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The focused ion beam technique: A useful tool for pharmaceutical characterization

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Abstract

Focused ion beam (FIB) instrumentation, a hybrid of the scanning electron micrsocope, ion milling and computer-aided design systems, has special uses in the electronic and semiconductor industries as a tool for failure analysis and device development. This paper examines the pharmaceutical applications of the FIB, particularly microscopic analysis of microspheres. With the FIB, microsphere structures were peeled off, layer by layer, and the structure of each layer was simultaneously observed under scanning microscopy. The particles had a wrinkled but non-porous surface. Going deeper, some holes appeared, with size and numbers increasing toward the particle center. This unique method precisely investigated the inner structure of particles, layer by layer. Then, by FIB milling, samples were extracted with an accuracy of localization of 50 nm from specific parts of the microspheres and prepared to a 200 nm uniform thickness film for examination under transmission electron microscopy. The FIB method also has the potential for a wide range of other quantitative and qualitative analysis of dosage forms and materials.

Keywords: Focused ion beam; Microspheres; Ion milling; Microscopy; Image analysis

1. Introduction

Microspheres have been considered in the pharmaceutical industry since the 1960's for different applications such as taste and odor masking, drug protection from the environment and vice versa, separation of incompatible materials, sustained release production, controlled release, and targeted medication (Burgess and Hickey, 2002).

Controlled release is an attainable and desirable characteristic of drug delivery systems. The rate of drug release from microspheres dictates their therapeutic action. Drug release from microparticles is governed by various properties of the polymer, the drug and the carrier system. Polymer-dependent factors include molecular weight and its distribution, the copolymer ratio and its distribution, and polymer crystallinity. Important drug-dependent parameters are drug solubility in dissolution or biological fluids, molecular weight and possible polymer–drug interactions. Carrier system-dependent factors comprise microparticle type (microsphere versus microcapsule), particle size and particle size distribution, and porosity and internal structure of the microparticles (Herrmann and Bodmeier, 1995; Washington, 1990), among which internal structure is one of the most significant features (Yang et al., 2000; Jeong et al., 2003).

Despite its importance, there are only a few articles regarding the study of microsphere internal structure, all of which prepared a cross-section of microsphere by random cutting with a blade or by breaking it under pressure (Ehtezazi et al., 1999; Wang et al., 2002; Ruan et al., 2002; Lambert et al., 2000). However, such techniques have the drawback of damaging the internal porous structure and of giving information only on a limited part of the internal structure. With these methods, it is impossible to exactly locate the investigated zone of the particle and to study the structure of particles at different depths. Also, they offer no chance to obtain a uniform and thin slice for further investigation by other techniques such as transmission electron microscopy (TEM).

The focused ion beam (FIB) has been widely exploited in the semiconductor industry for many years as a tool for failure analysis and device development (Melngailis, 1987). However,

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in more recent years, it has become well-established for broader materials science applications (Cairney and Munroe, 2003).

FIB instrumentation is a hybrid of the scanning electron microscope (SEM), ion milling and computer-aided design systems (Kim and Dravid, 2000). FIB systems operate in a similar fashion as SEM except that, rather than a beam of electrons, as the name implies, FIB systems use a finely-focused beam of metal ions that can be operated at low-beam currents for imaging or high-beam currents for site-specific sputtering or milling.

The ion beam is ejected from a liquid metal ion source (usually gallium), with a spot size smaller than 5 nm on modern systems. The gallium primary ion beam hits the sample surface and sputters a small amount of material, which leaves the surface as either secondary ions or neutral atoms. The primary beam also produces secondary electrons. As the primary beam hits the sample surface, the signal from the sputtered ions or secondary electrons is collected by SEM or scanning ion microscopy (SIM) coupled to the FIB to form an image (Kamino et al., 2004). It gives the opportunity to observe the exact structure and pores at different depths of the sample. Okayasu et al. (2001) tested the FIB method to study the pore size distribution of a coated layer on paper. By deploying ions as the scanned species, compositional imaging is possible via secondary ions (Anonymous, 2006).

