A Method for Quantifying Differential Expansion within Hydrating Hydrophilic Matrixes by Tracking Embedded Fluorescent Microspheres

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Abstract □ A method is described for quantifying the pattern of deformation within a matrix and is demonstrated by analyzing the expansion of polymer hydrophilic matrix tablets. The fundamental features of the method are the incorporation of nondiffusing markers into the matrix and the subsequent tracking of these markers during deformation. Since the markers are too large to diffuse, their individual movement reflects the translocation of the surrounding matrix, and the separation between pairs of markers reveals any perturbation in the intervening area. By tracking many markers, the pattern of deformation within a matrix can be ascertained. The method was demonstrated on hydrating hydrophilic matrix tablets, using fluorescent microspheres as nondiffusing markers which were observed with a confocal laser scanning microscope. Analysis of the tracks showed a wave of expansion moving from the exterior toward the core, with the greatest and earliest expansion found in the outer regions. The results also showed that even as deeper layers started to expand the outer layers continued to swell.

Introduction

Many materials undergo alterations in size during their production or use and while it is relatively easy to measure gross dimensional changes[1,2] it is much harder to establish the pattern of internal deformation. Deformation can vary from the isotropic expansion of an evenly heated metal bar to the contraction of drying gels or the hydration of hydrophilic matrix (HM) tablets, both of which are anisotropic, despite the homogeneity of the original material. The experimental difficulty lies in following and quantifying differential deformation, for which there appear to be no established methods.

Hydrophilic matrix tablets are used as controlled release formulations.[3] Their performance depends on the rapid formation of a coherent gel layer around the tablet on hydration. An intact gel layer restricts the further ingress of water and controls drug release by acting as a diffusional barrier and by limiting erosion. The gel layer is inhomogeneous,[4] varying between a water rich exterior and an initially dry core.[4] The common USP dissolution methods used to assess the performance of HM tablets measure only rates of drug release. However, the underlying processes are dynamic and complex, involving interactions between water, polymer, the drug, and ions, all in an expanding and eroding matrix. Similarly, in the food industry, syneresis of gels is estimated by reweighing[5] or from volume changes. These measures adequately describe overall performance but do not expose the underlying processes.

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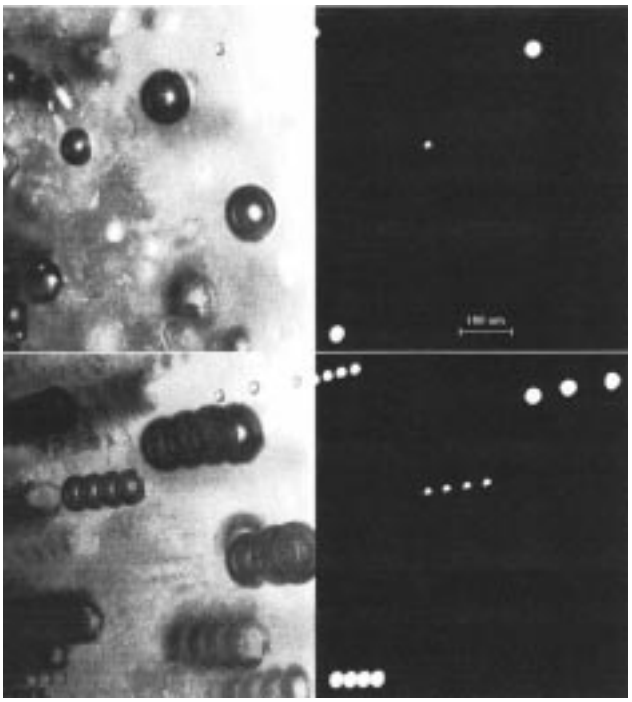

Figure 1—The left panels show (transmitted light image) air bubbles and microspheres, and the right panels (fluorescence image) microspheres only, in the gel layer of an HPMC tablet. The lower panels show a superimposed sequence of four images, showing movement. The bottom left image (transmitted light) is a minimum projection, and the bottom right (fluorescence) a maximum projection. Two sizes of fluorescent microspheres are apparent diameters of 2 μm and 22 μm.

