A novel micro-photogrammetric Instrument for visualizing in 3D small objects applied to the quantitative study of the dissolution behavior of a pharmaceutical dosage form.

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The work presented here proposes an innovative approach to 3D chemical mapping of solid formulations by micro-photogrammetry. We present details of a novel micro-photogrammetry apparatus and the first results for the application of photogrammetry to the dissolution analysis of solid pharmaceutical dosage forms. Unlike other forms of optical imaging, micro-photogrammetry allows a true 3D model to be constructed that includes direct observation of the sides of the sample rather than only top-down topographic imaging. Volume and structural changes are assessed quantitatively and related to chemical analysis by high performance liquid chromatography (HPLC). The recently introduce method of chemical identification by dissolution analysis, or chemical imaging by dissolution analysis (CIDA), is employed for the first time to obtain tomographic images of the dissolution process.

1. INTRODUCTION

Characterising the dissolution behaviour of pharmaceutical dosage forms is of crucial importance to developing effective treatments delivered by solid drug delivery systems (1). Solid dosage forms, i.e. tablets, are the most widely used method of delivering drugs to patients (2). There are various techniques for following the dissolution process of pharmaceuticals in 3D including solid-state nuclear magnetic resonance (SS-NMR), micro X-ray tomography and Raman spectroscopy (3), (4). These techniques are very useful but often expensive and, to be used effectively, require a high level of expertise in the relevant field.

Optical techniques such as interferometric surface profiling are difficult to use with the majority of dosage forms because of the light scattering caused by the usually transparent powders tablets are often made from (5). However, an optical method for following the dissolution process is desirable because of the clear intuitive connection between visual inspection and the phenomenon of interest. It is also possible that such a system could be low cost.

Photogrammetry is a well-established technique that is usually employed on the scale of meters and kilometers (6), (7). There are examples of it being used on smaller scales but to date it has not been used with optical microscopy. Although not impeded by scattering, there is the problem that objects with insufficient detail and texture can frustrate the reconstruction algorithms (8).This is often the case for dosage forms, especially when any coating has been removed; they are frequently white with little texture thus they present a challenge to photogrammetric imaging. We have sought to address this problem using a novel 3D reference structure. Photogrammetry has the advantage over other optical microscopy techniques in that it enables direct measurements of the sides and even the underside of the sample to be made; these observations are then used to construct the 3D model. Other forms of optical 3D imaging provide only top-down images.

In this article, we describe a novel micro-photogrammetry apparatus and associated components necessary for the construction of quantitative 3D models of dosage forms as they undergo dissolution.

2. HARDWARE AND SOFTWARE

A. Micro-photogrammetry apparatus

Figure 1 shows the basic imaging system. It has the following features; a small microscope is held by magnets in position on a semi-circular support. Several angles are possible with three positions at each angle giving different distances from the sample corresponding to different levels of magnification. The sample is placed on a rotating platform that moves under computer control as the images are taken.

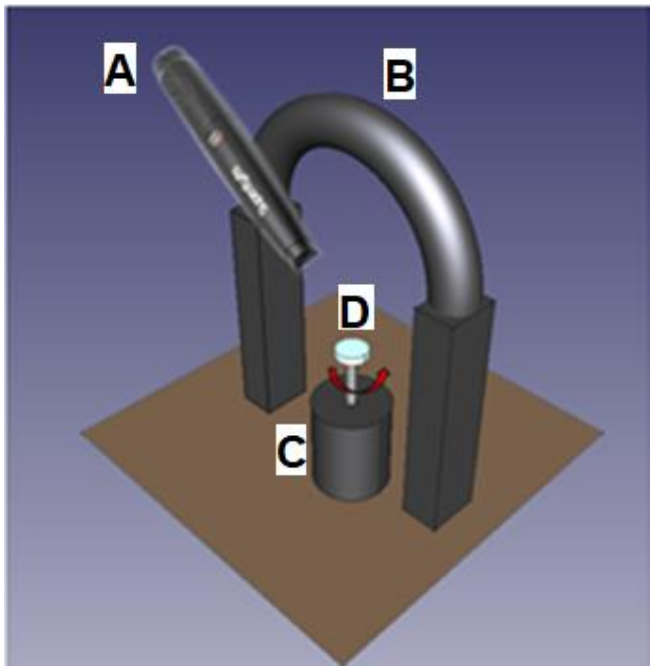

Figure 1. Apparatus used to collect images of the sample which comprises of an optical microscope (A) mounted into the semi-circular panel (B) of the camera stand held in place by magnets. During image collection the microscope is kept stationary at specific observation angles whiles the rotary motor (C) that supports the sample (D) rotates it so that the entire object is imaged.

