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Study on Pulp Fibers and Paper Morphology by ESEM and LTSEM*1

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ABSTRACT

The ESEM could be used in investigating the fibrous networks developed during handsheet-forming processes with the exception of the stages relating to the actual dispersion of the fibers and the drying of formed sheets. Also the cross-sectional images of swollen fibers were generated with the ESEM but the information given by the images was rarely fresh compared to the CLSM images. The LTSEM was extremely useful in generating images of the microfibrillar structure of a wet fiber with great resolution. However, pretreatment required in the LTSEM chamber was somewhat tedious due to the time consumed in sublimation of ice and sputter coating. For observation of lamellar structure of a hydrated fiber, the LTSEM exhibited greatly detailed structure with high resolution. Finally ESEM and LTSEM should be used in a finite field such as observation of surface morphology in detail.

Keywords : ESEM, CLSM, LTSEM, swollen fibers, sublimation, lamellar structure

1. INTRODUCTION

It is exceedingly significant to study morphological characteristics of pulp fibers, because they lead us to predict plentiful information on physical properties of fibers and paper. As new microscopy techniques, they have been introduced to the field of pulp and paper science, a category of information obtained from them become more plentiful than ever. Thus it is time to switch our standpoint to the new microscopy techniques using specimens in a natural state.

Kim & Wadhams (1998) and Kim *et al.* (1998) have already introduced some of the microscopes such as Confocal Laser Scanning Microscope (CLSM) and Scanning Probe Microscope (SPM). They exhibited great ability in studying refining effects on pulp fibers in a hydrated state. The CLSM showed a unique feature in obtaining non-destructive cross-sections of wet fibers and the SPM displayed the very fine-surface structure of wet fibers with greatest resolution.

On this paper, new types of Scanning Electron Microscopes (SEM) including Environmental SEM and Low-Temperature SEM are introduced. The environmental SEM is similar in type to a conventional SEM that allows the examination of specimens under natural environmental conditions. It has many advantages compared with the ordinary SEM, the main one being that specimens do not need any pre-

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treatment and modification. Wet and liquid specimens can be imaged in their own natural state and new detecting mechanisms, including that of secondary electrons (SE) emitted from true specimen surfaces, are available. The ability of ESEM to image materials without coating or other preparation also allows many faster sample throughputs. The microscope's flexibility and non-destructive operation makes it ideal for a plethora of applications in observing wet pulp fibers, such as the effects of continued rewetting and drying operation.

The techniques of the LTSEM in biological science were first demonstrated by Echlin *et al.* (1970). For the conventional SEM, all samples must be absolutely dehydrated before investigation, which usually requires rather elaborate and tedious sample preparation techniques due to dependence upon the use of organic solvents, critical point drying (CPD) or freeze drying (FD). Furthermore, the techniques for preparing specimens can cause shrinkage, collapse and distortion of samples to some extent. When most of the papermaking process is considered to be carried out in the presence of water, it is natural that the cryo-preparation techniques for Scanning Electron Microscopy have become essential for observing or understanding wet or beam sensitive materials. Using the technique for a cryogenic preparation removes the need for conventional preparation methods such as critical point drying (CPD) and freeze drying (FD) and allows observation of the sample in its natural state. Specimens for LTSEM are frozen-hydrated (FH) with liquid cryogens, usually nitrogen, within a very short time period.

This work aimed to show how ESEM and LTSEM could be used to study structural features, in detail, of pulp fibers and paper in a hydrated state.

2. MATERIALS and METHODS

2.1. Pulp

The Lapponia softwood bleached kraft pulp was used in the Lampen ball mill to obtain ESEM and LTSEM images. Before disintegrating, all pulps were soaked in water overnight. For making handsheets with unbeaten pulps, pulp equivalent to 24 grams of moisture free fiber was disintegrated in the British standard disintegrator, at a consistency of 1.2% for 75,000 revolutions. For the even treatment of pulp fibers in the chamber, 24 g (O.D.) of pulp were disintegrated and then thickened to 3% consistency for beating in accordance with the Second Report of Pulp Evaluation Committee to the Technical Section of the Paper Makers Association, 1936 (hereafter described as The Second Report).