The FIB is also practical for microanalysis of the interface between two different solid phases (Benzerara et al., 2005; Sugiyama and Sigesato, 2004). Another major ability of the FIB and dual-beam FIB-SEM systems is to analyze a 3D microstructure by taking sequential sets of 2D images (Steer et al., 2002; Inkson et al., 2001).

At low primary beam currents, very little material is sputtered, which is useful for imaging. On the other hand, high-beam operation serves to sputter or remove material from the surface. Indeed, the beam can be finely focused to a diameter of approximately 5 nm and deliver sufficient kinetic energy to sputter and remove materials with high positional accuracy (Ngo et al., 2000).

Another particularly helpful feature of the FIB is to prepare TEM specimens. Unlike many conventional techniques, the FIB allows TEM samples to be prepared from highly-specific areas with an accuracy of localization of 50 nm, and uniform thinning can be done routinely over large areas (up to 100 μm^2) (Volkert et al., 2004). The FIB has been used successfully to prepare TEM specimens from a wide range of materials, including galvanized steel (Giannuzzi et al., 1998), ceramic composites (Kim and Dravid, 2000), carbon-reinforced fiber (Mucha et al., 2005), apatite–protein composites, such as human tooth enamel (Hayashi et al., 1998) and human dentine (Hoshi et al., 2000).

This technique can give valuable information on the size, shape and arrangement of particles which make up the specimen. Particle interrelationships at the scale of atomic diameters are also attainable in terms of crystallographic or compositional information (if so equipped), i.e. the elements and compounds the sample is composed of and their relative ratios, in areas a few nanometers in diameter.

Despite these great advantages, to the best of our knowledge, the FIB technique has not yet been used in pharmaceutical sciences, particularly in drug targeting. In this article, the possibility of applying the technique to the structure analysis of poly (D,L-lactide) (PLA) microspheres is reported.

2. Materials and methods

2.1. Preparation of microspheres

Naltrexone-loaded microspheres were prepared by a double-emulsion, solvent-evaporation technique as reported previously (Dinarvand et al., 2005). Briefly, 400 mg PLA (Resomer LR708, inherent viscosity 3.6 g dl^{-1}) was dissolved in 10 ml methylene chloride. An aqueous solution containing 10%, w/w naltrexone hydrochloride (with respect to PLA) was prepared separately. This aqueous phase was emulsified into the oil phase (containing PLA) with a high speed homogenizer (T18 basic, IKA, Germany) at 18,000 rpm for 2 min. Afterwards, the primary emulsion was added to 100 ml of 0.5%, w/v poly vinyl alcohol containing 10%, w/w NaCl while stirring with a mechanical mixer at 800 rpm to form $W_1/O/W_2$ double emulsion. Mixing continued for 4 h at room temperature until complete evaporation of methylene chloride. The microparticles were collected by filtration, washed with water and finally freeze-dried.

2.2. Focused ion beam and transmission electron microscopy

2.2.1. Focused ion beam milling

FIB milling of samples was undertaken in a FB-2000A Hitachi focused ion beam system (Hitachi High-Technologies Corporation, Japan) at an accelerating voltage of 30 kV. For layer by layer microparticles peeling, a gallium beam current of 0.3 nA were used. For TEM sample preparation, rough thinning was performed with a beam current of 6.3 nA, and final thinning was performed with a beam current of 0.3 nA. More details are given under the results and discussion part.

2.2.2. Scanning electron microscope imaging

While the process of ion milling was carried out, the detailed structure of the sample was monitored by the built-in scanning electron microscope (SEM) at an accelerating voltage of 15 kV.

2.2.3. Transmission electron microscopy

Thin samples prepared by FIB milling were observed under a JEM-2100F Field Emission Electron Microscope (JEOL Ltd., Tokyo, Japan) operating at an accelerating voltage of 200 kV. Selected-area electron diffraction (SAED) patterns were obtained in microprobe mode under the same illumination conditions with a 30 μm selected-area aperture.