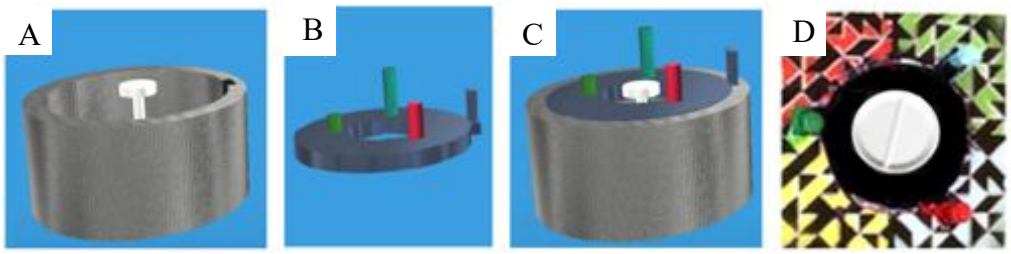

Figure 2. Representation of the sample mounted into the dissolution cell (A), the utilized annular reference with three reference posts (B), the assembled system comprising the sample located in the aperture of the annular reference (the slot and raised feature on the periphery means the reference structure is always relocated in the same place) (C) and the annular reference with the colored patterns used to facilitate the generation of 3D models (D).

During the analysis the sample is located in a sample holder that is mounted in the dissolution cell (Figure 2A). The sample is surrounded by an annular reference system (illustrated in Figure 2B and 2C), that is fitted into the dissolution cell (Figure 2D). The annular reference system comprises three posts of different sizes that enable calibration in all three axes.

The generation of 3D models of white and uniform objects (as pharmaceutical solid drug delivery systems) is challenging, because photogrammetry requires the presence of irregularities to assist the overlapping of the multiple images to create the 3D model. Therefore, another important feature is the coloured pattern applied to the reference system that surrounds the sample. It facilitates the alignment of the multiple images by assisting the recognition of overlapping areas because each colour/pattern combination is unique on the appropriate length scale.

B. Dissolution system

The sample is periodically subjected to dissolution. Before the dissolution step, the annular reference system is removed then half of the volume of the dissolution cell is filled with solvent. The removal of the reference structure means it does not come into contact with the solvent and also allows free access to the dissolution cell. Then, a tube (Figure 3B) is placed in the dissolution cell (Figure 3A) and the solvent is drawn into contact with the sample by suction. After a specified period of time, usually 30 seconds to a minute, the solvent is released and moved into vessels to be analysed.

At this stage, the sample is allowed to dry for a minute while it is imaged. The sample is never removed from its starting position so that all images are obtained with the sample and microscope(s) in the same relative positions. This does not mimic the process of swallowing a tablet which means that the sample has continuous contact with solvent, however, it does enable the dissolution behavior of different samples to be compared. Ultimately the objective is to have the sample immersed in solvent at all times viewed by the camera(s) through a transparent tube.