In studying the performance of gel or tablet matrixes, the real goal is to expose the underlying mechanisms and advance from using experiments to observe the performance of specific formulations, to the experimental validation of predictive mathematical models. To confirm and validate such models, experimental methods are required that generate data which fully describes their properties and behavior. With this in mind we have been developing techniques to examine the internal performance of matrixes.[6−8]

We have reported on the presence of air bubbles[9] in HM tablet gel layers and have subsequently observed that these bubbles move as the gel layer continues to expand (Figure 1). Similarly undissolved particulates within a gel are also translocated (Figure 2). In this paper we consider how this observable movement can be used to map the internal performance of matrixes, and we describe a novel method in which nondiffusing markers are embedded within a tablet matrix and tracked during hydration. These tracks are then used to establish where and when expansion occurs. A fundamental assumption is that the influence of Brownian motion on our markers is minimal and that any

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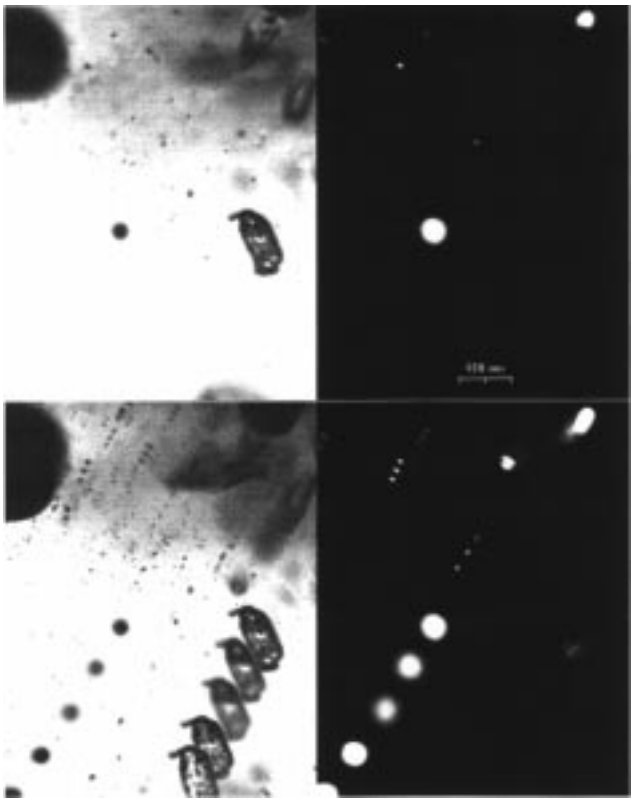

Figure 2—Left panels (transmitted light) show particulates and microspheres, and the right panels (fluorescence image) microspheres in the gel layer of a HPMC tablet. The lower two panels show a superimposed sequence of five images: the bottom left image is a minimum projection, and the bottom right image is a maximum projection. Two sizes of fluorescent microspheres are apparent, diameters of 22 μm and 2 μm.

movement of the markers therefore mirrors movement of the surrounding matrix.

Tracking the positions of individual embedded markers shows the changing location of these points within the matrix. However, their movement is not necessarily a consequence of a localized deformation: when a train moves, all the carriages also move but without any local or even overall deformation. Similarly a difference in velocity between markers does not necessarily reveal different degrees of local expansion: stretching a rubber band, with one fixed end, produces the greatest extension at the free end while the fixed end remains almost stationery, but the local expansion is nonetheless uniform. However, local expansion can be measured from the changing distance between a pair of markers, because their relative separation reflects any perturbation in the intervening zone, and it therefore becomes possible to move from measuring the translocation of markers to the measurement of localized deformation.

In summary, this study examines the use of nondiffusing markers to follow localized deformation within hydrating hydrophilic matrix tablets.

Experimental Section

Materials—Five millimeter diameter flat-faced circular tablets were prepared by direct compression of 40−63 μm sieve fractions of hydroxypropylmethylcellulose (HPMC) (Methocel K4M, Dow Europe, Germany) or xanthan gum (Satiaxane CX90, Sanofi Biopolymers France) containing 0.125% w/w latex microspheres, 2 μm and 22 μm average diameter, labeled with Nile red (Polymer Labs Ltd, UK). The microspheres, supplied in 10% w/v suspension in water, were dried by evaporation and then mixed with the dry polymer by trituration prior to tableting. Tablets were manufactured at a compression force of 1.2−1.5 kN using a Manesty F3 single-punch tablet press (Manesty machines, Speke, UK). Additional HPMC tablets were prepared containing different quantities of microspheres (0, 0.025, 0.05, 0.1, and 0.2% w/w).