3. Results and discussion

Fig. 1a presents the secondary ion image of a microsphere surface. The particle had a wrinkled but non-porous surface. Half of the microsphere was faced towards ion milling in a FIB machine. Just after peeling off a few layers, at a depth of 4 μm from the surface of the microsphere, some holes started to appear

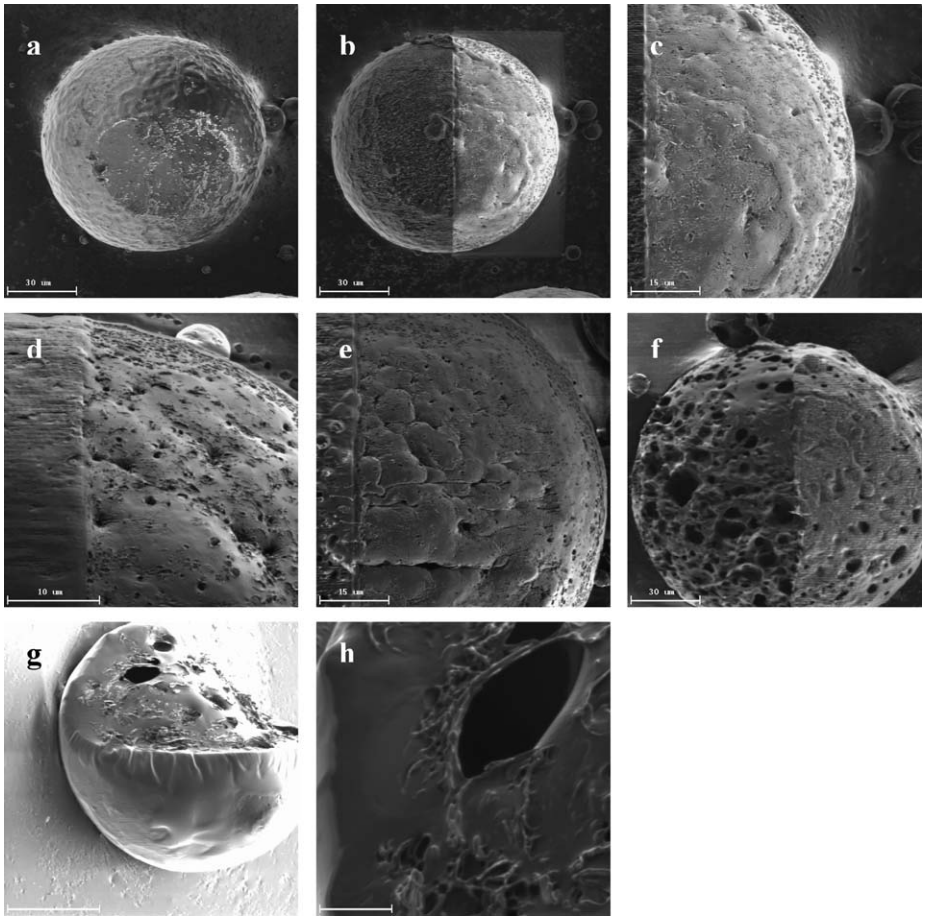

Fig. 1. Observation of the internal structure of a PLA microsphere, layer by layer, using FIB milling: (a) intact microsphere, (b–h) after milling to different depths.

on the surface (Fig. 1b). Going deeper, the number and size of the holes increased, clearly showing the porous structure of the microsphere (Fig. 1c–f). At a depth of 20 μm from the surface of the particle (Fig. 1g and h), some very large holes appeared.

As discussed in details elsewhere (Dinarvand et al., 2005), during the microsphere preparation by double emulsion technique, increasing the osmotic pressure of the outer aqueous phase by addition of 10% of NaCl resulted in microspheres with smooth and dense surface. In the absence of NaCl, water from the aqueous outer phase migrates towards the higher osmotic pressure medium of the inner aqueous phase. This movement of water creates microspheres with a highly porous surface. Adjusting the osmotic pressure of the aqueous outer phase by using NaCl prevents the migration of water to the non-solidified droplets and leads to a dense surface (Dinarvand et al., 2005). FIB milling revealed, despite the non-porous surface of the particles, the presence of pores inside the microspheres, which were prepared using NaCl in the external aqueous phase. It is thought that the porous structure of the microspheres of this formulation results from the inclusion of primary emulsion droplets within the polymer layer. It is conceivable that a pore would develop at the site of an aqueous droplet upon solidification of the polymer matrix.