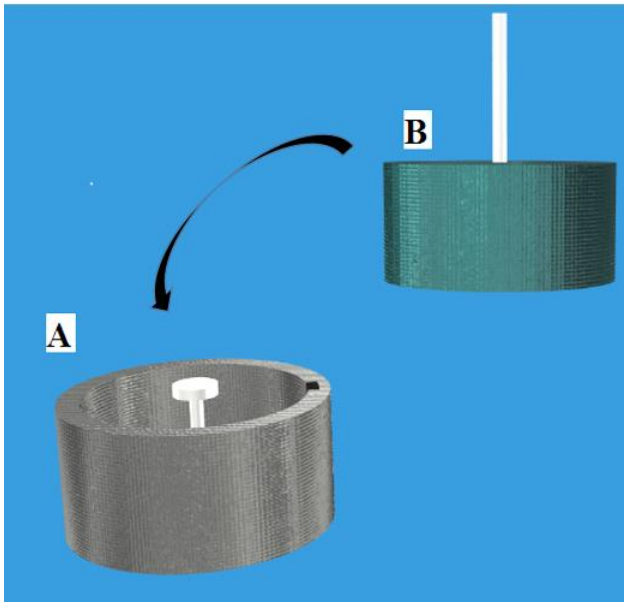

Figure 3. Representation of the dissolution cell with sample support (A) and the tube which is inserted into the dissolution cell to bring the solvent in contact with the sample (B).

C. Software

The software used was the commercially available package Agisoft Photoscan Professional was selected due to its simplicity of use, high capability of processing close range images and accuracy of measurement of distances, area and volume of the object.

Data processing was realised with a computer with 16 GB RAM (the minimum required to rapidly process the numerous images of the sample), a fast Intel i7 3.5 GHz processor and a graphic card dedicated to the image processing, specifically a 4 GB Nvidia GeForce GTX 950.

In addition, we have used software package that was developed to enable the recently introduced method of chemical imaging by dissolution analysis or CIDA; this is described in (9). We have added the additional capability to take the three posts on the annular reference system and use them to calibrate the measurement in the x, y and z axes. This is illustrated in the “system evaluation” section.

3. EXPERIMENTAL

The samples under analysis were the commercial tablet, Anadin Extra (batch number: CNZ022, expiration date: March 2019, purchased in March 2016). The tablets, produced by Pfizer Consumer

Healthcare, contain the following active ingredients: Aspirin BP, Paracetamol Ph Eur, Caffeine Ph Eur. The sample was chosen to evaluate the feasibility of the application of the developed method in the characterization of complex pharmaceutical specimens where multiple active ingredients are delivered.

Samples were placed in the dissolution cell; the lower surface of the specimen is glued to the sample support. In future experiments only a small area of the sample will be glued to the holder thereby improving the fidelity with which the real-world situation is simulated by exposing over 90% of the sample to the solvent (currently it is approximately 80%). For the present, this study validates the principle novelty of the experiment which is the process of constructing quantitative 3D models of a dissolving dosage form despite its optical properties and appearance. During image collection, which is completed within a minute, samples were surrounded by an annulus where a coloured and patterned sample support with Z reference posts were used as calibration points as shown in Figure 2.

After image collection samples were put in contact with the selected solvent for 50 seconds. This contact time was identified as being appropriate with initial scouting studies carried out on the adopted formulation. In these studies specimens were subjected to different contact times to identify the duration of the dissolution step that would guarantee drug release above the limit of quantification while avoiding significant disaggregation of the formulations.

After contact with the sample the solvent was removed from the dissolution cell, collected in volumetric flasks, then stored at 4ºC for the duration of the experiment before the high performance liquid chromatography (HPLC) analysis. Stability of the components was tested over the time required to complete the experiment, only the Aspirin showed traces of degradation which were detected but not quantifiable (below limit of quantification). Once the dissolution cell was washed, images of the sample were collected to be used for 3D model construction.

The HPLC assay was performed with an Agilent Technologies system, configured with quaternary pump, auto-sampler and multi-wavelength detector 1200 series. The software used for data analysis was ChemStation (version B.03.01, 317).

For the analysis of Anadin Extra experiments were performed with a Hypersil® BDS C18 column (150 x 4.6 mm, id 5 μm) with isocratic flow (1 ml/min) of a mixture comprising water, methanol and glacial

acetic acid (69:29:3). The diode array detector was set to identify the absorbance at two different wavelengths:

- 226 nm, which was used for the quantification of Aspirin. Aspirin has two distinct maximum wavelengths in water, of 226 and 270 nm. It was experimentally found that the best results and peak shape were at the wavelength detection of 226 nm.
- 270 nm for the quantification of Caffeine and Paracetamol as they have a maximum absorbance in water of 270 and 277 nm respectively.