2.2. Embedding and microtoming

The resin used for embedding was JB4 hydrophilic resin (glycol methacrylate) kit produced by TAAB. For a preparation of catalysed resin, 0.5 g of dry catalyst C was added to 50 ml of JB4 solution A. The mixture was stirred by a magnetic stirrer until dissolved in the fume hood. As stable for 2 weeks at 4℃, the infiltration solution should always be kept cold in the refrigerator. This infiltration solution was added to the pulp suspension in a small jar through an solution A + C series: 75% pulp: 15% solution → 50% : 50% 15% : 75% → 100 % solution → 100% fresh solution → 100% solution → 100% solution → 100% fresh solution. The time consumed for each infiltration stage was 2 days. After all infiltration stages, the pulp fibers should be filled with a 100% fresh resin. For replacing resins in pulp fibers with a mixture of A + C + B, stable for 10 minutes,

exactly 1 ml of solution B(hardener) was added to 25 ml of cold fresh catalyzed solution A + C. Beforehand, the resin-filled pulp fibers were carefully filtered through a filter paper in order to keep fibers from collapsing. The collected fibers were transferred to an embedding mould and then solution A + C + B was added. The moulds were covered with an aluminium foil during the polymerization, which was completed at room temperature in around one day.

Optimal sectioning was performed with 5 ㎛ in thickness by the LKB ultramicrotome III.

2.3. ESEM

The ESEM used for this study was Electro-Scan 2020. The ESEM uses differential pumping techniques to link a series of chambers at increasing pressures along the beam path by small apertures (Figure 1). In the ESEM, specimen temperature (-200 to 1,000℃), moisture content and gas environment were manipulated precisely and at will, while continuously observing the sample. In addition, real-time processes such as wetting and drying were stored on the computer and then transferred onto floppy diskettes (1.44″). When inspecting large specimens such wet sheets, the ESEM's computer driven 5-axis stage (with 1 ㎛ resolution in the *xy* plane) was used to make accurate long-distance measurements and for automatically returning to exact spatial co-ordinates on the specimen after removal for experimental treatments.

2.4. LTSEM

The LTSEM used for this study was Hexland LTSEM. The Hexland cryosystem was first used by Howard (1987) and subsequently Moss *et al.* (1989) in the field of pulp and paper science.

To avoid unacceptable artefacts during specimen preparation, the cryopreparation technique was most commonly preferred. The hydrated fibers were rapidly cooled and maintained below -135℃, then examined, without water loss, for extended periods under vacuum. The internal structure of cooled specimens could be revealed by freeze- fracturing techniques using the cold knife inside LTSEM. From this, a great detail of specimen surface was exposed by carefully raising the temperature of the freeze-fractured specimen to a point at which water began to sublime at a controlled rate (-80℃). Right after sublimation of the specimen, the specimen was coated with a conducting metal at low temperature to prevent charging occurring during examination and photography.

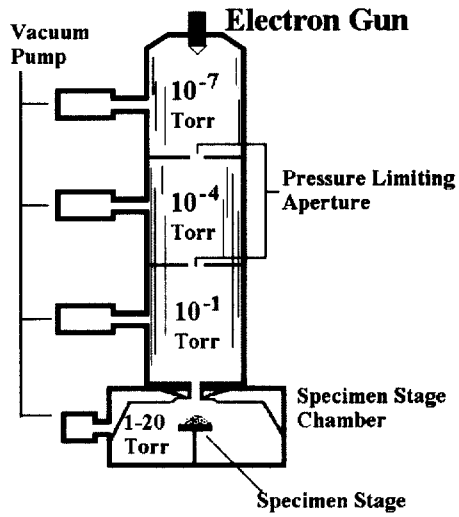


Fig. 1. A schematic system of ESEM redrawn from Danilatos' work (1988).

