Evaluation of Correlation between Dissolution Rates of Loxoprofen Tablets and Their Surface Morphology Observed by Scanning Electron Microscope and Atomic Force Microscope

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We observed the surface morphological structures of 60 mg tablets of Loxonin®, Loxot®, and Lobu® using scanning electron microscope (SEM) and atomic force microscope (AFM) to evaluate the dissolution rates. We found a significant difference among the initial dissolution rates of the three kinds of loxoprofen sodium tablets. Petal forms of different sizes were commonly observed on the surface of the Loxonin® and Loxot® tablets in which loxoprofen sodium was confirmed by measuring the energy-dispersible X-ray (EDX) spectrum of Na$K\alpha$ using SEM. However, a petal form was not observed on the surface of the Lobu® tablet, indicating differences among the drug production processes. Surface area and particle size of the principal ingredient in tablets are important factors for dissolution rate. The mean size of the smallest fine particles constituting each tablet was also determined with AFM. There was a correlation between the initial dissolution rate and the mean size of the smallest particles in each tablet. Visualizing tablet surface morphology using SEM and AFM provides information on the drug production processes and initial dissolution rate, and is associated with the time course of pharmacological activities after tablet administration.

Key words atomic force microscope; scanning electron microscope; energy-dispersible X-ray; loxoprofen sodium tablet

It was recently reported that there is a significant difference in dissolution rates between brand-name and generic drugs, although most generic versions passed the official dissolution test standards.[1)] We examined the dissolution rates of 60 mg tablets of Loxonin®, Loxot®, and Lobu® and confirmed a significant difference among them. The dissolution rate of an active pharmaceutical ingredient greatly depends on the surface structure and the constituent particle size of the tablet. These three tablets include loxoprofen sodium hydrate, $C_{15}H_{17}NaO_3 \cdot 2H_2O$, so dihydration is the same.

The atomic force microscope (AFM) can be used to obtain 3D images with nanometer resolution and quantitatively measure surface morphology. Furthermore, AFM was recently used to measure adhesion force between glidants and pharmaceutical fillers and particle friction in a pharmaceutical system.[2,3)] The mechanism of drug particle formation has also been investigated by scanning electron microscope (SEM) and AFM.[4)] AFM was also used to investigate adhesion problems during tablet manufacturing relative to run time on the tablet press and the influence of mechanical milling time on the surface stability of salbutamol sulfate.[5,6)] Therefore, we investigated the morphological surface structures of the three tablets using SEM and AFM and found that SEM and AFM image analyses can be used to correlate surface structure and dissolution rates.

Experimental

Materials The samples used in this study were Loxonin®, Loxot®, and Lobu® tablets. The dissolution rate and behavior of loxoprofen sodium were examined by following the dissolution test manual and the loxoprofen sodium tables of the Japanese Pharmaceutical Codex (JPC), part 3. The apparatus used for the dissolution test was an NTR-6200AC (Toyama Industry Co, Toyama, Japan). Briefly, the dissolution test was conducted with 900 ml of aqueous solution at 37 °C with stirring at 50 rpm by the paddle method. About 20 ml of the eluate was promptly filtered with a 0.45-μm membrane filter (Millipore, Bedford, MA, U.S.A.); the first 10 ml of eluate was removed, the remaining 10 ml was diluted to the regulation quantity, and was prepared to measure absorbance. The same process was performed at a time interval of 5 min. The absorbance of the loxoprofen sodium was measured at 5 min intervals at wavelengths of 223 nm and 340 nm with a U-1900 spectrophotometer (Hitachi, Japan).

AFM The AFM instrument was a NanoScope IIIa (Digital Instruments, Santa Barbara, CA, U.S.A.).[7,8)] A tablet sample fixed with adhesive tape on a stainless steel plate was placed on the scanner unit of the instrument. AFM images were measured using the tapping mode in air. A cantilever (SSS-NCH-50) made of n^+-silicon was used. The scan rate was 0.5—0.7 Hz, and the tapping frequency was about 330 kHz.

SEM SEM images and the elemental analysis of the tablet surface were conducted with an S-800 (Hitachi) and JSM-5200 (JEOL, Tokyo, Japan) equipped with an energy-dispersible X-ray (EDX) diffractometer JED 2001, respectively. The sample tablets were coated with Au using an ion sputter apparatus E-1030 (Hitachi).