The active compounds' concentration was evaluated using calibration curves for the single ingredients, with a concentration range between 10 to 250 μg/ml. Validation of the developed HPLC method was performed and the tested parameters of linearity, peak symmetry and repeatability of retention time and area, were within the food and drug administration (FDA) prescribed ranges (10) .

The software assisted image processing starts with the reconstruction of the camera position around the object so that many data points are generated. Those represent projections onto the virtual space of elements identified on the sample structure from the processed images. The obtained cloud of data points will be interconnected by linkages, procedure called triangulation, to form the solid structure object.

To achieve reliable models partial overlapping between the collected pictures is required to reduce projection error that can significantly influence the measurement outcome. Hence, to guarantee a robust triangulation overlapping images are recorded by rotation of the sample of 5 to 10 degrees at each step (Figure 4).

Different observation planes are required for the complete reconstruction of the object structure, in this study this was achieved by fixing the camera in different positions on the microscope stand. Five observation planes were adopted, the camera angles in respect with the samples were: 0°, 25°, 45°, 60° and 85°. The generation of data points into virtual space is achieved by the software by the identification from peculiarities such as colour changes or topography.

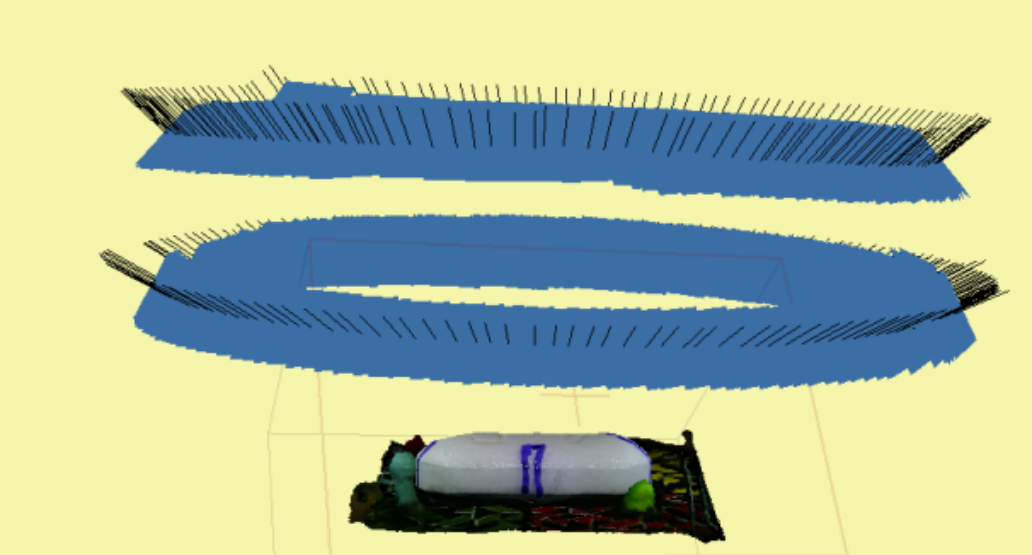

Figure 4. Representation of the virtual space with the 3D model of the native specimen mounted in the patterned sample support with the coloured pillars rising above the plane of the sample holder, these are visible at the two ends of the tablet (Z posts). The blue rings are a schematic representation of the of the overlapping images collected around the sample whiles the black lines represent the center of each image and the angle the microscope had in respect to the object.

The analysis was conducted in triplicates where the specimens were subjected to 6 dissolution steps followed by images collection for 3D model generation. Hence, the proposed method not only provides information about drug release, as the conventional pharmacopoeia dissolution tests, but it has the advantage of visualizing the dissolution process and measuring the variation of the specimens' volume and shape. While this experiment does not allow an exact comparison with the standard test it represents a step toward a system that will duplicate this test while providing the additional structural information.

4. SYSTEM EVALUATION

A. Imaging

Figure 4 gives the series of 3D images as a function of time of exposure to the solvent after correction using the CIDA software. The method of aligning in x and y axes is described in (9), the three cursors are placed on the tops of the three pillars. The tomographic in the z axis is enabled by selecting by means of the cursor the top of one of the pillars and the bottom then typing in the known value of the height of the pillar.

