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International Journal of Pharmaceutics 186 (1999) 99–108

international journal of pharmaceutics

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Characterization of particle deformation during compression measured by confocal laser scanning microscopy

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Received 27 September 1998; received in revised form 8 April 1999; accepted 11 April 1999

Abstract

Direct compression of riboflavin sodium phosphate tablets was studied by confocal laser scanning microscopy (CLSM). The technique is non-invasive and generates three-dimensional (3D) images. Tablets of 1% riboflavin sodium phosphate with two grades of microcrystalline cellulose (MCC) were individually compressed at compression forces of 1.0 and 26.8 kN. The behaviour and deformation of drug particles on the upper and lower surfaces of the tablets were studied under compression forces. Even at the lower compression force, distinct recrystallized areas in the riboflavin sodium phosphate particles were observed in both Avicel PH-101 and Avicel PH-102 tablets. At the higher compression force, the recrystallization of riboflavin sodium phosphate was more extensive on the upper surface of the Avicel PH-102 tablet than the Avicel PH-101 tablet. The plastic deformation properties of both MCC grades reduced the fragmentation of riboflavin sodium phosphate particles. When compressed with MCC, riboflavin sodium phosphate behaved as a plastic material. The riboflavin sodium phosphate particles were more tightly bound on the upper surface of the tablet than on the lower surface, and this could also be clearly distinguished by CLSM. Drug deformation could not be visualized by other techniques. Confocal laser scanning microscopy provides valuable information on the internal mechanisms of direct compression of tablets.

Keywords: Confocal laser scanning microscopy; Direct compression; Riboflavin sodium phosphate; Microcrystalline cellulose; Compression force; Plastic deformation

1. Introduction

Solid particles have been compressed into tablets for a very long time, but the underlying theory has proved to be extremely complex. A number of difficulties which arise during compression can be related to the behaviour of particles

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00141-6

under a compressing force (Armstrong, 1982). There is now a greater tendency to investigate particle deformation rather than bulk deformation of the powder bed to better explain the mechanisms of tablet formation (Monedero, 1994). In order to understand in greater detail the behaviour of the drug particles under a compressing force, non-invasive techniques that can obtain drug information from the tablets are required.

Confocal laser scanning microscopy (CLSM) is widely used in cell biology and medicine but also in material science where it has become a recognized part of paper science research (Moss, 1998). It can generate high-resolution images, as out-of-focus interference is essentially absent from confocal images, and also visualize structures in three dimensions through materials. Thus, it allows profiling of the surfaces of 3D objects and multilayer structures (Sheppard and Shotton, 1997). Its non-invasive nature avoids the generation of surface defects and contamination, and allows imaging through non-conducting passivation layers, in contrast to SEM. This will give valuable information for investigating behaviour and changing structure in pharmaceutical solid dosage forms. There is only one report on quantifying drug release processes within controlled release dosage forms using CLSM (Cutts et al., 1996). However, no studies on the deformation of particles during compression by using a non-invasive, 3D-generating technique, have been reported.

1.1. Theory of CLSM microscopy

The basic principle of confocal microscopy is shown in Fig. 1. In CLSM, excitatory laser light from the illuminating aperture passing through an excitation filter is reflected by the dichroic mirror and, through an x-y deflection mechanism, this light is turned into a scanning beam, focused to a small spot by an objective lens on to a fluorescent specimen. The mixture of reflected light and emitted fluorescent light is captured by the same objective and is focused on to a photomultiplier via a dichroic mirror. The reflected light is deviated by the dichroic mirror, while the emitted fluorescent light passes through in the direction of the photomultiplier. A confocal aperture (pinhole) is placed in front of the photomultiplier, such that the fluorescent light from points on the specimen that are not within the focal plane where the laser beam was focused will be obstructed by the pin-