3. RESULTS and DISCUSSION

3.1. ESEM images

3.1.1 Fiber surface morphology

In Fig. 4, the photomicrographs show ESEM

(a) Disintegrated fibers

(b) Refined fibers

Fig. 2. ESEM images of pulp fibers in a hydrated state.

images of disintegrated and refined fibers of softwood bleached kraft pine pulps. They were generated at an accelerating voltage of 5 kV in the ESEM. The morphological features of the disintegrated fibers (Fig. 2a) showed not only that their fibrillar strands were bonded to the neighbouring fiber, but also that the fiber surface was relatively even. On the other hand, due to the mechanical treatment, the image of the refined fibers (Fig. 2b) obtained at 5 kV appeared to have a more uneven surface than the disintegrated fibers. Moreover, the image showed that fibrillar or film-like materials were bonded to the neighbouring fiber below. The beaten fibers also looked flat in appearance and they were in close contact, causing fiber-to-fiber bonding. Because water should be removed to a certain extent (4~5 torr) in order to generate images at 5 kV, this seemed to cause pulp fibers to collapse and to start interfiber bonding between fibers, drawn by the surface tension of the receding water.

Most works (Danilatos, 1988) relating to ESEM have been generally carried out at 20 kV, but the pictures taken at 5 kV in Fig. 4 could surely afford more effective advantages in obtaining detailed information on morphological change of pulp fibers which occurred before and after mechanical treatment than those obtained at 20 kV. Although most of ESEM work was carried out at 20 kV, it is generally true that many of the interesting features were not visible. The 5 kV voltage could be a far more suitable accelerating voltage for imaging structural details arising from mechanical actions that the fibers received. Since there appeared to be some evidence of moisture loss at this lower level which might have led to some measure of fiber collapse, however, it was concluded that the method was unsuitable for use in cases where fibers were swollen in water.

For the ESEM, the method of preparing specimens for examining the fiber surface structure was the simplest compared to the works of Kim & Wadhams (1998, 1999) and Kim *et al.* (1998), just inserting non-conductive or wet pulp fibers directly into the sample chamber without any complicated sample preparation procedures such as chemical fixation, dehydration, and/or sputter coating. Since all observations under the ESEM were carried out in a fully water-saturated state, the water existing around and in the very fine fibrils might be able

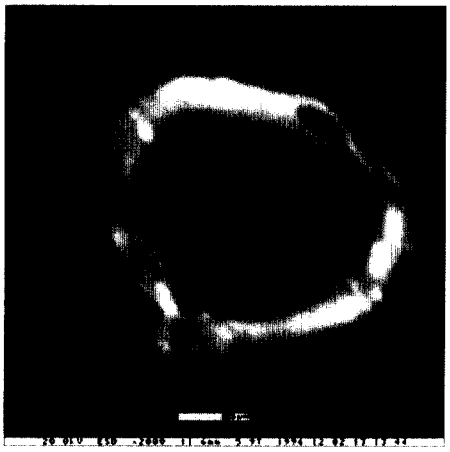
to prevent the gaseous detector of the ESEM from distinguishing the ionizing electrons emitted from the fibrils and water at 20 kV. This appeared to result in a losing of information from the specimen. On the other hand, observing at 5 kV requires us to have water between and within fibers removed to a certain extent by the low vacuum pressure (4 or 5 torr). In addition to removing water, the *z* height of the sample stage in the specimen chamber should be controlled to be closer to the detector (3 mm), too. This leads to a change of depth of an electron beams penetration from a few μm to a few *nm*. In general, finer surface images in SEM can normally be obtained with lower accelerating voltages. At higher accelerating voltages, the beam penetration and diffusion area become larger, resulting in unnecessary signals (e.g. backscattered electrons) being generated from within the specimen, as suggested by JEOL (1996). These signals reduce the image contrast and veil fine surface structures. On the other hand, at 5 kV, the microstructures of the specimen surface were clearly seen because the penetration and diffusion area of incident electrons were shallow. Therefore, from this work, it was also ascertained to be especially desirable to use a low accelerating voltage for detailed observation of the wet fiber surface.