Results and Discussion

Dissolution Test The dissolution rate of the three kinds of tablets was over 85% after 30 min, which conformed to the loxoprofen sodium standards written in the Japanese Pharmaceutical Codex (JPC), part 3. The dissolution rates for the three tablets after 10—20 min, were ranked as follows: Lobu®>Loxonin®>Loxot® (Fig. 1). This difference may be reflected in the surface morphology of the tablets.

SEM Images and X-Ray Spectra Figure 2a is an SEM image (×150 magnification) of the central surface of Loxonin® tablets and shows a bright powdery and dark region and cellulose structure. Figure 2b is an optical image from a charge coupled device (CCD) camera equipped as a monitor on the AFM instrument. The black rod and shadow on the right side of this image are a cantilever (125 μm length) and its holder, respectively. Both images were recorded from the same area on the Au coated surface. When comparing the

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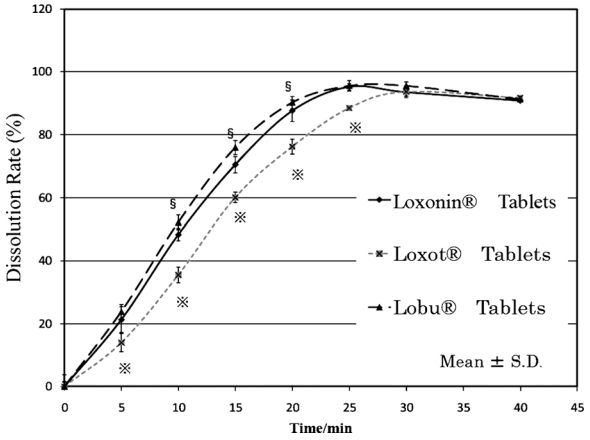

Fig. 1. Dissolution Profile of Loxoprofen Sodium Tablets

The values are presented as mean±S.D. of six replicate assays. * $p<0.05$ for *t*-test of Loxot® tablets *vs.* Loxonin® tablets; § $p<0.05$ for *t*-test of Lobu® tablets *vs.* Loxonin® tablets.

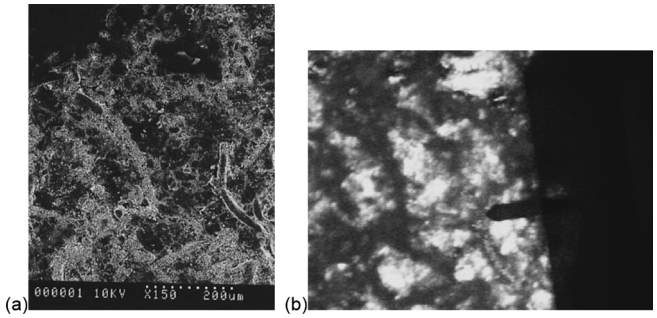

Fig. 2. (a) Scanning Electron Micrograph of the Surface of Loxonin® Tablets Coated with Au and (b) Optical Image of the Same Surface from a Charge Coupled Device (CCD) Camera

images, the bright and dark are reversed. The dark region in Fig. 2a was flat because an SEM image generally indicates an edge as bright and the flat plane as dark due to secondary electron scattering.

SEM images (×5000 magnification) and X-ray spectra of the three tablets are shown in Fig. 3. Based on Fig. 3a, the bright powdery region in Fig. 2a consisted of petal patterns. Figures 3a and c show a fine and wide petal pattern structure for Loxonin® and Loxot®, respectively. The presence of loxoprofen sodium in the petal pattern was confirmed by X-ray spectra obtained with SEM-EDX, as shown in Figs. 3b and d. X-ray lines due to Na$K\alpha$, Mg$K\alpha$, Al$K\alpha$ and the Au atom were confirmed from the inset in Fig. 3b. However, the Na$K\alpha$ X-ray line was not obtained on the flat region of the tablet surface. As listed in Table 1, the three tablets use lactose as a diluent, hydroxypropylcellulose as a binder, magnesium stearate as a lubricant, and low-substituted-hydroxypropylcellulose as a disintegrator. The additives include no sodium atoms.