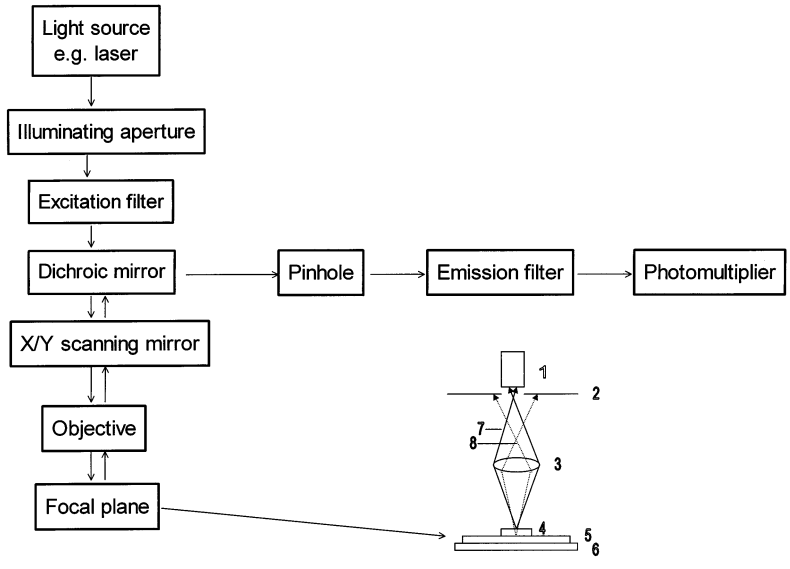

Fig. 1. Diagram of the confocal laser scanning microscopy principle: (1) photomultiplier, (2) confocal pinhole, (3) objective lens, (4) sample, (5) cover glass, (6) slide, (7) in-focus light, and (8) out-of-focus light.

Table 1
Compositions of tablet samples compressed at different compression forces

Sample	Component (45 mg)	Compression force (kN)
T1	Riboflavin sodium phosphate	23.0
T2a	Riboflavin sodium phosphate + Avicel PH-102	1.0
T2b	Riboflavin sodium phosphate + Avicel PH-102	26.8
T3a	Riboflavin sodium phosphate + Avicel PH-101	1.0
T3b	Riboflavin sodium phosphate + Avicel PH-101	26.8

hole. In this way, out-of-focus information (both above and below the focal plane) is greatly reduced. The key feature of confocal imaging is that only what is in focus is detected. This will only happen when the illumination and detection are confined to the same spot in the specimen at any one time. If the spot is so small that its limits are set by diffraction, the resolution in confocal microscopy is greater than in conventional microscopy (Brakenhoff et al., 1979; Wilson and Shappard, 1984). The lateral resolution (*xy* resolution) may approach close to the theoretical optimum of 0.7 times the conventional resolution.

In our work, using an autofluorescent riboflavin sodium phosphate as a model drug we introduce the CLSM technique as a non-invasive method to visualize the underlying mechanisms of particle deformation under different compression forces.

2. Materials and methods

2.1. Materials

Riboflavin sodium phosphate (Ph. Eur.) was used as a model drug. Two grades of microcrystalline cellulose, Avicel PH-102 and Avicel PH-101 (FMC, Ireland) were used as the direct compression fillers. Magnesium stearate (Ph. Eur.) and acetone (E. Merck, Germany) were used for preparing the lubricant suspension for tablet compression.

2.2. Tablet preparation

A 1% riboflavin sodium phosphate powder was mixed with Avicel PH-102 and Avicel PH-101 individually in a Turbula mixer (W.A. Bachofen, Switzerland) for 10 min. The mixtures were examined by CLSM. The quantities of each mixture (45 mg) were weighed individually, and for each tablet the die was hand-filled with powder to ensure uniformity of weight. The upper punch, the lower punch and the die were lubricated with 5% magnesium stearate acetone solution. Tablets were obtained by direct compression in a Korsch EK-0 single-punch tablet machine (Erweka Apparatebau, Germany) equipped with 5-mm flat-faced punches and fitted with strain gauges. The press was operated at a speed of 35 rpm. The tablets were prepared at a compression forces of 1.0 and 26.8 kN. Compression forces of 1.0 and 26.8 kN were chosen to see the effects of low and extremely high compression forces on the compression behaviour and particle deformation of riboflavin sodium phosphate. The compositions and compression forces applied to the tablet samples are shown in Table 1.

2.3. Confocal laser scanning microscopy (CLSM)

The confocal microscope used was a BioRad MRC 1024 (BioRad, UK) equipped with krypton-argon ion laser (excitation lines: 488, 568 and 647 nm) mounted on an Axiovert 135M inverted microscope. Confocal images were obtained using a Zeiss Plan-Neofluar $10\times$/0.30 N.A. air lens. Image processing was done using Lasersharp software (BioRad).

The riboflavin sodium phosphate tablets were individually placed in a cover glass without further preparation. The riboflavin sodium phosphate was autofluorescent compared to the background matrix when excited at 488 nm and the laser power was 1.5 mW. The box size was 512×512 pixels and the images were stored at 8-bit resolution.

The tablet samples could not be focused using $40\times$ water and $63\times$ oil objective of higher magnification. This difficulty was overcome by using a zoom facility. Higher magnification was reached

by means of the zoom facility without loss of resolution. At higher zoom factors, a smaller area of the sample was illuminated with the same laser intensity. The image produced was a thin section of precisely those structures that were in focus. The plane of focus was moved along the z-axis by focusing the microscopy with a stepper motor. The z-series was an automatically collected series of optical sections through the tablets, which was saved as a single multi-image file. The depth of imaging was $15 \sim 30$ μm, the thickness of the resulting optical slice was about 10 μm and the confocal aperture setting was 1.1 mm. By projecting Z-series, the images were subsequently combined. This produced a maximum brightness projection of the data set, based on the true intensities in the data. The images from the vertical section had inferior resolution and showed no details of drug deformation.