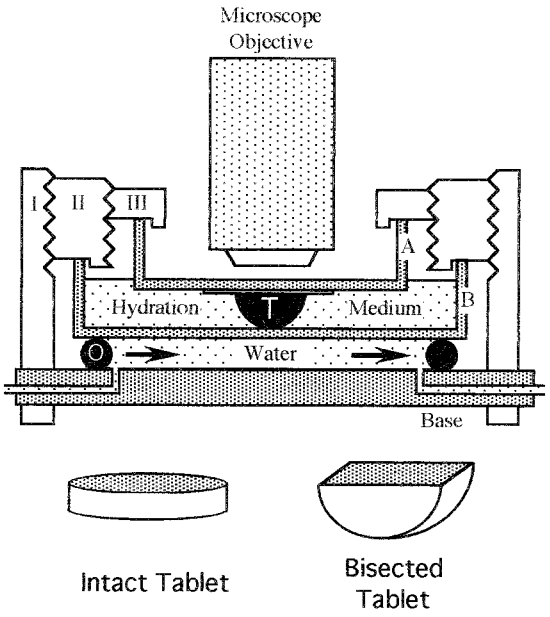

Figure 3—Tablet hydration cell: A bisected tablet (t) is shown placed between two Petri dishes (A and B) and held in place by three concentric brass rings (I, II, III). There is a silicon rubber membrane between Petri dish A and the tablet surface. The lower chamber, formed by compression of an O ring between the base and Petri dish B, can be perfused to maintain a constant temperature. Intact or bisected tablets were mounted in the cell, with a flat surface presented to the microscope objective.

Tablet Hydration Cell—Consisted of three interlocking brass rings (I, II, and III), two Petri dishes, and a transparent Perspex (poly(methyl methacrylate) base (Figure 3). Rotation of the upper two rings fixed a tablet between the two Petri dishes, enabling the observation of the upper surface of the tablet during hydration. The hydration medium was added to the chamber formed between the two Petri dishes. To prevent the hydration medium penetrating between the upper Petri dish (diameter 35 mm) and the upper surface of the tablet, a 200 μm transparent silicon rubber membrane was inserted between the tablet and the underside of the upper Petri dish. Temperature control was achieved by (i) preheating the hydration medium and (ii) circulating water through a bottom chamber formed by the compression of a silicon rubber O ring between the lower Petri dish and the Perspex base.

Tablets were hydrated at 37 °C in degassed distilled water. Two different orientations (Figure 3, bottom) of the tablets were used: (i) intact tablets face down for examination of radial expansion and (ii) bisected tablets with their exposed surface facing upward for both radial and axial expansion.

Confocal Laser Scanning Microscopy (CLSM)—Sequential images (768 × 512 pixels with 8-bit intensity) were generated using a MRC600 confocal microscope (Bio-Rad, Hemel Hempstead, UK) based on a Nikon Labophot upright microscope using the YHS filter with excitation at 565 nm and a ×1 microscope objective. Images were taken with the microscope focused 100 μm below the silicone/tablet interface.

The method relies on following individual microspheres through a series of images, but small movements in the *z* axis can take a microsphere outside the plane of focus, making tracking difficult. This problem was ameliorated by generating each image from a maximum projection of four almost synchronous images taken at increasing (20 μm step) depths. In a projection, homologous pixels from each image are compared and either the most intense (a maximum projection) or the least intense (a minimum projection) retained in the final image.

The confocal microscope was also used to generate transmitted light images which showed the initial edge of the dry tablet and the outer limit of the gel layer after hydration. These images were obtained using a light collector beneath the hydration cell. Changes in the extent of the gel layer were used to compare water uptake

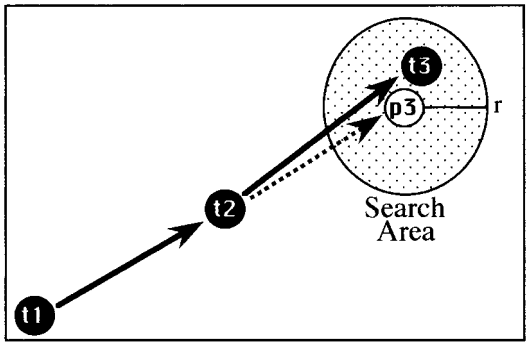

Figure 4—The tracking method: the two previous positions (t1 and t2) are used to predict the next location (p3), which is used as the center of a search area (radius r) for the actual subsequent location (t3).

in the series of tablets containing different fractions of microspheres.

Image Processing—In the CLSM images, the fluorescent microspheres appeared as areas of high intensity against a dark background, with each microsphere covering several pixels. Ideally each microsphere should be tracked from its center to the next center; however, it is possible that the brightest single pixel may not occur at the center. To ensure that the tracking was from center to center, a local averaging of the image preceded each search. For a bright object of a similar size to the distance used by the local area average, the brightest pixel is forced to center. The fluorescence image of the microsphere typically covered about 20 pixels and an equally weighted local average over a 5×5 pixel block was used. The centers were located to the nearest pixel.

