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The diffusion, size and location of added silver grains in the cell walls of Swedish pine, *Pinus sylvestris*

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Abstract The size and location of silver particles in K-glycerate/$AgNO_3$ impregnated Swedish pine, green wood as well as high temperature dried, have been studied using TEM micrographs.

The diameter of the silver particles was found to be 2–20 nm in the impregnated green wood and as large as 1000 nm (major axis) for the ellipsoid-shaped silver clusters in the impregnated dried wood. Studying the projected area of the silver particles in impregnated green wood indicated that there are a lot of particles (40%) in the compound middle lamella with fewer particles in the S_2 (6–8%), S_1 (4%) and S_3 (2%) layers. The average distance between the silver particles, 50 nm (S_2-layer), in impregnated green wood shows that the impregnant is distributed in the cell wall at the microfibrilar level. Experimental results show that the fastest diffusion path into the cell wall is from the lumen over the pit membrane through the compound middle lamella and not from the lumen through the secondary wall layer S_3.

Introduction

In wood, which is a porous material, there are two categories of internal voids; relatively large voids, such as cell lumina and pit openings and the cell wall microvoids or microcapillaries (Panshin et al. 1980). The size of the cell wall capillaries limit the maximum size of impregnant molecules that can penetrate the cell wall.

There are no permanent capillaries in the cell walls (Davies 1968). When water is desorbed during drying, the microfibrils in the cell walls move closer together (shrinking). After re-wetting the microfibrils move apart again (swelling), but not nescessarily to the same positions as before, with the water occupying all the spaces between microfibrils.

Kellogg et al. (1969) found a porosity of around 2% for three different species of pine in the oven dried condition.

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The free volume in an amorphous polymer below its glass transition temperature (Tg) is around 2% (Sperling 1992). This could also be the case in wood. In other words, what Kellogg et al. described as porosity in oven-dried wood might also be called the free volume of the amorphous wood polymers. The free volume increases above Tg for polymers.

Berlyn (1969) reported that cell wall porosity in *Pinus resinosa* is about 25% in the swollen, water saturated, state and Boutelje (1973) found that the calculated maximum volume shrinkage of the cell wall in *Pinus sylvestris* is 26–27% for early wood and 29–30% for late wood.

Much work has been carried out to measure capillary sizes, but unfortunately, direct visual measurement of their size has not been possible. Table 1 shows results obtained, in the swollen state, by using indirect measurement techniques such as X-rays, diffusion, nitrogen adsorption, inverse size exclusion and metal precipitation.

The aim with this work was to determine the sizes and the distribution of silver grains, believed to be attached to previously impregnated glycerate molecules, in the different cell wall layers of Swedish pine. The distribution was expected to give information about the penetration of the impregnant into the cell wall.

Experimental

Materials

Specimens of Pine sapwood (*Pinus sylvestris*) with dimensions 40 × 2 × 2 mm (longitudinal × tangential × radial), of green wood and high temperature dried quality, oven-dried at 103 °C for 24 h, were impregnated.

The specimens were first impregnated with potassium stained glycerol, K-glycerate. The impregnation scheme was 15 minutes in vacuum followed by 4 h at 0.5 Mpa. The specimens were then kept in the impregnation bath at atmospheric pressure for a period of 8 weeks followed by air drying.

Table 1. A summary of some work done to measure capillary pore size in wood

Name, year	Method	Microcapillary diameter (or particle diameter) Meanvalue, (nm)
Frey-Wyssling (1937)	Silver precipitation	10
Stamm (1946)	Flow of liquids	8, (max. 100)
Thode et al. (1958)	Nitrogen adsorption	3.2–4.4 (total range) 3.8 (most common)
Stone et al. (1965)	Nitrogen adsorption	3.2–3.8 (median value) 1.6–2.0 (most common)
Rudman (1966)	Silver precipitation	6–100 (total range) 70 (in pit membranes)
Davies (1968)	Silver precipitation	5
Saiki (1973)	Silver precipitation	5–10 (in all cell wall layers) 9–15 (S_2-layer) 8–9 (intercellular layer)
Yata et al. (1972)	Silver precipitation	1.5–20
Berthold (1996)	Inverse size exclusion	<2.2 (71%) 2.2–7.0 (15%) >7.0 (14%)

These air dried specimens were then impregnated with a water solution consisting of 20 w/o glycerol and 5 w/o silver nitrate, $AgNO_3$.