2.4. Scanning electron microscopy (SEM)

The morphology of riboflavin sodium phosphate powder was studied by scanning electron microscopy (DSM962, ZEISS, Germany). Preparation of the riboflavin sodium phosphate was accomplished by placing a very small amount of the powder on to a specimen holder. The samples were coated with platinum using a vacuum evaporator. Electron micrographs were obtained at an acceleration voltage of 5 kV.

3. Results and discussion

Microcrystalline cellulose (Avicel PH-101 and PH-102) was chosen as a filler, because it is frequently used in direct compression, and its flow properties and predominating compression mechanisms are well known (Armstrong and Cham, 1986). According to the literature, Avicel PH-101 has a matchstick-like or rodlike structure, whereas Avicel PH-102 is a mixture of primary particles and agglomerates (Bolhuis and Lerk, 1973).

The morphology of riboflavin sodium phosphate powder is shown in the scanning electron micrograph (Fig. 2) and confocal laser scanning micrograph (Fig. 3A). This shows that riboflavin sodium phosphate powder is composed of spherical particles, which cohere, forming aggregates of different shapes. Fig. 3B shows one section image of Fig. 3A.

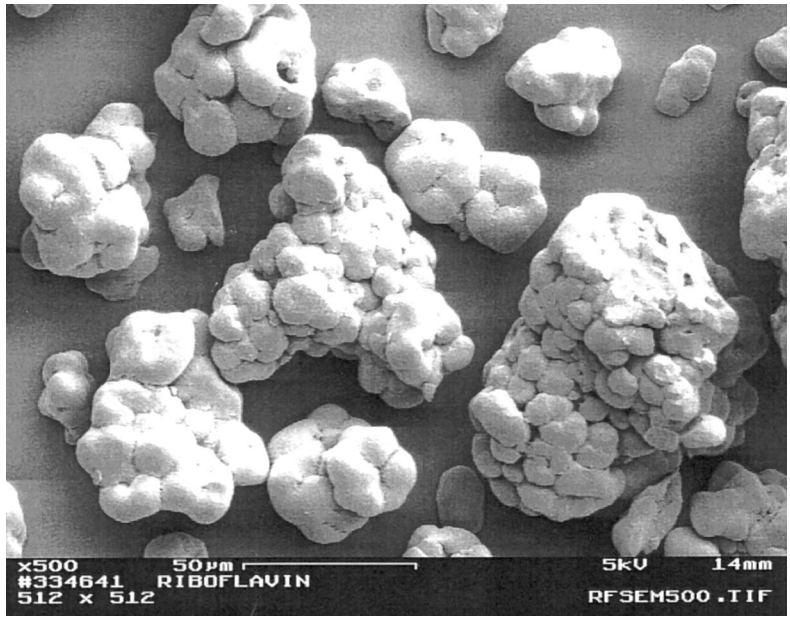

Fig. 2. Scanning electron micrograph of riboflavin sodium phosphate powder. Scale bar = 50 μm.