An interesting point of the FIB technique is the possibility of precisely observing the inner structure of particles, layer by layer. Potentially, the images of the microsphere layers allow quantitative measurement of the pore size and total porosity of particles by image analysis software. Okayasu et al. (2001) examined the precision of the FIB method for the measurement of pore size distribution of a coated layer on paper. The FIB method and mercury porosimetry were also compared in samples with known pore structures. The results indicated that the FIB method did not cause significant thermal damage to the paper and was a valuable tool to analyze porosity.

Another possibility with this milling technique is to map drug distribution or to realize compositional analysis in a predetermined depth or part of the dosage form by transferring the sample after different steps of milling to another analyzing machine equipped with X-ray, fluorescence, confocal systems, etc. It could help to have a deeper understanding and more precise interpretation of microsphere structure–property relationships, especially their release properties.

A common and useful technique in materials science is TEM because it allows observation of the internal structure of a specimen with atomic resolution. Another factor in the heightened interest in TEM is that it now allows elemental analysis of microareas smaller than 1 nm. TEM resolution, however, requires thin-sectioning of the specimen. Sample preparation for TEM has been a topic of extended interest and study in different industries, especially for metallic and ceramic samples. But until

now, the only technique for TEM sample preparation of pharmaceutical samples has been cutting the dosage form or material in an ultramicrotome. It is not very usable for small particles such as microparticles, and usually they are observed directly (without cutting) under TEM, which causes some problems and limitations because of high sample thickness. Another main disadvantage of ultramicrotomy is the impossibility of examining and precisely choosing the location of the cross-section.

In the next step, the potential of FIB for polymeric pharmaceutical samples has been examined. Fig. 2 shows the different steps for TEM sample preparation of a microsphere by the FIB lift-out technique. The first step in FIB milling for TEM sample preparation was to determine the area of interest, in this case by observing the sample by FIB imaging. Then, tungsten was deposited for 10 min over the area of interest to form a layer (3 μm × 15 μm) to prevent milling or damage of that region during subsequent steps (Fig. 2a). This tungsten strip will also reveal itself necessary at a later stage when connecting a probe to the sample.

Next, a 30-kV gallium beam operating at 6.3 nA (M0-50 beam) excavated material from one side of the tungsten strip to mill a 15 μm × 20 μm stair-shaped trench 1–2 μm beside the tungsten strip (Fig. 2b). This procedure was necessary to obtain stable and precise excavation and to allow the further step of undercutting while saving milling time. The same procedure was repeated on the other side of the tungsten strip to form a