The impregnation scheme was 15 minutes in vacuum followed by 4 h at 0.5 Mpa (for the 144 hour-specimens only). The specimens were then kept in the impregnation bath at atmospheric pressure for 144 h, 2 h or 10 minutes followed by air drying.

Before TEM-specimen preparation, 10 mm of the specimen length was cut away.

TEM-specimen preparation

An acrylic polymer (Unicryl) polymerized at 55 °C, was used as a lumen filling material. The polymer was used as a cell wall supporting material when the final wood specimens were cut from blocks, 2 × 2 mm in cross section, using an LKB 2088 Ultratome V equipped with a diamond knife. Thin specimens, 100–200 nm, were cut from a mesa of about 1 × 1 mm and placed on 200 mesh copper grids.

TEM-observation and analysis

The distribution and sizes of the silver particles was examined with a Transmission Electron Microscope (TEM), JEM-2000 EX. The magnification was corrected by using a calibration grid (2120 lines/mm) resulting in a magnification error of less than 3%.

The size and projected area of the particles were measured on photographic paper by using a magnifier at 0.1 mm intervals and point counting.

The silver concentration (intensity, measured as inverted intensity) measurements were aided by *Image* 1.61, a public domain program for the Macintosh PC for digital image processing and image analysis.

Results and discussion

Re-impregnation with an aqueous solution of glycerol containing 5 w/o silver nitrate into previously glycerate impregnated Swedish pine (green wood) results in very few silver particles in the ML and the secondary walls after 10 minutes, Fig. 1.

Figure 2 shows half a bordered pit and the torus. The concentration of silver in the torus is high even for the short impregnation time (10 min).

The diffusion lengths for the torus and S_3 would be considered similar and, given the same diffusion speed, the torus and S_3 layer should have the same amount of silver. This is not the case, the torus having much more silver.

Silver salt distribution in wood cell wall has been reported earlier (Davies 1968, Bailey et al. 1969, Yata et al. 1972 and Saiki 1973).

For the present work it must be remembered that there is an impregnant in the cell wall, K-glycerate, when the silver nitrate is impregnated. The location of the K-glycerate in the cell wall has been reported earlier (Wallström and Lindberg 1999).

There is no significant difference in the stabilizing behaviour between glycerol and its K-carboxylate (K-glycerate), unpublished work done by the Swedish Institute for Wood Technology Research (Trätek). According to these results the solubility of the K-glycerate molecule, in between the cell wall polymers, should not differ much from the solubility of glycerol.

The glycerol molecule has high solubility in hemicellulose and pectin; in fact glycerol is used as a promotor for water uptake in pectin in the preparation of jellies, Merck Index (1989).