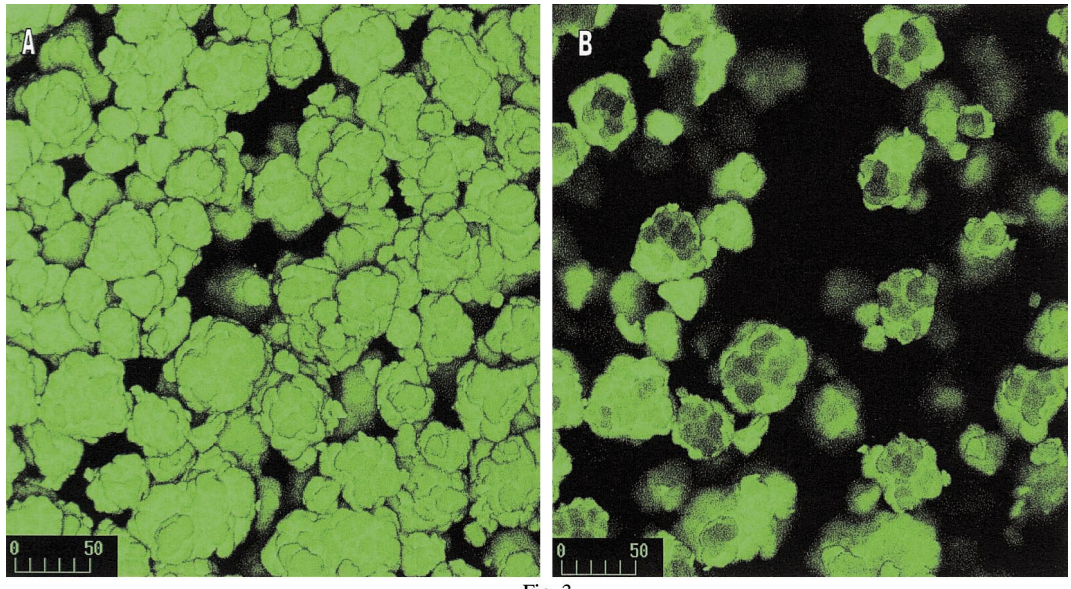

Fig. 3

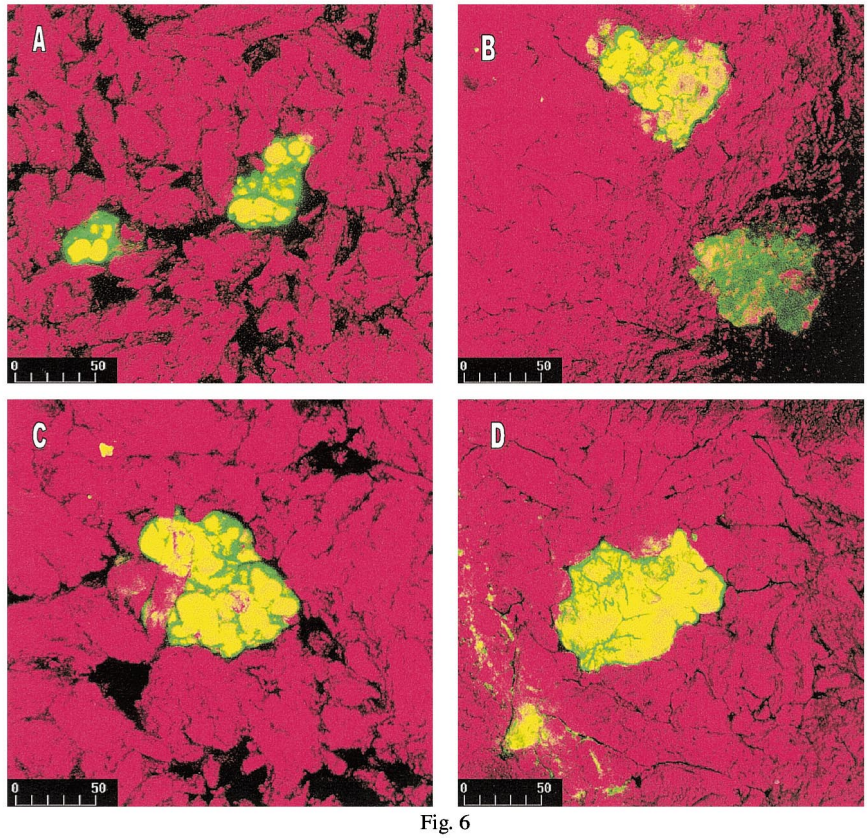

Fig. 6

Fig. 3. Confocal laser scanning microscopy micrograph of riboflavin sodium phosphate powder (A) and one section of z-series (B). Scale bar = 50 μm.

Fig.6. Confocal reflection and fluorescence images performed simultaneously using reflection mode from the lower surface of T3a (A), T3b (B), T2a (C) and T2b (D) tablets. Scale bar = 50mm.

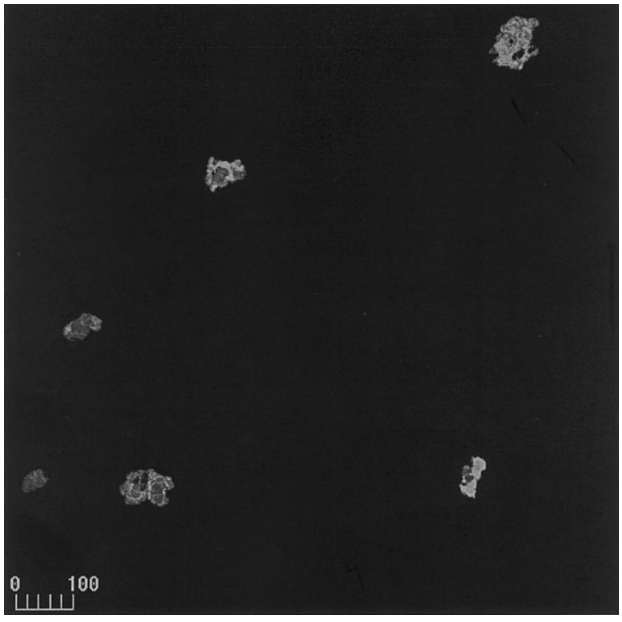

Fig. 4. Confocal microscopy image of distribution of 1% riboflavin sodium phosphate particles within powder mixture of riboflavin sodium phosphate and Avicel PH-102. Scale bar = 100 μm.