Tracking Microspheres—Software was developed around a Semper 6.4 (Synoptics, Cambridge, UK) kernel operating on a Sprynt 40 processing board (Synoptics, Cambridge, UK). The objective was to determine the *x,y* coordinates of identified particles throughout a sequence of images. Figure 4 shows how the location of a microsphere in two preceding images (t1 and t2) was used to predict the next location (p3), and this predicted location then became the center for a local search (radius *r*) for the particle, producing the actual location (t3). Once the location of the microsphere had been established in several images, the predicted location was then based on the preceding three images, which proved to be more accurate. The initial location of each particle (t1) was input using a mouse from a prehydration image, and the second location (t2) was also input manually from either the first post-hydration image or from a maximum projection image, which sometimes made it easier to establish the direction of a track. After the first two locations had been established, the subsequent tracking was undertaken by the software alone. The search radius around the predicted location was either 8 pixels or half the length of the predicted movement, whichever was the greater. This choice of search radius depended upon the density of microspheres and was a compromise between checking a small enough area to exclude a second microsphere, and using a sufficiently large area to accommodate substantial changes in velocity.

Provision was made in the tracking software for automatically discontinuing the search for microspheres that exceeded the limits of the image (once part of the search area fell outside the image) and also for the rejection of manifestly incorrect tracks. These can occur when a second more intense microsphere appears in the search area and the search algorithm then latches onto an incorrect path.

Analysis—After tracking was completed, the initial location and subsequent path taken by each microsphere was stored as a sequential list of *x,y* coordinates from which the speed of each microsphere was obtained. However, our primary interest lies in determining where and when the matrix deforms. As discussed previously, the movement per se of a single microsphere does not reveal the local rate of expansion in the matrix, but this can be determined from the relative position of a pair of microspheres. However, when considering the radial expansion of flat-faced cylindrical tablets, which are radially symmetrical, the distance of each microsphere from the center of the tablet can replace the *x,y* coordinates. This then permits the grouping of microspheres into bands of similar radii. Within each band the radius of each microsphere was expressed as a ratio to its original radius, and the mean ratio multiplied by the nominal radius of the band then produced the average radius. The changes in the average radius of each band and difference in radii between adjacent bands were used to measure overall expansion and local expansion. The coordinates of the tablet center were derived from the curvature of the tablet edge. When bisected tablets were observed, the movements of individual microspheres were separated into radial and axial vectors.

Results

The images obtained showed that it was practical to incorporate and detect fluorescent microspheres in HM tablets. The compression used during tableting did not seem to fracture or fragment the microspheres, and their distribution appeared to be random. Sequential CLSM images of hydrating HM tablets showed that individual microspheres could be followed for long periods.

In Figures 1 and 2 individual fluorescent microspheres and their movement are apparent. The individual microspheres, polymer particles, and air bubbles appeared to move linearly and in concert. The speed (the distance between successive occurrences) of microspheres within a few hundred micrometers of each other differs, which we attribute to different rates of local expansion of the matrix. In the lower panels of Figure 2 a blurring of the polymer particle and the large microsphere is apparent, and in the maximum projected image (bottom, left) there are five occurrences of the large microsphere but only three occurrences of the smaller microspheres. We attributed these variations to small movements in the *z* axis that took smaller objects outside the focal plane and defocused the larger objects. Problems associated with small movements in the *z* axis were minimized by using only the larger 22 μm microspheres in subsequent experiments and by using a maximum projection of four images taken at different depths.

Figure 5 (upper) shows that individual microspheres can be clearly seen in a dry tablet. The maximum projections (Figure 5 center and Figure 9 upper) contain the combined sequence of images and revealed linear tracks, apparently radiating away from the center of the tablet. The tracks of individual microspheres, shown in the maximum projected images in Figures 5, 8, and 9 are either linear or gently curving and are in the direction of expansion. Their linearity shows that Brownian motion had minimal influence. The overall pattern of movement and the continuing proximity of microspheres to the edge of the gel layer indicate that the microspheres were carried along with the hydrating polymer.

Individual microspheres, shown by their paths in the maximum projected images in Figures 5, 8, and 9, cover markedly different distances depending on their initial position in the tablet. The actual distance covered is the path length minus the diameter of the microsphere, but this is an overall measure taken over the full duration of the experiment; a more detailed assessment requires tracking individual microspheres through the whole sequence of images.

The tracking software proved able to follow a substantial number of microspheres. However, some microspheres moved outside the plane of focus of the confocal images and were lost; others were not apparent in the first image and only appeared in subsequent images, either by moving into the plane of focus or becoming visible as a transparent gel layer replaced the relatively opaque dry polymer. The greatest problems occurred with microspheres on the edge of the tablet, which move very rapidly in the initial 2 min after hydration making them difficult to track, and with