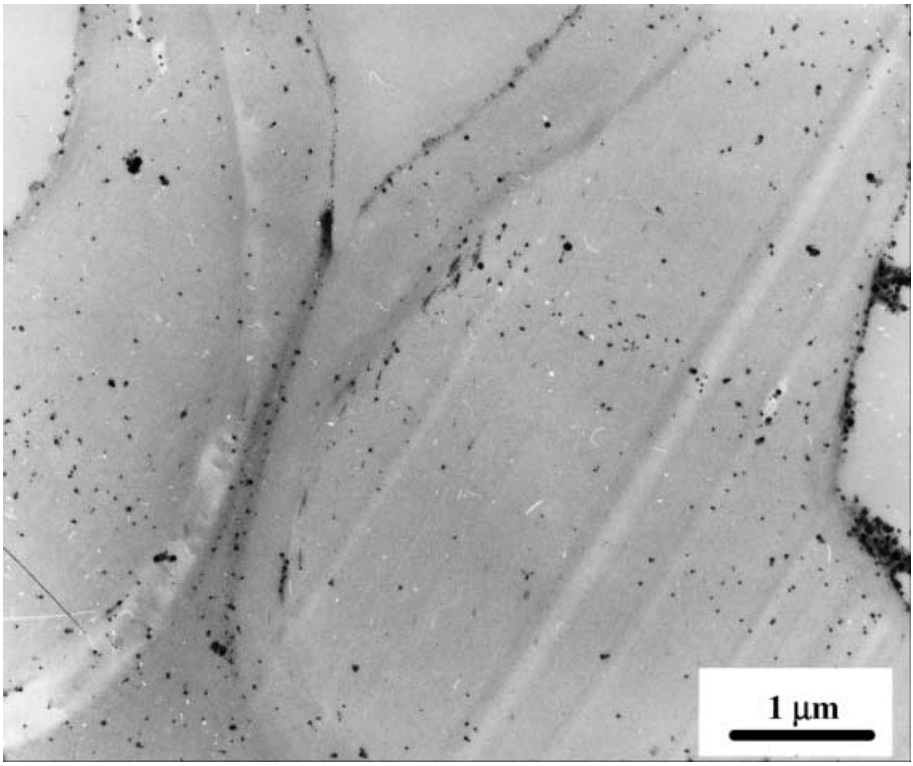

Fig. 1. Impregnation for 10 minutes in an aqueous solution containing glycerol and 5 w/o $AgNO_3$

Impregnation with only an aqueous solution of $AgNO_3$ or $AgNO_3$ impregnated into earlier glycerol impregnated wood did not result in silver precipitation. The silver grains are thus expected to be located with the glycerate molecules in the cell wall, Wallström et al. (1998).

One explanation for the high silver concentration in the torus is that there is a high concentration of glycerate because of the high amount of pectin present (Saka et al. 1983 and Fengel 1984). It is believed that the torus has a large available space due to its pectin content.

However, by increasing the re-impregnation time to two hours, a lot of silver particles were found in the true ML (intercellullar layer), the primary walls and the secondary wall close to the pit chamber, see Fig. 3. In this Figure, the pit chamber, with part of the torus, is shown in the middle to the right. The darker ML and primary walls close to the pit show that the diffusion of $AgNO_3$ is time dependant.

A measurement of the silver concentration close to the torus (in the radial part of the ML) and in the tangential part of the ML show that the concentration decreases relatively fast with the distance from the torus, see Fig. 4.

More silver particles were found in the ML close to the pit compared to the ML, a short distance away from the pit. This result shows that the impregnation chemical diffuses faster from the pit chamber via the ML compared to the route through the secondary wall from the lumen. Lange (1947) pointed out that pulping medium (studying the lignin concentration) reached the layer close to the