The powder mixtures of riboflavin sodium phosphate and MCC were studied at drug concentrations of 0.1, 1 and 5% (w/w) by confocal laser scanning microscopy. Fig. 4 shows the distribution of riboflavin sodium phosphate particles at a concentration of 1% in a powder mixture. This concentration was considered acceptable for direct compression studies. As seen in Fig. 4, after 10 min of Turbula mixing, the riboflavin sodium phosphate particles were still in aggregates and did not adhere to Avicel PH-102. Thus, a random mixture was formed.

The slice of pure riboflavin sodium phosphate powder was manually compressed without a die, in order to examine the behaviour of the drug under the high compression force. The images from CLSM are shown in Fig. 5A. The original boundaries of riboflavin sodium phosphate had disappeared and their original was lost when compared with Fig. 2 or Fig. 3A. A new crystal shape had been formed. This phenomenon can probably be explained by recrystallization and/or sintering phenomena. Firstly, riboflavin sodium phosphate contains a variable amount of water, and, during

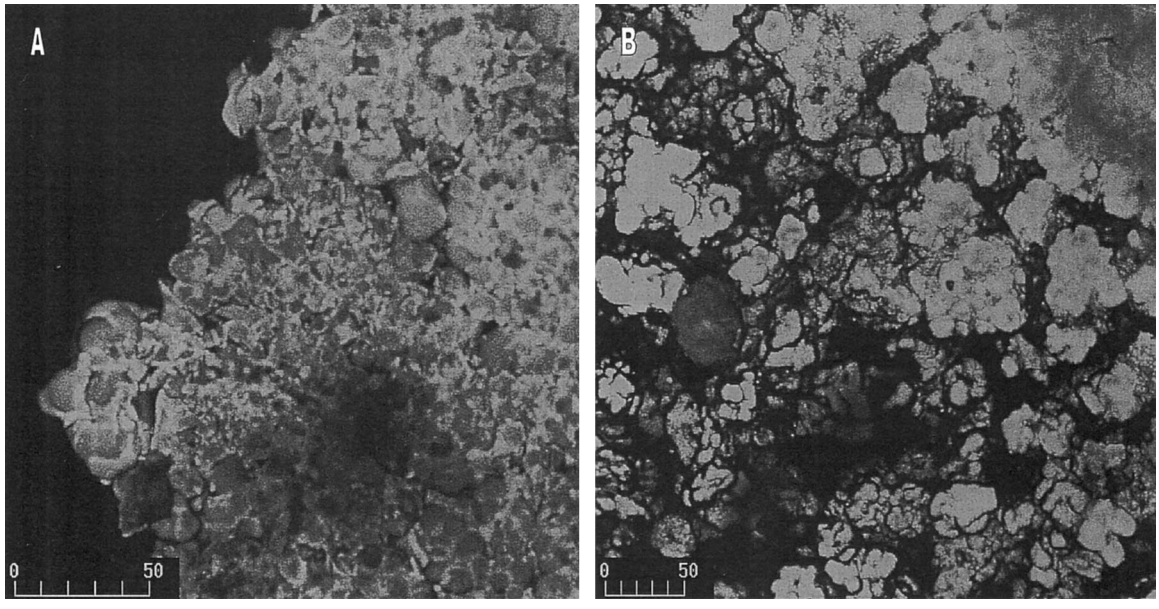

Fig. 5. Confocal microscopy images of a slice of riboflavin sodium phosphate under manual compression without die (A, scale bar = 50 μm), and upper surface of the tablet T1 under mechanical compression (B, scale bar = 50 μm). This is the result of a high compression force.

