during formulation and manufacturing processes and help correlate coating thickness with drug dissolution profiles. RCI technology is especially valuable for multilayer beads or for troubleshooting manufacturing processes.

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BIOGRAPHIES

Dr. Oksana Klueva started at ChemImage in 2004 as a Senior Scientist. After providing technical support for Air Quality and Biothreat applications on government projects, she moved to developing new applications and supporting marketing. After graduating from Boston University in 2002, she worked as an Application Scientist for Horiba Jobin Yvon, Optical Spectroscopy Division. Her responsibilities were to provide technical support for customers and during the sales process. Dr. Klueva earned her PhD in Physical Chemistry from Boston University and her MS in Organic Chemistry, Moscow State University.

Dr. Ryan J. Priore joined ChemImage Corporation in 2008 as a Senior Scientist and is the operational leader of the applications group, where he is responsible for exploring and developing pharmaceutical chemical imaging applications as well as delivering high-quality chemical imaging contract services. He earned his BS in Chemistry from the University of Pittsburgh and his PhD in Analytical Chemistry from the University of South Carolina for his development of real-time, optical computing technology. Dr. Priore then joined Ometric Corporation, where he led the application development of optical computing based, in-line process measurement instrumentation for the pharmaceutical, food, beverage, and pet nutrition industries. He is also the author of a dozen publications and patents on spectroscopic applications and instrumentation.

Brian K. Jensen has been with Vector Corporation for 25 years, previously as Senior Scientist, Process Development, and as Laboratory Manager since 2005. He is a graduate of Iowa State University and has been involved with a large number of Vector Corporation's customers, providing product and process development as well as providing guidance for product line improvements and equipment modifications. He holds several patents associated with Vector's fluid bed line and is a member of AAPS and CRS.