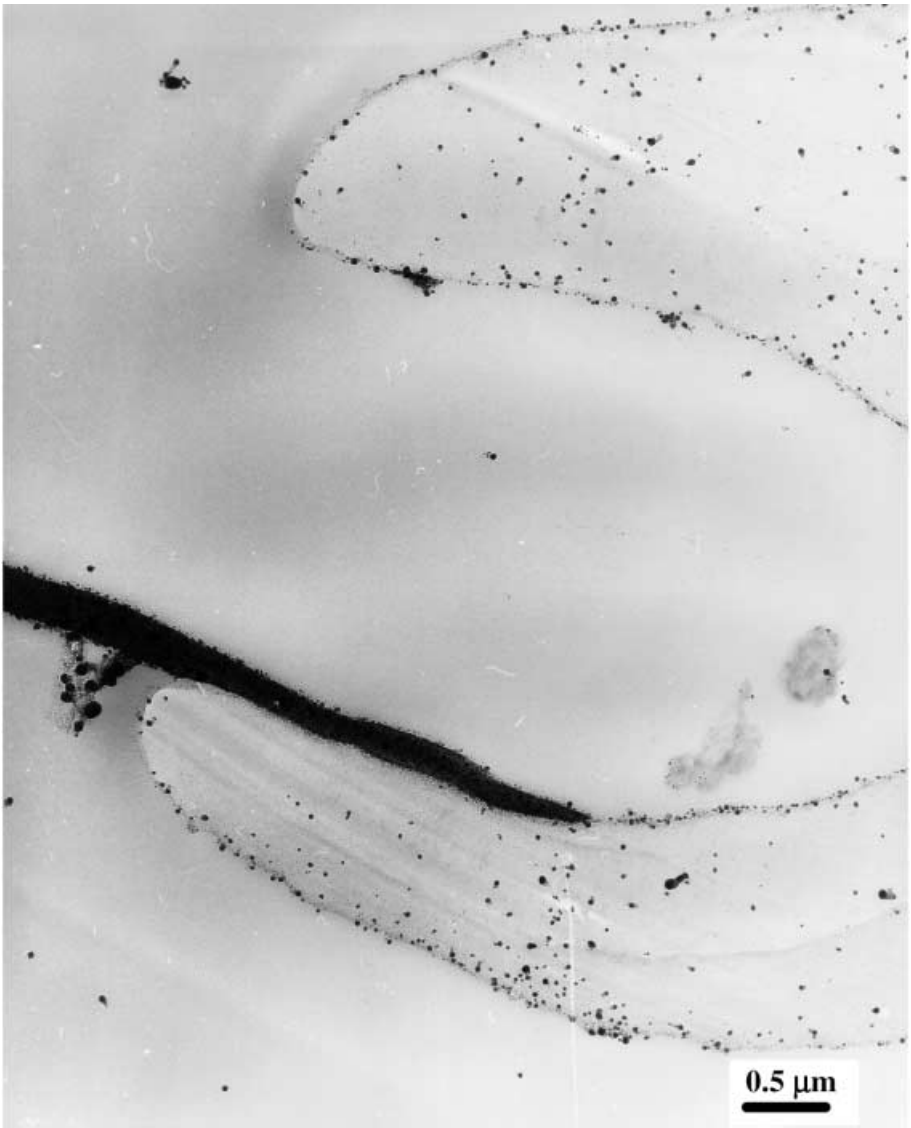

Fig. 2. TEM micrograph showing the torus with a high Ag concentration after ten minutes impregnation in an aqueous solution consisting of 5 w/o $AgNO_3$

middle lamella earlier than the layer close to the lumen, and therefore suggested that diffusion took place into the middle lamella zone from the pit chamber. On the other hand, he did not dismiss the fact that pulping medium could reach the middle lamella from the lumen via the secondary wall. Fengel et al. (1971) also suggested that one way for the impregnation solution to reach the secondary walls is through the middle lamella via the pit chambers.

Increasing the time that the specimens are immersed in the impregnant solution results in a more even distribution of silver with relatively high concentrations in the thickest secondary wall layer, S_2 and less in the S_1 and S_3 layer. This distribution has been shown earlier in Wallström and Lindberg (2000).

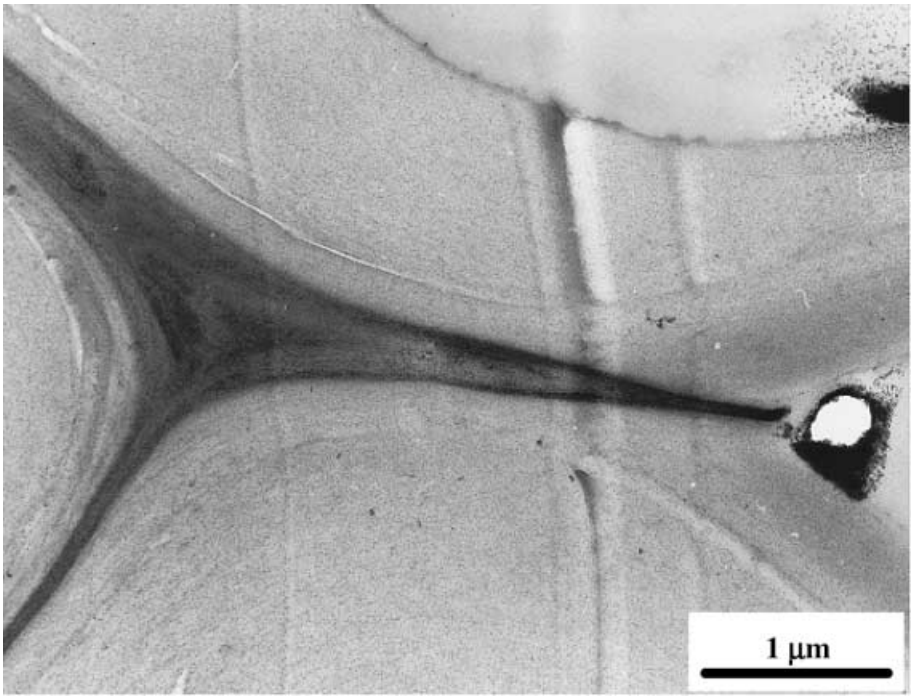

Fig. 3. Impregnation for 2 h in an aqueous solution consisting of 5 w/o $AgNO_3$

Figure 5 shows the silver distribution after 144 h in the impregnation bath.

The Figure indicates that the concentration of silver grains in the ML close to the pit chamber is about the same as in the ML in the tangential cell wall.

Figure 6 shows that the silver concentration in the cell wall ML is about the same, independant of location, relative to the pit chamber after $AgNO_3$ impregnation for 144 h.