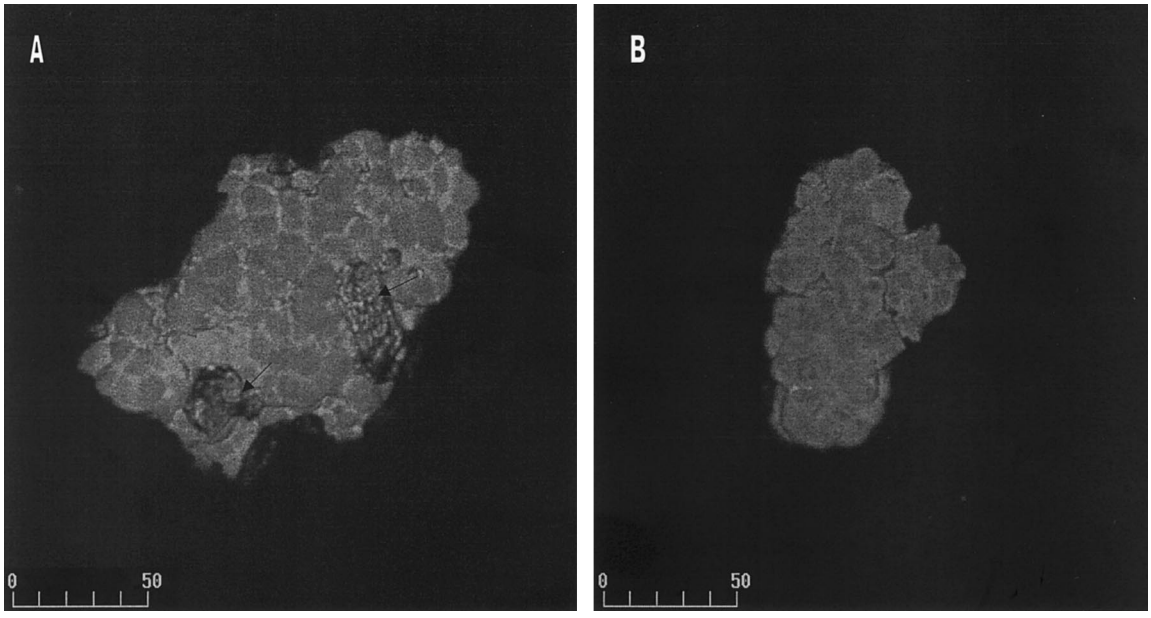

Fig. 7. Confocal microscopy images of riboflavin sodium phosphate particles on the upper surface (A) and lower surface (B) of tablet T2a at a compression force of 1 kN. Scale bar = 50 μm. The recrystallization area is shown by arrows.

compression, the released water dissolves the drug, forming a liquid film separating particles, followed by recrystallization (Stewart, 1986). Secondly, under the high compression force, heat is produced by internal friction, and it will cause a state of fusion in riboflavin sodium phosphate. After passing the highest energy level, the heat will decrease, and sintered forms of the drug may be observed during the ensuing energy decrease (Fuhrer, 1996). The same phenomenon also appeared in mechanical compression of pure riboflavin sodium phosphate with a compression force of 23.0 kN (Fig. 5B).

Riboflavin sodium phosphate tablets compressed with and without lubrication were examined by CLSM. It was observed that magnesium stearate did not affect the image of the samples.

Fig. 6 shows confocal reflection and fluorescence images performed simultaneously using reflection mode, the particle deformation of both microcrystalline celluloses and riboflavin sodium phosphate on the lower surface of the tablets. At a compression force of 1.0 kN, the original Avicel PH-101, Avicel PH-102 and riboflavin sodium phosphate particles could be clearly distinguished (Fig. 6A and C). At 26.8-kN compression force, two grades of MCC and drug particles were deformed and lost their individuality (Fig. 6B and D).

To examine the morphology of riboflavin sodium phosphate separately on the upper and lower surfaces of the tablets, the riboflavin sodium phosphate samples in the confocal images were randomly selected using fluorescence mode from the upper and lower surfaces, depending on their clarity. In the case of riboflavin sodium phosphate crystals, at a compression force of 1.0 kN the original shapes of the crystals on both the upper and the lower surfaces of the tablet T2a can be clearly seen (Fig. 7). The particles underwent rearrangement to form a less porous structure. This takes place at very low forces, with the particles sliding past each other (Armstrong, 1982). The upper surface of T2a tablet showed a small area of spotted crystals (Fig. 7A) as a result of recrystallization. No recrystallization was observed on the lower surface of the same tablet (Fig. 7B). The particles on the lower surface had more voids than those on the upper surface, apparently due to the fact that the compression

force received by the lower punch is always less than that applied by the upper punch (Armstrong, 1982). The same results can be observed in the images of tablet T3a (Fig. 8), except that the recrystallization appeared on the lower surface.

At the compression force of 26.8 kN, the upper and lower surfaces of riboflavin sodium phosphate particles in the T2b and T3b tablets (Figs. 9 and 10) showed the original crystal boundaries, the porosity of the particles disappeared and in-