3.1.2 Cross sectional views

In order to obtain cross sections of wet fibers in the ESEM, complex embedding work using hydrophilic polymer (methacrylate-based resin) had to be done before microtoming. Cross sectional images obtained by physical sectioning techniques and ESEM are shown in Fig. 3. In the disintegrated fibers, shown in Fig. 3a, the fiber wall thickness looked very thin due to less swelling than that of the refined fibers in Fig. 3b. This was due to the fact that, before refining, no cell wall delamination occurred in the disintegrated fibers. On the other hand, in the refined fiber, it was seen that their fiber wall thickness due to refining was almost doubled compared with that of the disintegrated one. As the primary wall and S_1 layer of the secondary wall were removed during refining, the delaminated layers of the S_2 layer in the secondary wall were more likely to allow water to be imbibed easily. Unfortunately, the cross-sectional images of the refined fibers (Fig. 3b) obtained from ESEM did not reveal the concentric lamellar structure in the delaminated cell wall proposed by Stone & Scallan (1965, 1968) and later microscopically confirmed by Page & DeGrace (1967) and Moss *et al.* (1989). Only through comparison of fiber wall thickness before and after refining, the extent of fiber wall delamination could be assumed. This might be due to the amount of water actually contained within the fiber wall making it appear as a continuum. Comparing the time required for obtaining the cross-sectional images, the information was not so significant as the CLSM images by Kim & Wadhams (1998). Furthermore, some of the images showed that distortion occurred during dehydration or microtoming, leading to inaccuracy in the measurement of cross sectional dimensions.

The appearance of cross-sections before and after refining became slightly modified. The cross section of the disintegrated fiber (see Fig. 3a) was usually irregular or angular but that of the refined fiber was circular and oval in appearance (see Fig. 3b). Internally delaminated during refining, the fiber walls swelled toward the lumen. This was because water entrained in the cell wall layers contributed to expansion of the fiber walls themselves.

ESEM provided a rapid and simple technique for studying fiber surfaces before and after beating. The low accelerating voltage used was more beneficial in studying the fibers morpho-



(a) Disintegrated fiber

(b) Refined fiber

Fig. 3. ESEM images of fibers in cross-sections.

logy, generating highly detailed microstructure information. However, since dehydration of a specimen was needed during sample preparation at the low voltage, this did not seem to be a good microscopy technique to use in observing specimens in their natural state.

3.2. LTSEM image

3.2.1. Surface morphology of pulp fibers in a hydrated state

Fig. 4 shows an image of disintegrated soft-

Fig. 4. LTSEM image of microfibrils on fiber surfaces.

wood bleached kraft fibers generated by the LTSEM. The microfibrils became evident on fiber surfaces and were observed in the form of web-like fibrillar material. The most striking feature of the LTSEM image was that the surfaces of the disintegrated fiber looked like refined fibers examined under the conventional microscopes like light microscope (LM) or Scanning Electron Microscope (SEM). This indicated that the maximum detail of the fiber surface texture could be obtained with the LTSEM. However, it should be born in mind that the microfibrils shown in the LTSEM image could be an artefact of the cryofixation process due to the presence of the dissolved carbohydrates, observed in Moss *et al.* (1989). During observation, great care had to be taken in distinguishing the artefacts and the microfibrils.

Despite such limitations, it has been well recognized that the application of the cryogenic preparation technique in the SEM, with its great depth of field for wet pulp fibers, was ideally suited for preserving and observing microfibrils or fibrillar bundles of wet fibers.