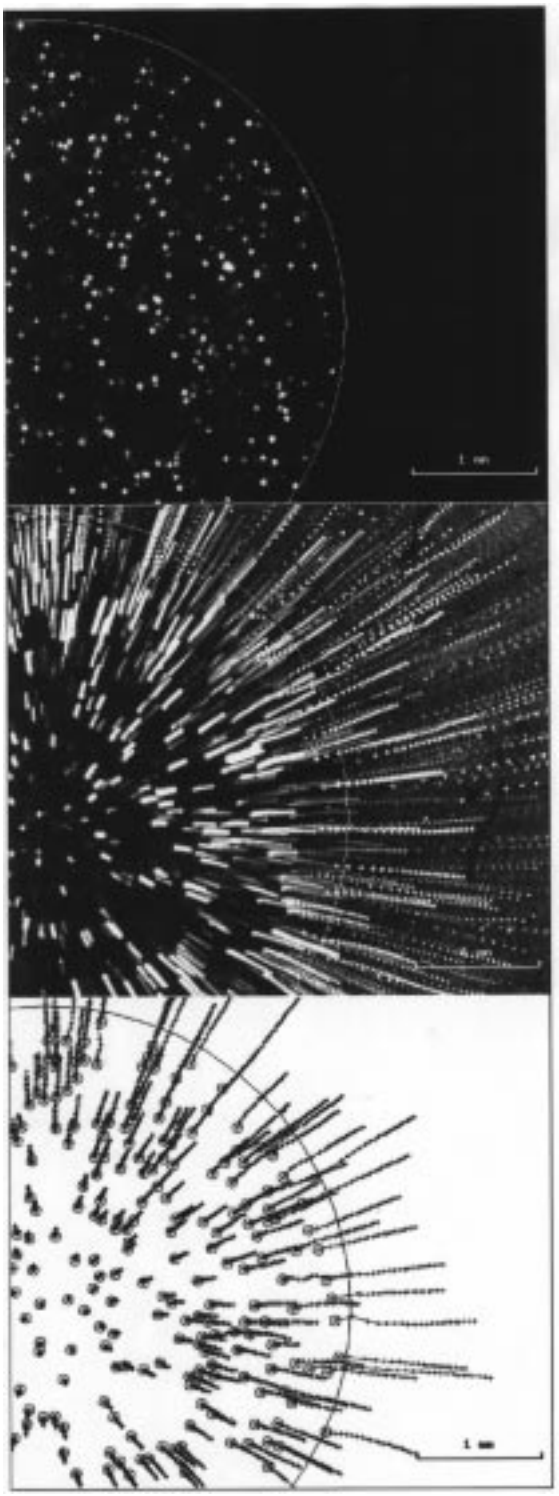

Figure 5—Radial expansion of a xanthan tablet. Upper panel: a confocal microscope image showing fluorescent microspheres within a dry tablet. Center panel: a maximum projected image showing the paths taken by microspheres during the 40 min following hydration. Lower panel: the mapped tracks of identified microspheres starting from their original locations. The original edge of the tablet is superimposed on each image.

pairs of microspheres that were too close to be easily separated. The complete tracks of 211 microspheres that were followed from the first image appear in Figure 5 lower panel.

It is apparent that the movement of individual microspheres depends on their proximity to the initial edge of the tablet and declines further into the tablet. This is made clear in Figure 6 when the individual movements are

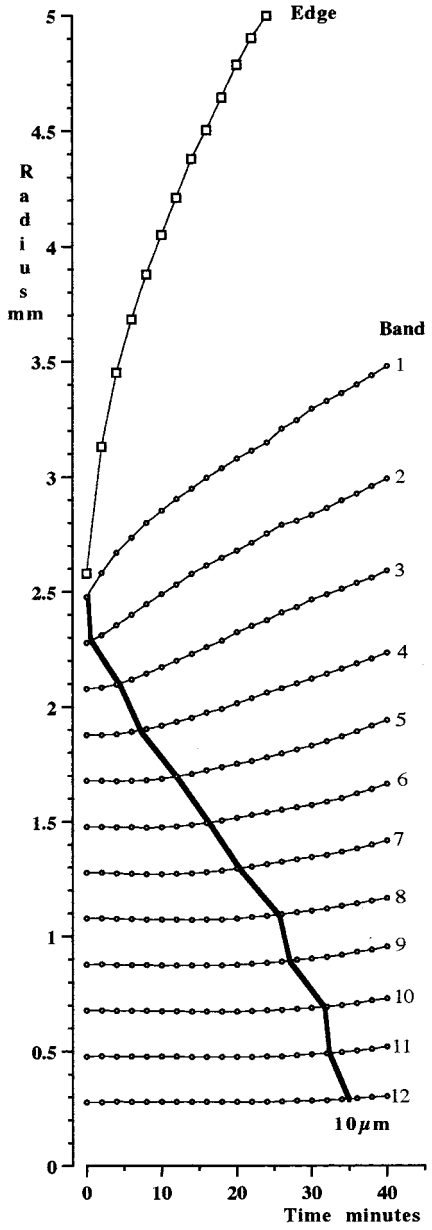

Figure 6—The expansion within a xanthan tablet undergoing hydration, shown as the growth of a series of 200 μm concentric bands radiating from the tablet center. Band 1 is the outermost and band 12 the innermost. $y = 0$ mm is the tablet center. The movement of the outermost edge of the tablet is also shown. The thick line links the time points when each band first moved by 10 μm.

pooled by assigning microspheres into a series of 200 μm wide bands at increasing distance from the tablet edge. The outer bands move earlier and faster, but even the deepest bands finally undergo some movement. An estimate of when each band began to move was obtained by marking the time when a 10 μm displacement first occurred. This reveals the ingress of a wave of expansion which reached the innermost band after 36 min. This was a surprising finding since the gelatinous layer formed around the tablet only extends over the first few bands.