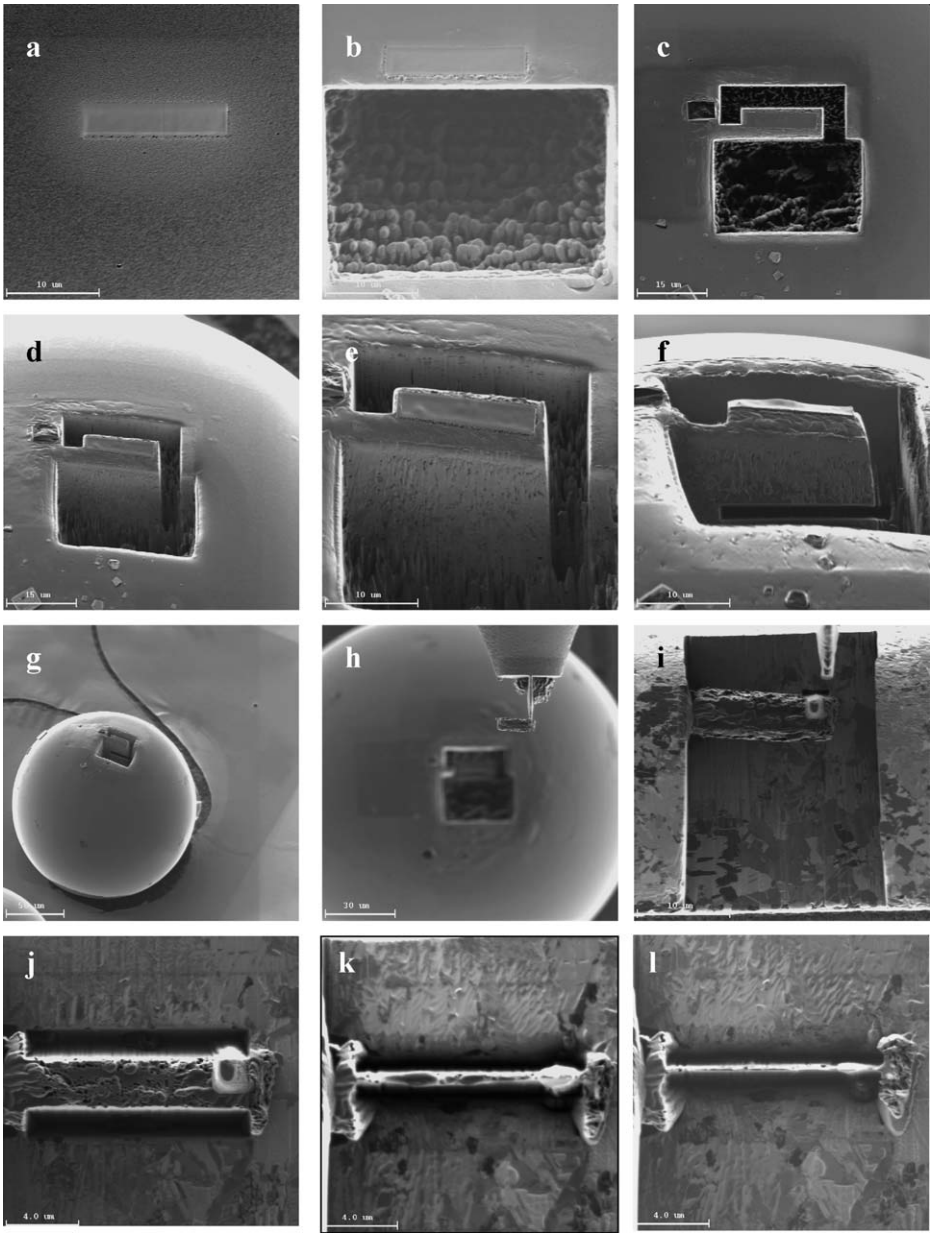

Fig. 2. Secondary ion images of a PLA microsphere at different stages of FIB sample preparation for TEM studies. Images reveal the surface after the following preparation stages: (a) deposition of the tungsten strip; (b and c) ion milling of the trenches; (d and e) tilting of the specimen; (f and g) full undercutting and partial side cutting; (h) lifting out of the sample; (i) fixing the sample at the TEM copper grid; (j–l) final thinning of sample to a thickness of 0.2 μm.

smaller trench. The depth of the trenches was around 15 μm. Then, the right side and a portion of the left side of the sample were cut (Fig. 2c). The next step was to undercut the sample. The microsphere was tilted to a steep angle around 45–60° (Fig. 2d and e), and the undercutting was done by ion beam perforation of the sample along the bottom edge (Fig. 2f). During all these steps, the sample remained connected to the whole microsphere from one corner (Fig. 2g). Then, it was tilted back to its starting position. The next step was to lift out the sample from the microsphere and position it on the TEM copper mesh grid. For this transportation (Fig. 2h), a probe was connected to the sample by 5-min tungsten deposition. After that, the small bridge remaining between the sample and the microsphere was cut, and the free sample was moved to a TEM copper grid on which a 21 μm × 25 μm hole had been created before by a FIB M1-500 system. The sample was put inside the hole and connected to the wall of the hole by tungsten deposition for 7 min (Fig. 2i). Then, it was time for final ion milling and polishing of the sample to achieve 200-nm electron transparency suitable for TEM studies (Fig. 2j–l). This milling was done at 1–2° with respect to the plane of the specimen surface. Ion beam current was reduced to 0.3 nA to minimize possible damage to the sample by the ion beam. The sample was ion-polished on both sides to a nominal thickness of approximately 200 nm. Milling on such a nanometer scale demonstrates the precision that the FIB microscope micromachining system is capable of attaining. The site specifity and precision of this method make the FIB system a very useful tool for TEM sample preparation or when any milling on a microscopic scale is deemed necessary. With the procedure, a sample with an area covering a depth of 15 μm of a microsphere from its outer surface was prepared.