3.2.2. Cross-sectional view of refined pulp fibers

The cross-sectional images of softwood blea-

Fig. 5. LTSEM image of a fiber cross-section.

ched kraft and sulfate pulp fibers beaten in the Lampen ball mill to a wetness of 18° SR are presented in Fig. 5. The fiber cross-section was generated in the SEM chamber with the cold knife, which was proposed by Moss *et al.* (1989). The lamellar texture of fiber walls was readily observed in cross-sections. If the primary wall or thin S1 layer was supposed to be removed readily during refining, it became evident that cell wall delamination mainly took place in the S_2 layer of the secondary wall of fibers. The refined fiber appeared likely to have had the primary wall and S_1 layer successively peeled off. It could be assumed that these internally fibrillated fibers in water during refining would undergo a large increase in flexibility from the breakdown of the fiber wall structure into the thinner layers. However, the kraft pulp fiber showed a few planes of cleavage, with spacing between the layers, and the image confirmed the observation of Page & DeGrace (1967) and Moss *et al.* (1989 a, b) and the pattern of internal fibrillation proposed by Scallan (1974).

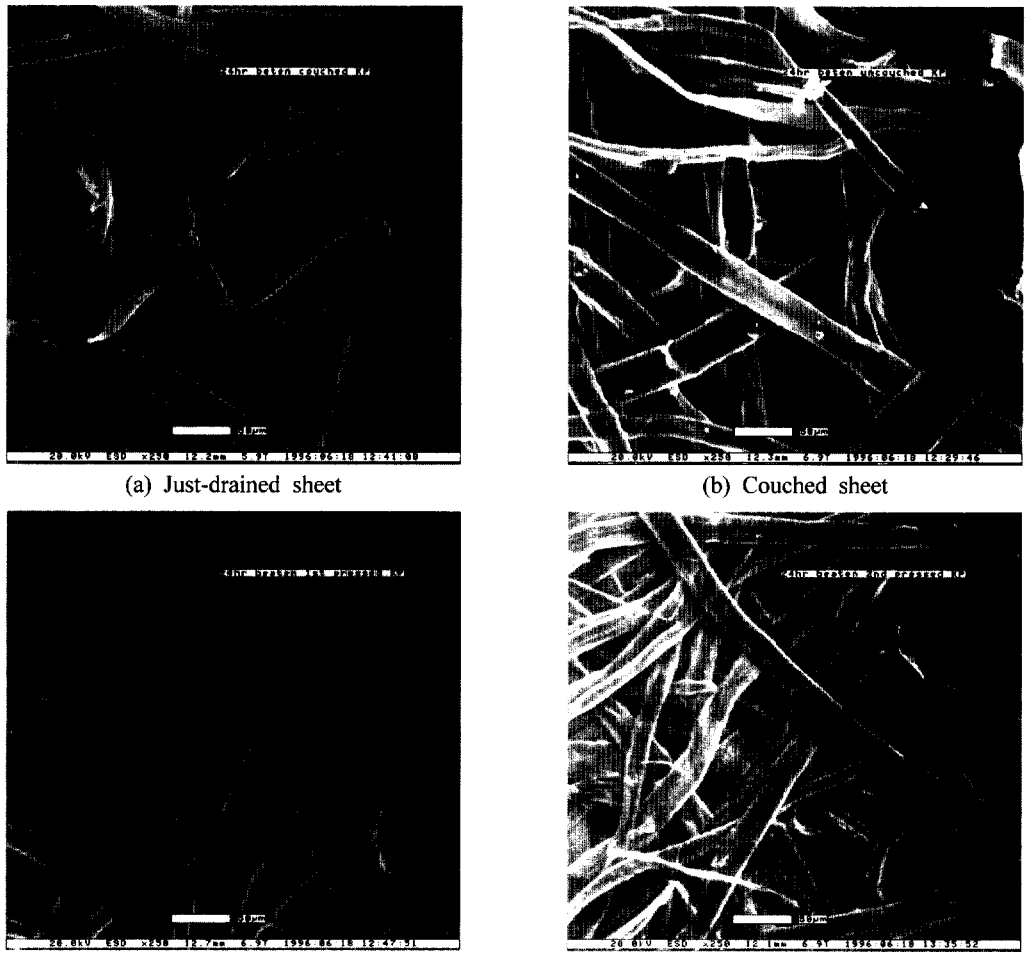
In this work, it has been demonstrated that the cryo-preparation method in preparing freeze-fracturing fibers was probably the best technique for observing the delaminated structure - honeycomb feature of the S_2 layer in the secondary wall of refined fibers.