We can then perform two different types of measurement; the total volume change as determined by the Agisoft package and then line profiles determined by the CIDA software. The volume change is given in Figure 5. Inspection and other analytical data (not shown) demonstrate the major loss of material begins after the 4th exposure, the dissolution profiles are presented in Figure 6.

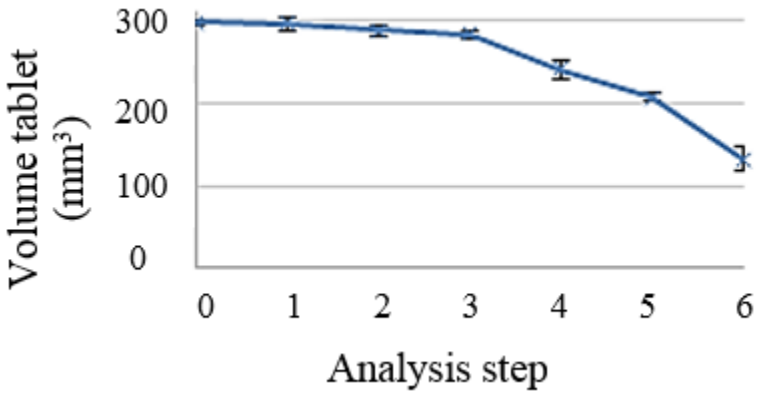

Figure 5. Variation of the specimen structure volume (n=3) induced by the dissolution process and quantified by Photogrammetry after exposure to the solvent (50 seconds each step).