It is well known that the impregnant reaches the large pore system (ray cells, lumen, etc) early in the impregnation process. From the rays and lumen the impregnant has to diffuse into the cell walls. This diffusion process is slower than just filling the rays and lumen. The diffusion spread is favoured by good solubility, availability of space for the diffusing molecule, temperature, concentration of the impregnant etc.

However in the present case, it is clearly shown experimentally that there is a fast diffusion path from the pit through the ML.

Considering the distribution in the S_2 layers, it would be natural for the silver precipitation to be continous along the elementary fibrils as a result of hemicellulose morphology. The silver in the S_1 and S_3 layers should then be seen as bars or rows of dots in the specimen cross section due to the fact that the microfibrillar angle relative to the fiber axis (tracheid direction) is 50° to 90°. In the S_2 the angle is 5° to 30° and the precipitated silver should be expected to be seen more as dots.

In the earlier investigation, (Wallström and Lindberg 2000), as well as in the present study mainly silver dots were found, with some areas with rows of silver particles, indicating no continous silver precipitation.

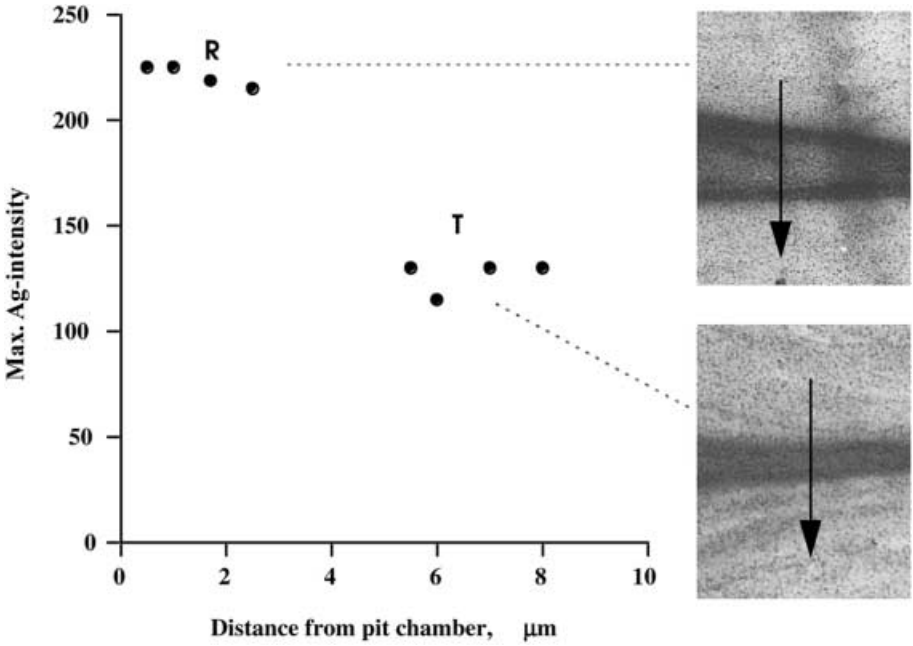

Fig. 4. To the right in the Figure are pictures of where the maximal silver intensity has been measured. To the left are the measuring points that show the intensity, in the radial (R) and the tangential (T) cell wall in Fig. 3, at different distances from the pit chamber