The outer edge of the gel, shown in Figure 6, appears to expand much more dramatically than the microspheres in the outermost 200 μm band. This is a consequence of underestimating the expansion of the outer band due to difficulties in tracking microspheres that originate close to the tablet edge. The rapid expansion made following these microspheres difficult.

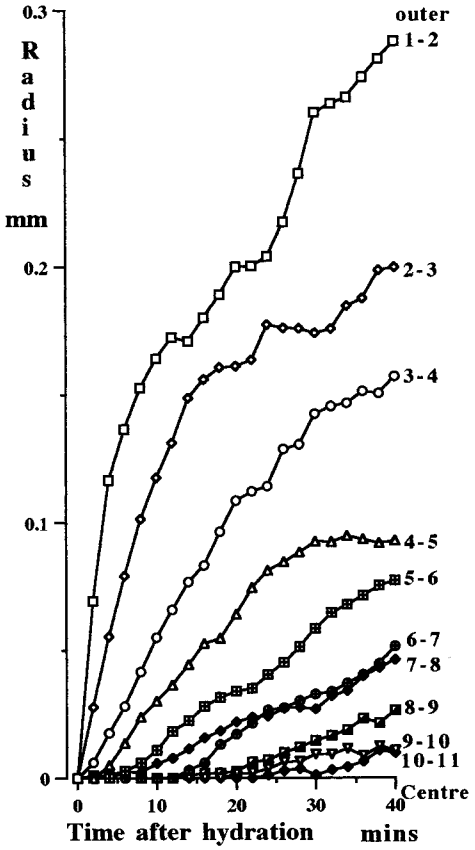

Figure 7—The differential expansion between adjacent concentric bands in a hydrating xanthan tablet, determined by subtracting the movement of the adjacent inner band. Each band is 200 μm thick and band 1-2 is the outermost.

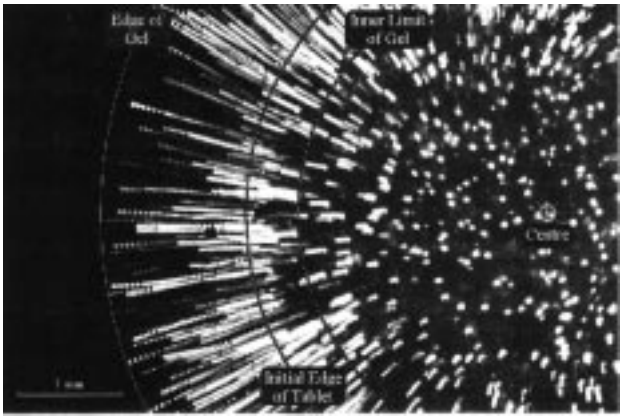

Figure 8—Tracks made by fluorescent microspheres in a xanthan tablet hydrating in 0.05 M NaCl over 80 min. The image is a maximum projection. Also marked is the initial location of the tablet edge and the final limits, inner and outer, of the gel layer.

The progressively increasing radius of each band could correspond to expansion within the band, to displacement following the expansion of deeper areas of the matrix, or to a combination of both mechanisms. In Figure 7 the analysis is extended by subtracting the radius of the adjacent inner band from each band, leaving the endogenous expansion. This shows that expansion progressively decreases deeper within the tablet and that the outer band is still expanding at the end of the experiment.

To determine the relative penetration of the gel layer into a tablet and the depths at which the movement of microspheres occurred, the gel layer of a hydrated tablet was gently removed with a small spatula and the size of the residual core measured. The core of the tablet was hard and appeared dry. In Figure 8 the initial tablet edge and the limits of the gel layer are superimposed on the tracks of the embedded microspheres. The extended tracks of microspheres beyond the inner limit of the gel layer shows that expansion had occurred deep in the core of the tablet though had not yet reached the center.