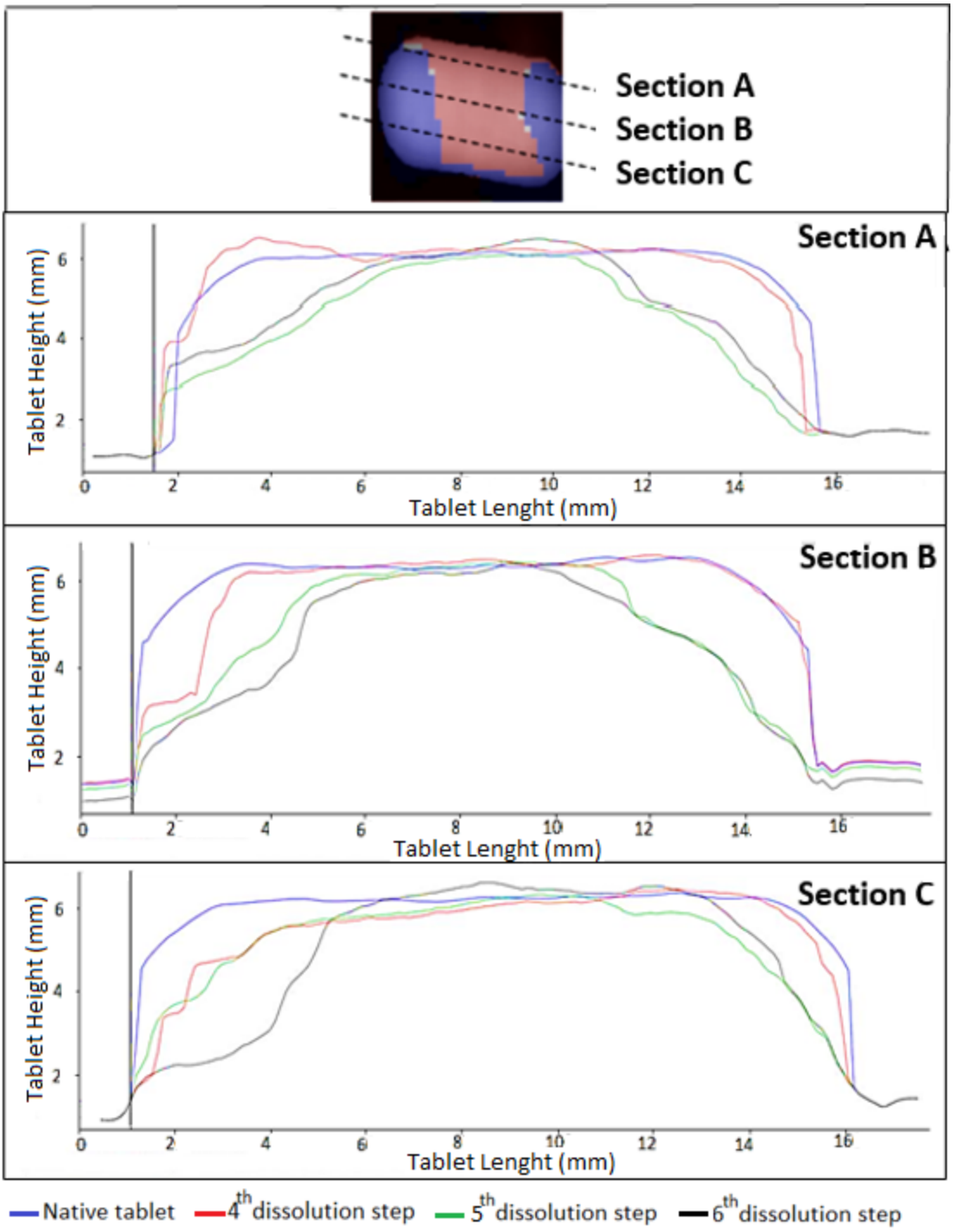

Figure 6. Mapping the dissolution/erosion behavior in 2D (top-down surface view) and 3D by slicing through the sample (tomography) section a), b) and c) are three different areas of tablet selected and analysed by looking at how tomography is changing with advancement of dissolution (native tablet and 4^{th}-6^{th} step of analysis).

The methodology used to create these images (Figure 6 (a-c)) and cross-sections is that described in reference 9. In brief; the height images (the grayscale images beneath the profiles) were aligned in x and y by selecting common features in each image (here the posts were used). The images were then aligned in the z axis by selecting points where the height is known not to change, in this case the surface of the annulus. With the cursor, one of the posts is selected and its height is entered into the software, in this way the z axis was calibrated. The height-loss map was then constructed in accordance with the method described in reference 9, we present a top-down color coded image that shows the area, red, where little material was removed and blue, where significant amounts of material were lost. Then, using the tomographic method described in reference 9, slices were constructed in three places as indicated in the colored image. These slices show how the removal of material was very inhomogeneous (Fig. 6).

5. CHEMICAL ANALYSIS

The results for the chemical analysis are given in Figure 7 co-plotted with the volume data.