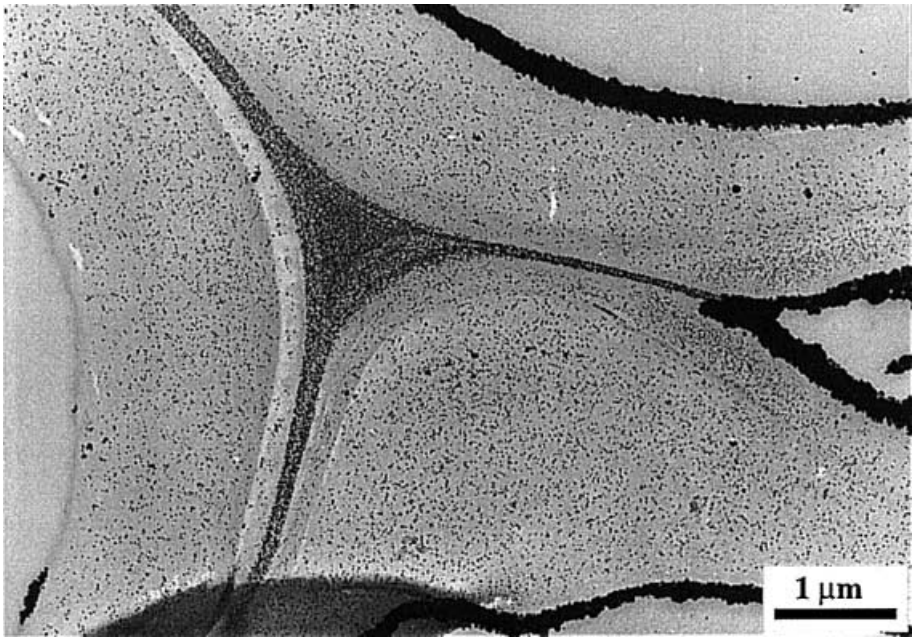

Fig. 5. A second impregnation for 144 h in an aqueous solution consisting of 5 w/o $AgNO_3$

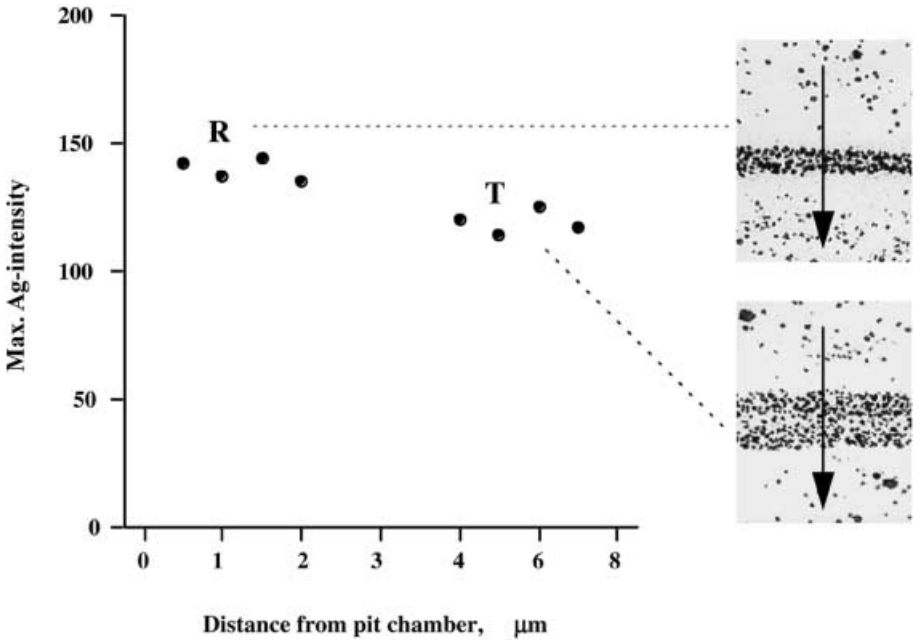

Fig. 6. To the right in the Figure are pictures of two areas investigated in the radial (R) and the tangential (T) cell wall, see Fig. 5. To the left are the measuring points that show the maximum silver intensity in the respective cell wall

Davies (1968), who investigated silver deposited in wood, *Eucalyptus regnans* (impregnated with 5 w/o silver nitrate), studied longitudinal sections of the cell walls and found that, like the cross sections studied in the present work, there was a dot pattern of silver particles.

It is believed that the hemicellulose is distributed continuosly along the elementary fibrils and microfibrils, so the impregnants will stay in the structure with larger free volume which have a morphology indicated by the silver precipitation in the Figures.

Another explanation for the discontinous silver precipitation could be that the driving force for creating silver particles is large, leading to diffusion of Ag and particle growth.

This is supported by the bimodal size distribution of silver particles shown in Fig. 7. The Figure shows the grain sizes in the middle of S_2. The most common and also the smallest particles are around 2–4 nm in diameter with a secondary peak at a maximum around 13 nm. A bimodal size distribution is uncommon in phase separating materials.

The diameters of the particles in S_1 and S_3 are about 2–5 nm. Figure 8 shows examples of representative areas in these cell wall layers. Distribution curves of the particle size are not presented because there are few larger particles present in S_1 and S_3. However, the distribution is probably bimodal.

Figure 9 shows the ML and the corner thickening. The particle sizes (diameters) range from 6 to 20 nm, most of the particles are around 15 nm.

The sizes of the silver particles found are close to the sizes of pores presented in earlier work, see Table 1.