To prepare a TEM sample extracted exactly from the centre of a microparticle, another microsphere was first milled to the centre of the particle by FIB equipped with secondary ion microscopy. Accordingly, the exact place of milling and subsequent extraction could be monitored by FIB microscopy. Then, the tungsten layer was deposited on the desired area at the centre of the particle, and all the procedures described above were repeated.

Fig. 3a and b, respectively shows TEM images of the surface and the centre of the microspheres. As can be seen in these high-resolution pictures, there is no atomic order in the sample, which is a sign of amorphous structure, both in the surface and centre of the microspheres. Selected-area electron diffraction patterns for samples from the centre and surface of the microspheres were obtained under the same illumination condition with a 30-μm selected-area aperture (Fig. 3c and d). The lack of distinct sharp and bright circles in these patterns confirmed the non-crystalline structure of both the centre and surface of the microparticles. The degree of crystallinity is important because the crystalline regions are less permeable to water than the amorphous regions and affect the degradation rate and consequently the drug release rate. The distribution of crystalline groups is also important to degradation. It will be much more difficult for the amorphous part to be degraded if it is blocked by the crystalline groups (Zhang et al., 2003; Freiberg and Zhu, 2004; Edlund and

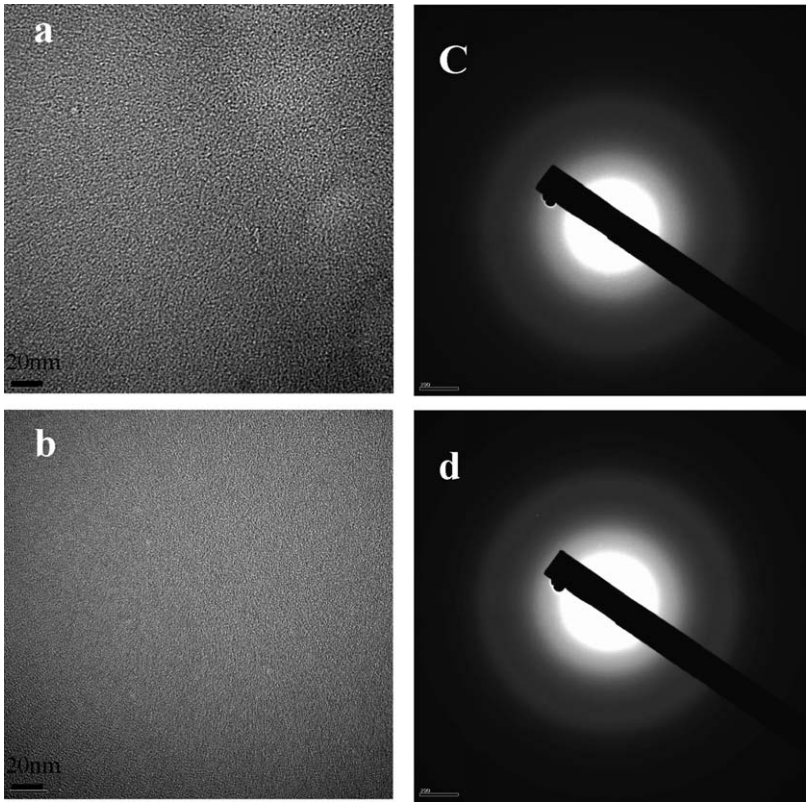

Fig. 3. Transmission electron micrograph of samples of a PLA microsphere from the surface (a) and centre (b) respectively; and corresponding selected-area electron diffraction patterns (c and d).

Albertsson, 2000). FIB-TEM combination represents a unique opportunity to study this matter further.

4. Conclusion

FIB milling and microscopy were used to characterize PLA microspheres. With this technique, it was possible to observe the exact internal structure of microspheres, layer by layer and see the shape and size of pores all around and inside the microspheres. Also, the technique was successfully applied for site-specific, uniform sample preparation for TEM. Samples had a localization accuracy of 50 nm. The technique also has the potential for a wide range of other quantitative and qualitative analyses of dosage forms and materials.

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