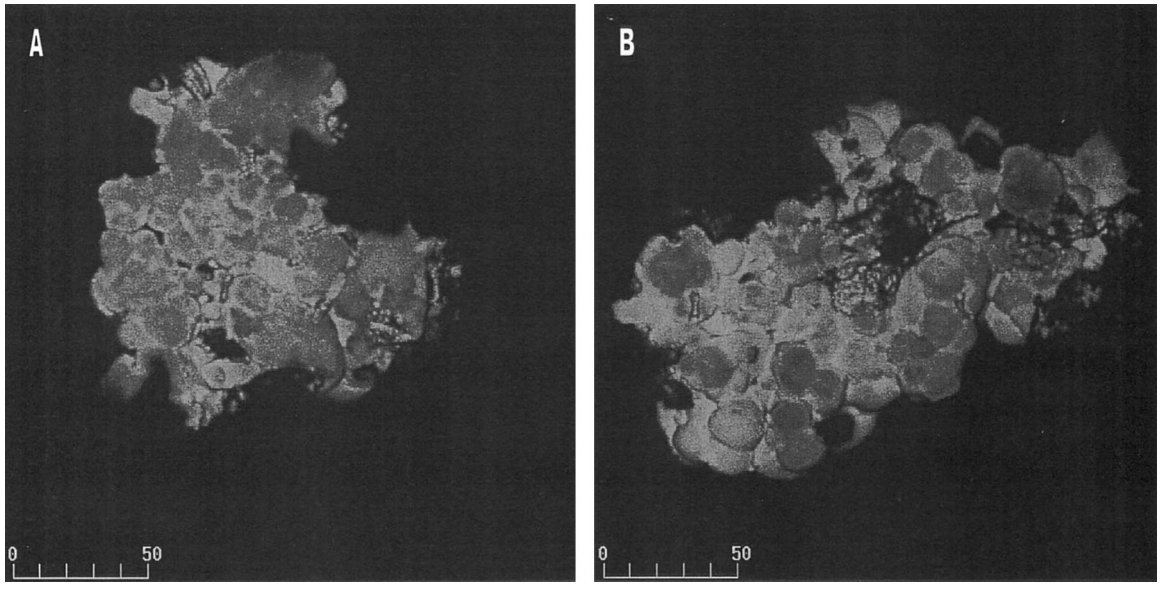

Fig. 8. Confocal microscopy images of riboflavin sodium phosphate particles on the upper surface (A) and lower surface (B) of tablet T3a at a compression force of 1 kN. Scale bar = 50 μm.

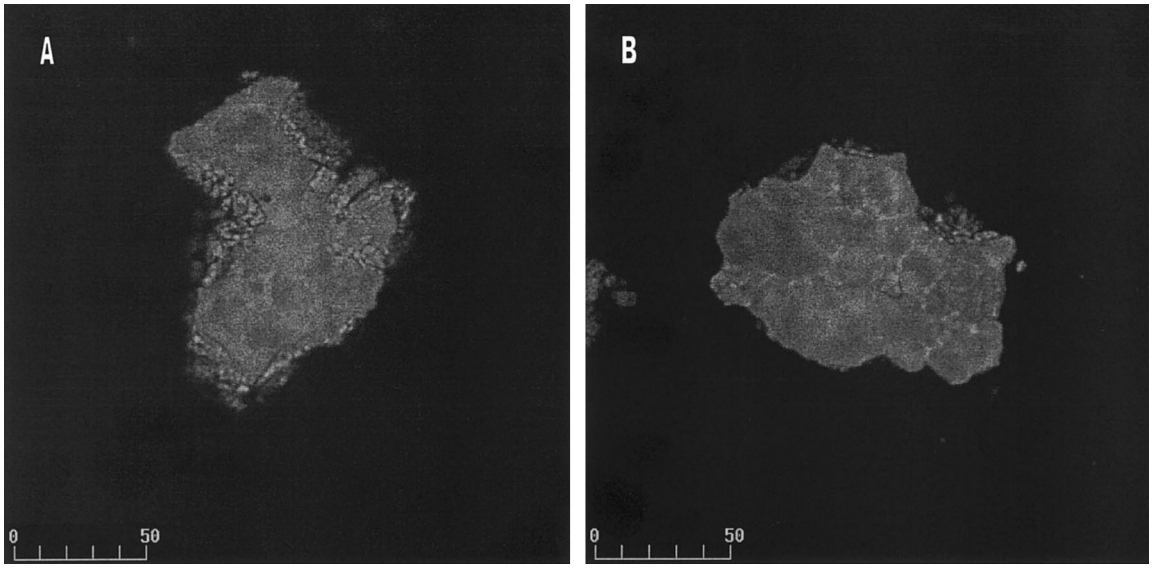

Fig. 9. Confocal microscopy images of riboflavin sodium phosphate particles on the upper surface (A) and lower surface (B) of tablet T2b at a compression force of 26.8 kN. Scale bar = 50 μm.