In contrast, the SEM image of the Lobu® tablets surface shows no distinctive petal pattern but a relatively flat plane, and there was no sodium $K\alpha$ X-ray line on SEM-EDX. Because Lobu® tablets are more fragile than the other tablets, we examined the fragments that remained in a specimen attached to the adhesive carbon tape. Micro-size particles were observed on the SEM image (Fig. 3e), and there was an Na X-ray line detected. Therefore, the form of the loxoprofen

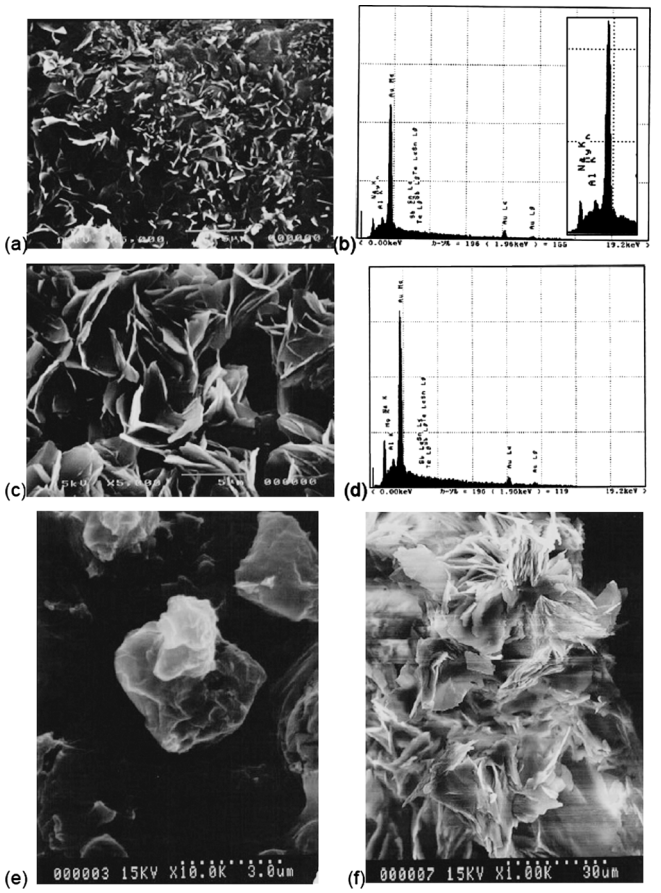

Fig. 3. (a) Scanning Electron Micrograph (SEM) and (b) X-Ray Spectrum of a Loxonin® Tablet, (c) SEM Image and (d) X-Ray Spectrum of Loxot® Tablet, (e) SEM Image of Lobu® Tablet, and (f) SEM Image of Loxoprofen Sodium after Freeze Drying

Each tablet was coated with Au.

Table 1. Loxoprofen Sodium Tablet Additives

Loxonin®	Loxot®	Lobu®
Low substituted hydroxypropylcellulose		Low substituted hydroxypropylcellulose
	Hydroxypropylcellulose	Hydroxypropylcellulose
Lactose monohydrate	Lactose	Lactose
Magnesium stearate	Magnesium stearate	Magnesium stearate
Red ferric oxide	Red ferric oxide	Red ferric oxide

sodium in the Lobu® tablets is different from that in Loxonin® and Loxot® tablets and resembles the form of raw refined loxoprofen sodium. The SEM image shown in Fig. 3f is similar to the petal pattern, and we verified that the petal patterns in Fig. 3f were created by freeze drying the aqueous solution of loxoprofen sodium. As a result, the difference among morphological surface structures of the tablets is probably dependant on different pharmaceutical processes.

AFM Images The AFM was used to observe superfine surface structures and surface roughness of the tablets. The bright region shown in Fig. 2b is relatively flat and within a height of 500 nm, whereas the roughness in the dark region is about 3—4 μm in height. AFM images shown in Figs. 4a and b are the loxoprofen sodium powder and the freeze dried loxoprofen sodium powder corresponding to Fig. 3f, respec-