3.3. Observation of a sheet-forming process by ESEM

An attempt was also made to observe changes of a fiber network which had occurred during handsheet-making processes using ESEM, including draining, couching, the first pressing, and the second pressing stage. No image of the dried sheet could be generated due to water absorption of fibers in the specimen chamber during observation. Furthermore, the dilute furnish could not be imaged, either. Fig. 6 shows the fiber networks that were formed after the four different stages in sheet consolidation. In a just-drained sheet (Fig. 6a), the fibers were well separated and fully saturated with water. In the couched sheet (Fig. 6b), as water was removed by blotters and a blotter roll, the voids or spaces between the fibers became smaller but still remained relatively large, and the fibers were drawn more closely together.

Just after the first press (Fig. 6c), interfiber bonding seems to have begun to take place and the fibers looked flat and dense. The web-like fibrils or microfibrillar bundles appears to bond to the adjacent fibers. The voids/spaces between fibers became much smaller than those at the previous stages, and the distinction between the fiber wall and the lumen was clearly made. After the second press (Fig. 6d), the distinguishable differences from the first pressed sheet were no longer found but the fibers seem to have become denser and flatter.

As mentioned above, the ESEM could not generate images of the diluted furnish and of the dried sheet. This is due to the well-known fact that water vapor should be used to emit electrons from the specimens during observation. Except for diluted stock and dried sheet, however, the ESEM images did not indicate any big difference from the CLSM ones presented in the previous paper by Kim & Wadhams (1998).



(a) Just-drained sheet (b) Couched sheet

(c) 1st pressed sheet (d) 2nd pressed sheet

Fig. 6. Changes of the fiber network in the handsheet making process examined by the ESEM.

Finally even though the ESEM was effectively applied to the limited fields of papermaking processes, it is not viable for observing all papermaking processes. Thus this work confirmed that the CLSM as an alternative to the ESEM was probably the most effective means of observing the sheet formation processes.

4. CONCLUSION

ESEM and LTSEM had their own unique abilities in studying morphological characteristics of pulp fibers in both wet and dry state. In particular, ESEM exhibited great features to investigate the handsheet forming process and cross sections of hydrated pulp fibers, but the resultant images were not so distinct as those from the CLSM. LTSEM was excellent to display microfibrils and lamellar structure (or honeycomb model) of the hydrated fibers. Finally, it was concluded that ESEM and LTSEM could be used only in a finite field such as observation of surface morphology with great resolution.

Table 1. Functional comparison of the modern microscopes used in studying morphological features of pulp fibers

Observing field	CLSM	SPM	ESEM	LTSEM
External fibrillation in inner fiber walls (lumen wall)	Excellent	Not available	Not available	Not available
Microstructure of fiber surface	Good	Excellent	Good at 5 kV	Good (artefacts considered)
Fibrillar texture on fiber surface	Poor	Excellent	Good at 5 kV	Excellent (artefacts considered)
Internal structure of a fiber by optical sections	Good	Not available	Not available	Not available
Drying or wetting sequence of fibers	Good	Not available	Good	Not available
Layered cross-sectional view of fiber wall	Possible	Possible	Possible	Excellent
Quantification of cell wall delamination	Excellent	Not available	Possible (by physical sections)	Possible (by classifying and counting)
Surface roughness of a single fiber	Not available	Excellent	Not available	Not available

The Table 1 summarized the characteristics of the modern microscopes capable of being used to study morphology of pulp fibers, based on the previous works including Kim & Wadhams (1998, 1999) and Kim *et al.* (1998).

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