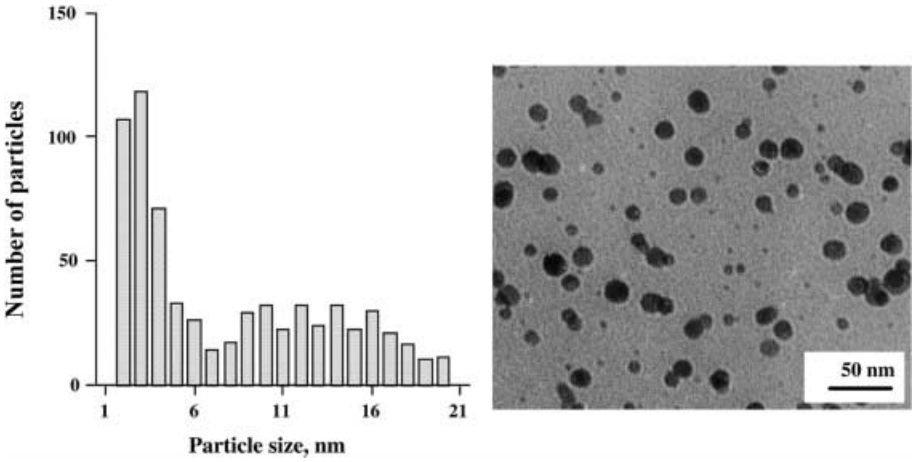

Fig. 7. Particle size (diameter) distribution in the S_2 middle layer and to the right a TEM micrograph showing silver particles in the middle of S_2

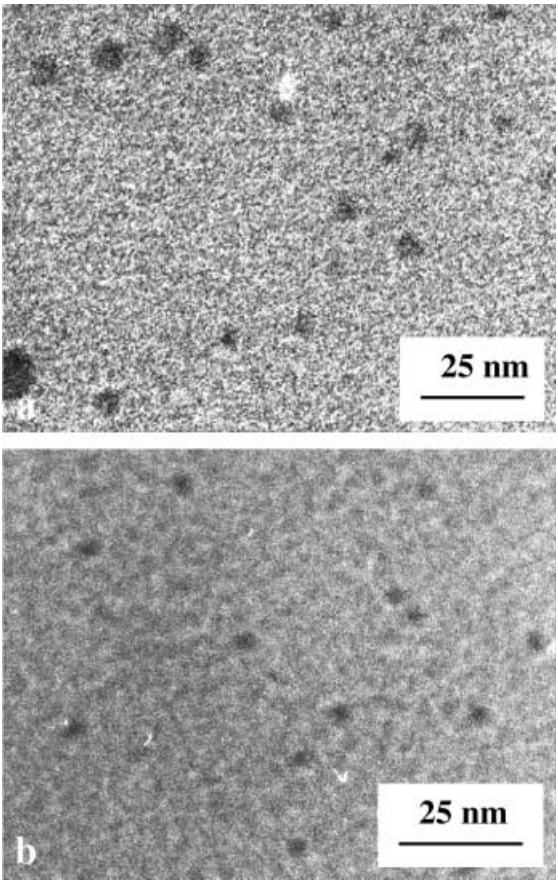

Fig. 8a, b. Particles in S_1 (a) and S_3 (b)