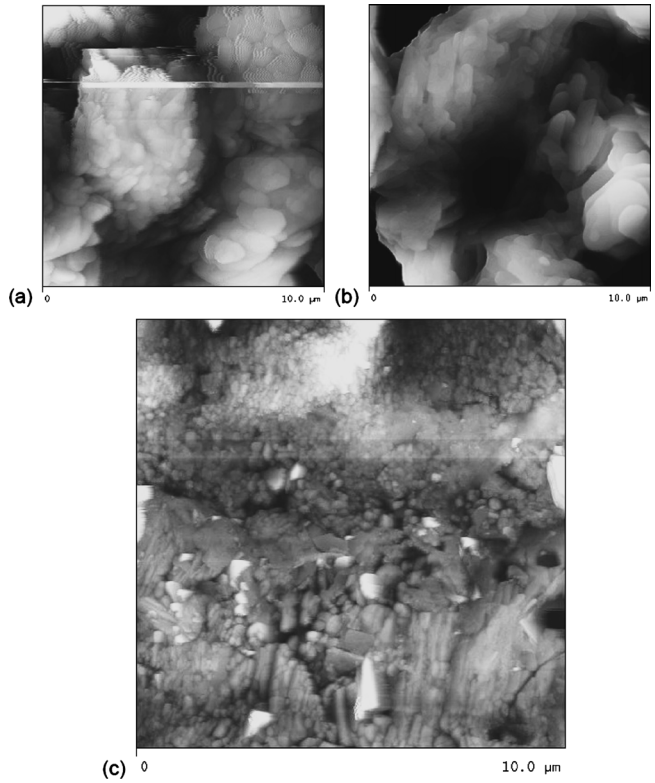

Fig. 4. AFM Images of Loxoprofen Sodium Powder (a), Loxoprofen Sodium after Freeze Drying (b), and Loxonin® Tablet Surface (c)

The size of the images is (a, b) 10.0×10.0 μm^2, and (c) 10.0×10.0 μm^2. The height range between the white and black areas is (a) 4 μm, (b) 3 μm, and (c) 0.8 μm.

tively. Figure 4a shows plane crystal (right down side) and aggregate of fine particles. It was confirmed from X-ray powder diffraction pattern that the loxoprofen powder was crystalline, though the pattern was omitted. Figure 4b shows irregular aggregate of plane nanoparticles. Figure 4c is the image of flat region of Loxonin® tablet surface. The upper part of Fig. 4c shows widely spread aggregate of nanoparticles. The nanoparticle aggregate is discussed by the following.

Most of the AFM images (5.0×5.0 μm^2) shown in Fig. 5 are the flat region on the tablet surface without an Au coat. Figure 5a is an AFM image of Loxonin® tablets, and Fig. 5b is an expanded section of the image (1.5×1.5 μm^2) marked in Fig. 5a. Because loxoprofen sodium powder AFM images exhibited a nanoparticle aggregate and the crystalline particles of Lobu® tablets shown in Fig. 3e show a plane nanocrystal, the images shown in Fig. 5b are similar to those of the loxoprofen sodium particle aggregate. Figure 6 is a histogram of particle sizes, which shows the frequency distribution of Heywood diameters for the 12 particles marked in Fig. 5b, and the mean diameter was determined to be about 294 nm.

AFM images of Loxot® and Lobu® tablets are also shown in Figs. 5c and e, respectively. Using the same analysis as for the Loxonin® AFM image, the Heywood diameter histogram of the nanoparticles for the Loxot® and Lobu® tablets is shown in Fig. 6. The mean diameters were 183 nm for Lobu® and 449 nm for Loxot®. Whether these nanoparticles are loxoprofen sodium or additives such as lactose was not clarified from the present AFM data. As a result, the nanoparticle

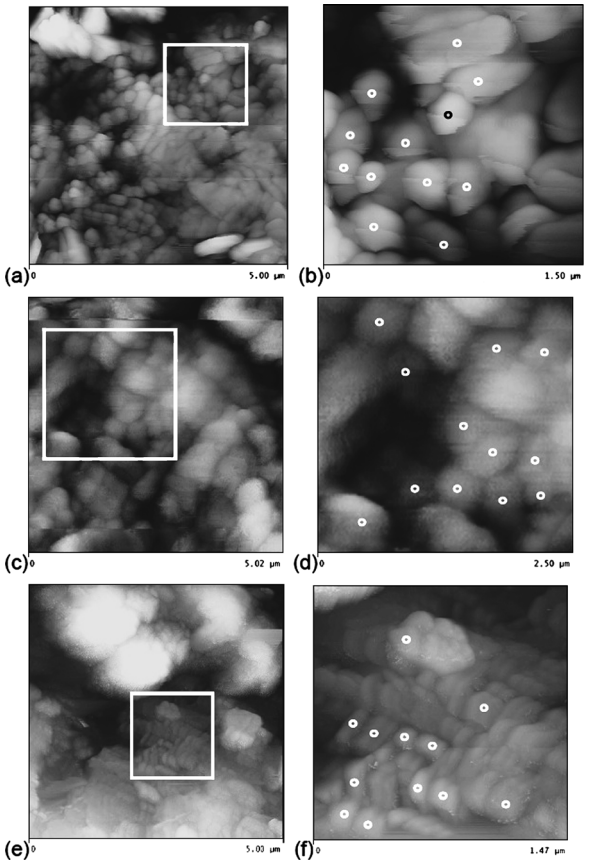

Fig. 5. AFM Images of Nanoparticles on the Surface of a Loxonin® Tablet (a, b), Loxot® Tablet (c, d), and Lobu® Tablet (e, f)

(a) 5.0×5.0 μm^2, (b) expanded section as marked in a, 1.50×1.50 μm^2, (c) 5.02×5.02 μm^2, (d) expanded section as marked in c, 2.50×2.50 μm^2, (e) 5.0×5.0 μm^2, (f) expanded section as marked in e, 1.47×1.47 μm^2.