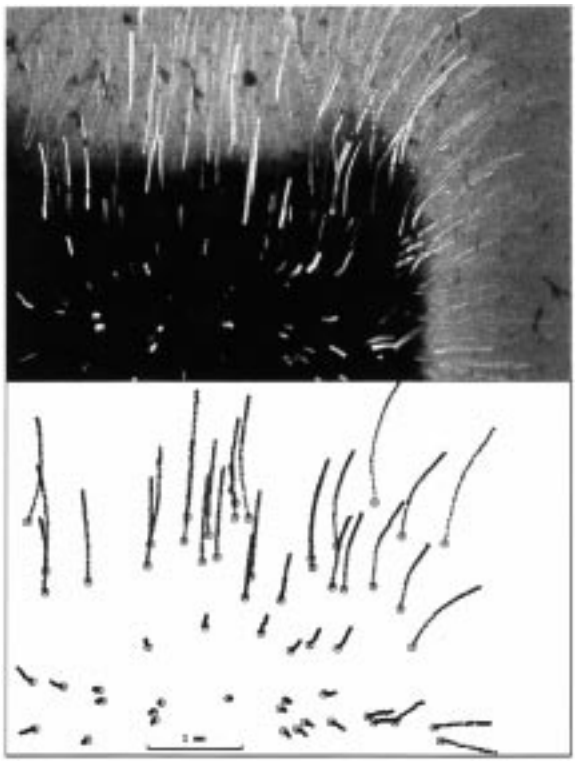

Figure 9—The expansion of a bisected hydroxypropylmethylcellulose tablet after hydration. Upper panel: a maximum projected image showing the paths followed by microspheres following hydration. The inclusion of the fluorophore Congo red in the hydration medium makes the gel layer apparent. Lower panel: the mapped tracks of identified microspheres, starting from their original locations. Axial movements are in the vertical plane and radial movements in the horizontal plane.

When a bisected hydroxypropylmethylcellulose (HPMC) tablet was examined (Figure 9), the pattern of microsphere movements was more complex, with some microspheres taking curved paths, rather than the linear trajectories seen with intact tablets. Curved tracks predominated at the corners of the tablet, reflecting the interplay between axial and radial expansion. More unexpected were the tracks of some microspheres that originated slightly away from the corners and, while predominantly moving axially, had an initial inward radial component that was later replaced by an outward component. We suspect that this pattern may be due to an inward pressure exerted by the more rapidly expanding corners of the tablet that cause a slight short-lived radial compression. The larger surface area at the corners increases their exposure to water and accordingly leads to a greater rate of expansion, which produces a dumbbell shape.[10]

Examination of tablets containing different amounts of microspheres (0−2.0% w/w) showed no discernible difference in their rates of expansion when recorded at 4 min intervals and followed over 60 min, with each performed in duplicate.

Discussion

We have demonstrated that it is possible to closely follow events within a hydrating tablet and to follow its expansion in the *x* and *y* axes but not concurrently in the *z* axis.

The hydration cell, by preventing direct access by water to the upper face of the tablet, effectively makes observation of only this face serve to cover a range of depths from the outer limit of gel layer to the center of the tablet. Prevention of direct hydration of the upper face is critical because (i) the formation of an overlying gel layer would quickly exceed the imaging depth of even a confocal microscope, (ii) the opacity of the unhydrated polymer would prevent imaging below the unhydrated surface, and (iii) the accurate measurement of depth[11] is especially difficult through layers of progressively changing refractive index. The prevention of direct hydration was achieved with a silicon rubber membrane between the upper surface of the tablet and the upper Petri dish and is further enhanced once a gel layer has formed around the edge of a tablet, which seals any residual gaps and prevents water flowing across the upper surface. By imaging 100 μm below the silicon rubber/tablet interface, we probably avoid any interference between the polymer and the membrane. However, an interaction can only be precluded either by imaging at much greater depths, which is unfortunately impractical, or by comparison of the experimental observations with similar observations from an unrelated technique, which is currently unavailable.

The use of intact tablets measures radial expansion while bisected tablets permit the measurement of both radial and axial expansion. Intact tablets, when confined in our hydration cell, model a tablet of infinite axial thickness in which only radial expansion can occur. The curved and faster trajectories seen with microspheres at the corners of bisected tablets is probably due to their exposure to external water from two surfaces and generates the dumbbell shape commonly observed with flat-faced circular tablets. The bisected tablet is a better model of in vivo hydration since it shows both axial and radial expansion; however, the analysis of the tracks is much more difficult, and this approach requires the physical cutting of tablets, which may alter their performance.

Our measurements of expansion were made in unstirred water, unlike the standard USP procedures for measuring drug release which place tablets in a rotating cage or a medium stirred by a paddle. Our experiment was therefore a simplification of a tablet undergoing drug release since erosion of the tablet was greatly reduced; however, it does demonstrate that complex measurements are possible within these tablets. The hydration cell could be modified to permit both agitation of the hydrating medium and also to allow fluid sampling to determine drug release rates, thereby providing a more complete picture of in vitro release.