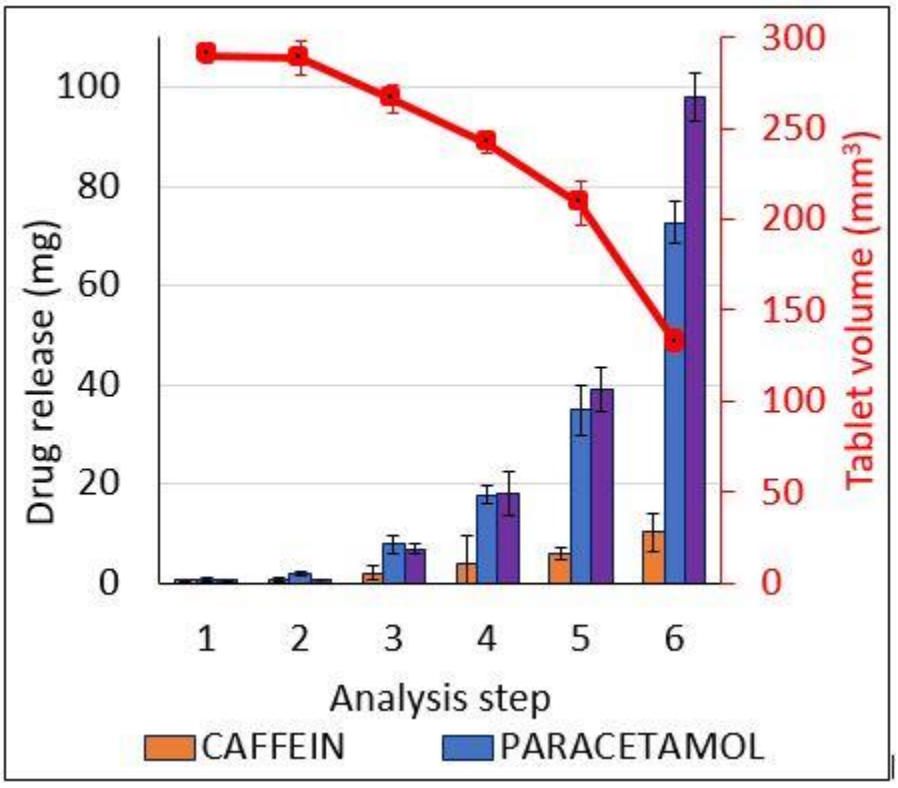

Figure 7. Correlation between volume alteration of the formulation and components release measured during the dissolution of the analysed sample in water (n=3).

6. DISCUSSION

We can see by inspection three important characteristics of the dissolution data; there is a good correspondence between the volume lost and the amount of material detected by the analysis, the relative amounts of the active ingredients change as the tablet dissolves and the dissolution is highly inhomogeneous with the ends of the tablet dissolving before the middle. The internal calibration for the x, y and z axes has worked well and the error bars are small.

In a previous study we have shown how sequential dissolution of a sample combined with 3D imaging of its surface and chemical analysis of the solvent can be processed, with appropriate software, to create 3D chemical maps. The imaging technology was atomic force microscopy and the analytical technique was FTIR spectroscopy. We were able to create the first nanoscale chemical tomographic images using this approach which we called chemical imaging by dissolution analysis or CIDA. The principle can be applied to any length scale and in this study we have made important advances toward achieving the same goal on the scale of mm. Our interest is pharmaceutical dosage forms. Having failed to obtain satisfactory measurements using optical profilometry and z stacking due to the optical properties of our samples, we devised a novel approach that is capable of constructing quantitative 3D images of dosage forms, namely micro-photogrammetry used with a novel reference structure. We have shown how this approach can create quantitative maps of how the surface of a dosage form changes as it is dissolved. We have also shown that this can be combined with chemical analysis. In terms of the instrumentation and software we have the elements that enable the type of measurements we require to be made on a scale of mm. In this case we have used the CIDA methodology to Map in 3D dissolution behavior and this has given important insights as have the analytical results. Now that that the basic imaging instrumentation has been shown to work, we are working toward the goal of chemical mapping.

In this case it seems probable that there is something in the manufacturing process that causes the ends of the tablet to dissolve at a different rate to the middle. To elucidate the cause the localised dissolution method described in (9) could be used on the middle then the ends of the dosage form. We intend to employ micro-photogrammetry at higher magnification for these experiments supplemented by AFM imaging. The localised and global dissolution options provide a powerful combination for understanding kinetics and structure. Analysing the contents of a medium used to dissolve a sample as a function of time

is commonplace. Being able to correlate this with changes in the structure of the sample is, as mentioned above, much less common but can provide valuable insights as in this preliminary study.

In the analysis steps 1 to 2 the formulations appeared to be subjected to wetting where the volume was not altered significantly. Thereafter a substantial erosion of the formulation was visible from the variation of the profile, analysed with CIDA software, moreover volume reduction was quantified by photogrammetry. Erosion appeared to influence the release of the active ingredients as presented in Figure 6.

7. CONCLUSION

We have constructed a low cost system capable of characterising the dissolution behavior of solid state dosage forms in 3D. To the best of our knowledge this is the first time this has been achieved with an optical system and the first demonstration of photogrammetry with a microscope. The volume of material removed from the dosage form can be quantified and compared with conventional analytical measurements. Tomographic slices of the progress of dissolution can be obtained thereby characterising how homogeneous the dissolution process is. Here we have focused on pharmaceutical dosage forms but the apparatus can be applied to any small sample. In this study we have used a sample with mm dimensions. In future work we intend to study submillimeter features. Furthermore, we will use multiple microscopes in parallel to image samples in a transparent tube while they are kept permanently sub-merged. The use of multiple cameras will speed up the image acquisition process. In this way, we will more closely mimic how tablets behave when in the stomach.

Our technique is relatively inexpensive and will ultimately provide chemical imaging in 3D. As mentioned above, this has already demonstrated at the nanoscale using atomic force microscopy (9) and we are working toward this goal on the scale of microns to millimeters using optical techniques.

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