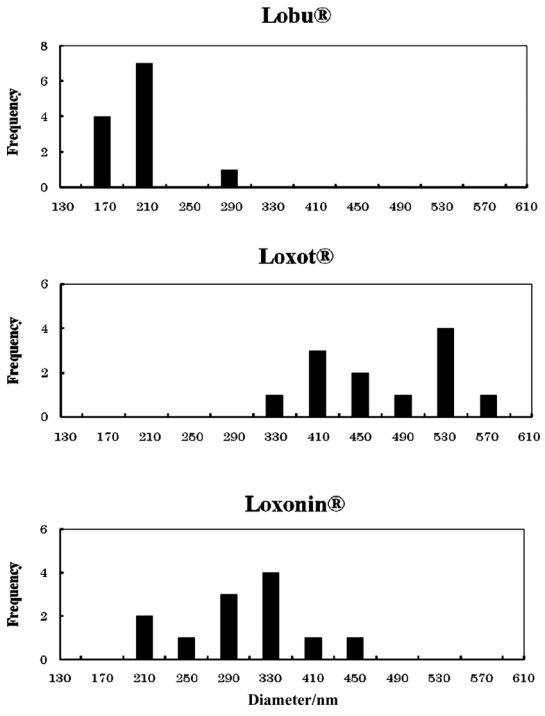

Fig. 6. Frequency Distributions of Nanoparticle Heywood Diameters for Each Tablet

diameters increase in order of Lobu®<Loxonin®<Loxot®. This order is reverse to the ranking of tablet dissolution rates. Surface area and particle size in tablets are important factors for dissolution rate. It is known that the dissolution rate changes by crystal polymorphism and crystal habit. This examination will be further subject.

Conclusion

Approximately 10—20 min after the dissolution test started, the dissolution rates of the Lobu® and Loxot® tablets were significantly different when compared to that of the Loxonin® tablets, and the rank in order of decreasing dissolution rate was: Lobu®>Loxonin®>Loxot®.

The SEM images in Figs. 3a (Loxonin®) and c (Loxot®) revealed several micrometer-sized petals, and there was a high sodium distribution. The petal size was larger in Loxot® than in Loxonin®, *i.e.*, the surface area of the fine petal pattern was larger than that of the wide petal pattern. The faster dissolution rate of Loxonin® than that of Loxot® was consistent with the tablet surface areas. No petal pattern and only a few micrometer-sized crystalline particles were observed in the Lobu® tablets, and the particles showed a Na X-ray line. The particle surface area of the Lobu® tablets was the largest of the three tablets. The nanoparticle sizes of the tablets were examined using AFM. The form and size of loxoprofen sodium varied among the tablets. From these results, there was a correlation between dissolution rate and tablet surface morphology. The results suggest that the surface morphology observed by SEM and AFM provides important information on dissolution rate and the drug production process.

References

1) Miyamoto E., Kawaguchi A., Hamaguchi N., Oshima T., Maida C., Saito K., Wakiya Y., Mutoh K., Kanamori K., *Jpn. J. Pharm. Health Care Sci.*, **33**, 942—947 (2007).
2) Ohta K. M., Toyoshima K., Fuji M., Takei T., Chikazawa M., *J. Soc. Powder Technol. Jpn.*, **41**, 169—176 (2004).
3) Bunker M. J., Roberts C. J., Davies M. C., James M. B., *Int. J. Pharm.*, **325**, 163—171 (2006).
4) Moribe K., Yamamoto K., *Pharm. Tech. Jpn.*, **24**, 2363—2369 (2008).
5) Begat P., Young P. M., Edge S., Kaerger J. S., Price R., *J. Pharm. Sci.*, **92**, 611—620 (2003).
6) Wang J. J., Li T., Bateman S. D., Erck R., Morris K. R., *J. Pharm. Sci.,* ***92***, 798—814 (2003).
7) Utsuno K., Tsuboi M., Katsumata S., Iwamoto T., *Chem. Pharm. Bull.*, **49**, 413—417 (2001).
8) Utsuno K., Tsuboi M., Katsumata S., Iwamoto T., *Chem. Pharm. Bull.*, **50**, 216—219 (2002).