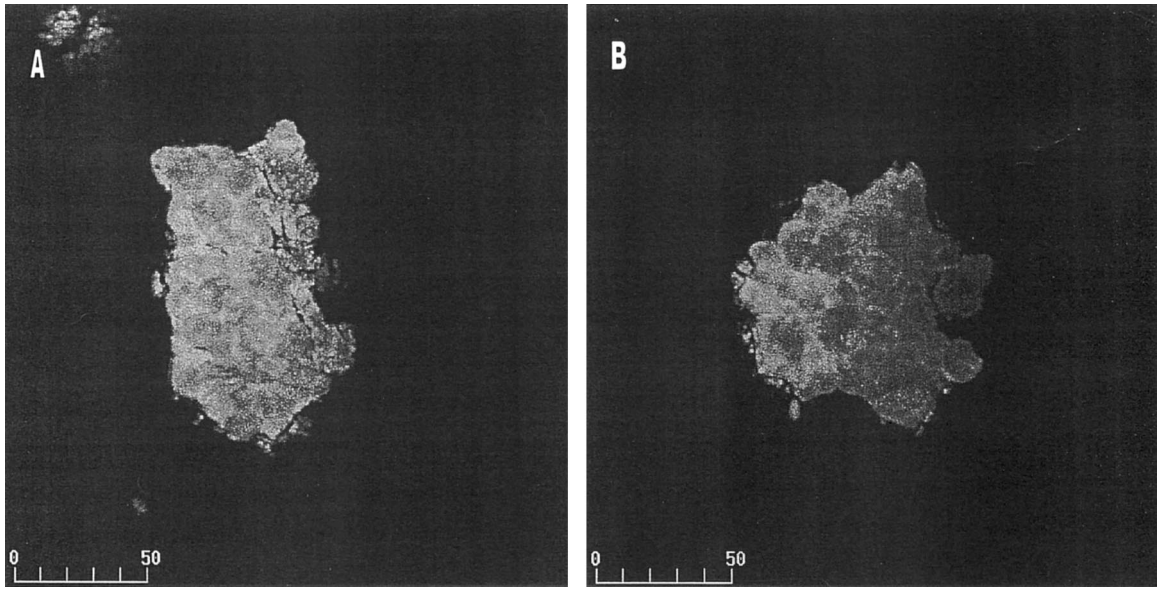

Fig. 10. Confocal microscopy images of riboflavin sodium phosphate particles on the upper surface (A) and lower surface (B) of tablet T3b at a compression force of 26.8 kN. Scale bar = 50 μm.

terparticulate contact increased. A crystalline compact of particles was formed. For the T2b tablets containing Avicel PH-102 as filler, recrystallization occurred around the crystalline compact of drug particles on the upper surface of the tablet (Fig. 9A). The higher compression force caused more recrystallization than the lower compression force. Deformation of the riboflavin sodium phosphate can also be seen on the lower surface of the same tablet (Fig. 9B). The original edges of the particles can still be identified on the lower surface. Only a small amount of recrystalline structure was formed. This is because the upper punch has a stronger force than the lower punch, and the warmest spots are located on the upper tablet surfaces (Ketolainen et al., 1993). In the case of Avicel PH-101 tablets, recrystalline structure was formed on the upper surface of the tablet to a smaller extent (Fig. 10A) compared to tablet 2b (Fig. 9A). This is apparently due to the elastic recovery of Avicel PH-101 at the higher compression force (>10 kN) (Szabo-Revesz et al., 1996) and the more porous structure of Avicel PH-101 (Landin et al., 1993), which may somewhat impede some recrystallization of riboflavin sodium phosphate.

It can be concluded from the images that the behaviour of riboflavin sodium phosphate is plastic. On the upper surface of the tablet, the riboflavin sodium phosphate particles were more tightly bound than on the lower surface. At the same time, it was noticed that the MCC influenced riboflavin sodium phosphate deformation. No drug fragmentation tendency has been observed. When comparing the shapes of riboflavin sodium phosphate particles compressed with or without MCC, it seems clear that the plastic deformation properties of the filler protect the riboflavin sodium phosphate from fragmentation. This agrees with the view that the almost isodimensional form of pyridinolcarbamate crystals reduces the deformation with MCC when both are compressed (Pintye-Hodi et al., 1989). Scanning electron microscopy (SEM) has been used for studying direct compression of tablets (Duberg and Nyström, 1982), but by this technique it is not possible to see the deformation behaviour of individual particles so clearly.

4. Conclusions

Confocal laser scanning microscopy (CLSM) is a non-invasive technique producing three-dimensional images of the internal structure of tablets. CLSM can be used in characterizing the behaviour and deformation of drug particles (i.e. riboflavin sodium phosphate) and excipients (i.e. MCC) under compression forces. The compression behaviours of both fluorescent drug and non-fluorescent filler can be visualized simultaneously by using fluorescence and reflection modes. Riboflavin sodium phosphate particles have a clear tendency to undergo recrystallization during direct compression. Both compression force and filler affect the deformation of riboflavin sodium phosphate particles. The plastic deformation properties of the filler (MCC) may protect the drug from extensive fragmentation.

Acknowledgements

The authors would like to express their sincere thanks to Helena Vihinen, M. Sc. (Technology), and Jyrki Juhanoja, Lic. Sc. (Physics), for technical assistance in CLSM and SEM, respectively.

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