A major assumption underlying the use of markers is that they remain trapped within the matrix and do not move independently. The linearity of their tracks, in contrast to the random walks seen with Brownian motion, confirms our expectation, based on their mass and the viscosity of the gel that diffusion does not contribute significantly to their movement. Also we found no evidence that the initial inrush of water carried microspheres deeper into the matrix. An additional assumption was that the presence of the microspheres does not alter the performance of the matrix; the small fraction of the matrix occupied by the chemically inert microspheres makes this assumption plausible, and experiments showed that the expansion of tablets was unaffected by the presence microspheres.

Early experiments tested silver granules, observed by reflection, as nondiffusing markers, but their lower intensity and irregular shape made tracking difficult and they proved less satisfactory than fluorescent microspheres. Undissolved particles of polymer and air bubbles could potentiallly be used as markers, but air bubbles are only found in hydrated regions of the gel layer and are unevenly distributed, while the irregular shape of undissolved polymer particles makes precise tracking difficult. Both air bubbles and polymer particles can only be observed with transmitted light which would preclude observation in the poorly hydrated tablet core which is opaque.

The tracking software combined with CLSM imaging was able to follow individual microspheres. Similar tracking of particulates has been employed to measure the motion of individual particles on cell surfaces,[12] sperm motility,[13] and rates of mucocillary clearance.[14] The use of a predictive tracking strategy is only appropriate because the random walks seen with simple diffusion are not seen. Our analysis requires that each microsphere is tracked from its initial location, prior to hydration, over the whole image sequence. This proved difficult for microspheres originating close to the tablet edge, because the dramatic initial swelling makes finding the subsequent location very difficult. Increasing the frequency of images over the first few minutes reduces this problem, though the changing time intervals need to be incorporated into the predictive part of tracking strategy.

To summarize the pattern of expansion, the tracks of individual microspheres were grouped into bands of similar depth and the normalized movements of each microsphere were combined to obtain the changing radius of each band (Figure 6). This treats each band as homogeneous, which is reasonable given the radial symmetry of the expansion and the relatively macroscopic level of investigation. Within each band microspheres appeared to be randomly distributed and each band contained many microspheres; however, it should be noted that the shrinking area covered by bands closer to the center of the tablet progressively reduces the number of associated microspheres and therefore the accuracy of the measurements. This could be ameliorated by calculating the location of each microsphere to subpixel accuracy.[15] In the outermost band the assumption of homogeneity of deformation is combined with the difficulty of tracking the outermost microspheres and produces an underestimate of the expansion. While this is lessened by the ability to follow the outer gel edge, it could be reduced by using thinner bands and by using more frequent imaging in the initial period after hydration.

Overall we are able to quantify deformation throughout a HM tablet and confirm that expansion reaches deep into the tablet, well in advance of the limits of gel layer. Expansion of the core, especially in HPMC matrix tablets has been reported previously,[16] but this is the first direct observation made of the internal dynamics of the process. The underlying mechanism is unclear, but we suspect either the effect of water vapor moving ahead of the gel front or that the gelation of the exterior reduces the mechanical integrity of the tablet, releasing stresses stored during compression. Prolonged exposure to water vapor alone has been reported to cause axial expansion of HM tablets[1] albeit over a longer time scale.

It is also apparent that the outer layers of the these tablets expanded dramatically and still continued to expand as deeper layers began to hydrate. In tablets made from HPMC we have observed how rapid water uptake of the constituent polymer particles then retards the total water uptake by the whole tablet,[17] but the time scale for this process may differ between polymers. In addition, we would anticipate finding, and being able to experimentally differentiate between, hydrated matrixes with similar gel layer thicknesses, but which have resulted from different patterns of internal expansion. This could result in different polymer distribution within the gel layer and altered controlled release properties.

Our method has similarities to studies of HM tablets with magnetic resonance imaging that were used to follow gross changes in the size of the gel layer[18] and map water mobility[19] but cannot establish the local expansion within the gel or dry core. At present, MRI imaging also has poorer temporal and spatial resolution than CLSM.

There is considerable scope for extending the use of embedded microspheres. We have used them to measure syneresis in gels,[20] and the technique would be suitable for examining dehydration shrinkage. The method could also be used to examine selected areas at much higher magnifications, to isolate localized inhomogeneities that undoubtedly exist. The development of techniques for imaging deep within matrixes would make it feasible to measure deformations in three dimensions.

Through measuring internal events within HM tablets we believe it will become possible to elucidate the complex events that underlie their performance. Although the suggestion that "I have yet to see any problem, however complicated, which when looked at in the right way, did not become still more complicated"[21] might be considered to apply, we submit that the dynamics of drug release from HM tablets are inherently complex and require models of similar complexity founded on similarly detailed experimental observations.

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