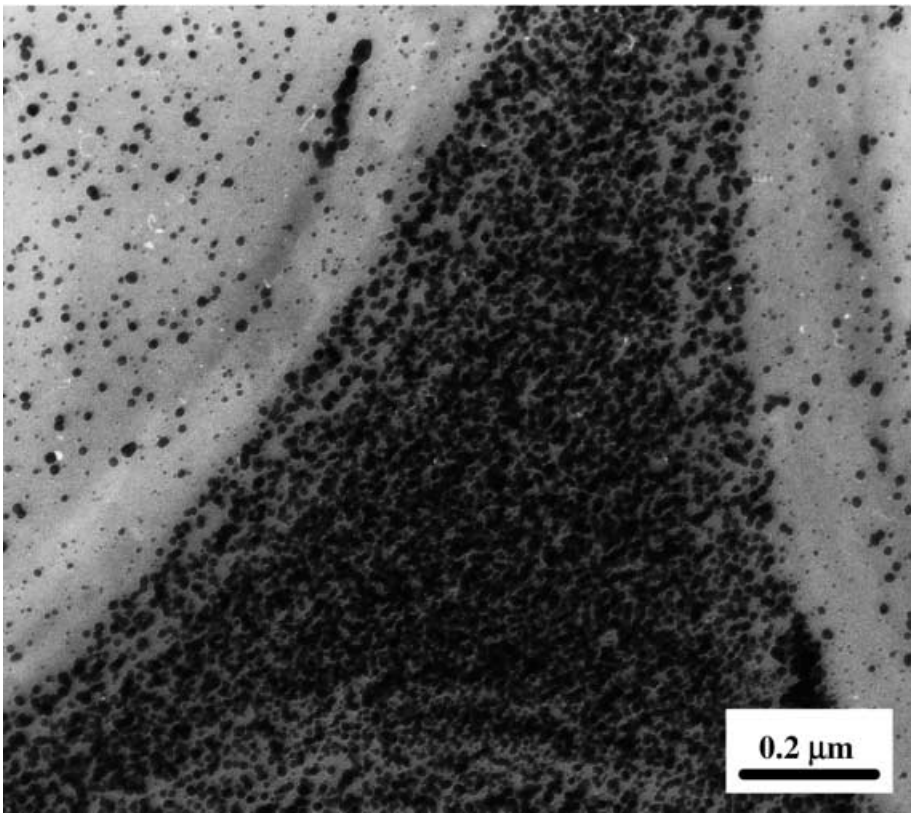

Fig. 9. TEM micrograph showing the ML, the corner thickening and S_1

The projected area distribution of silver particles in the different cell wall layers are presented in Fig. 10.

Looking at the inset picture it can be seen that at the left hand side of the picture, there are many silver particles (black areas), which have been adsorbed at the lumen surface.

There are a lot of particles, about 40% projected particle area in the ML, (seen to the right of the picture) and less particles in S_1 and S_3 compared to S_2, about half and quarter of the particles projected area in S_2 respectively. Davies (1968), also observed that the S_3 layer appeared to be fairly free from silver particles.

The S_2 layer is divided into an outer, middle and inner part and shows a decrease in particle projected area in S_2 closer to S_1 and S_3.

Line scans across double cell walls using STEM/EDX have been presented earlier, (Wallström and Lindberg 2000). The large quantity of silver particles in the ML might be the result of superpositioning of particles because of the larger thickness of the ML resulting from surface tension effects in thin sections.

There might also be superpositioning of silver particles in the S_2 layer, but in the S_1 and S_3, the possible error caused by this phenomenon might be considered as small because fewer particles are present.

The high pectin content in the true ML (Dinwoodie 1989), and especially in the primary wall (Siau 1984), might also contribute to the relatively high silver content in the ML.

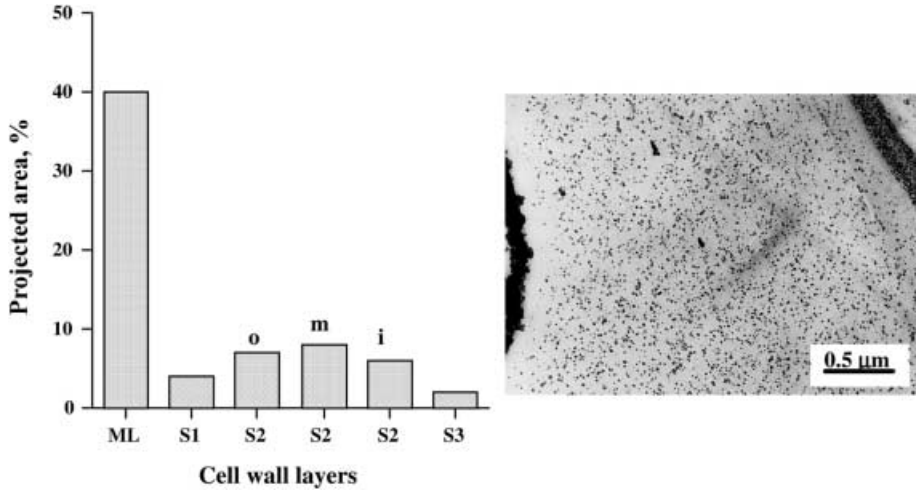

Fig. 10. Projected particle area in the different cell wall layers in Swedish pine, impregnated in the green state. To the right, one of the investigated areas

The grain sizes in wood that has been high temperature dried prior to the same impregnation procedure as above are shown in Fig. 11. There is a significant difference compared to the grain sizes in the impregnated green wood. The smaller particles, as seen in the green wood, are more or less spherical in shape and the larger particles, clusters of spherical particles, are more or less elliptic in

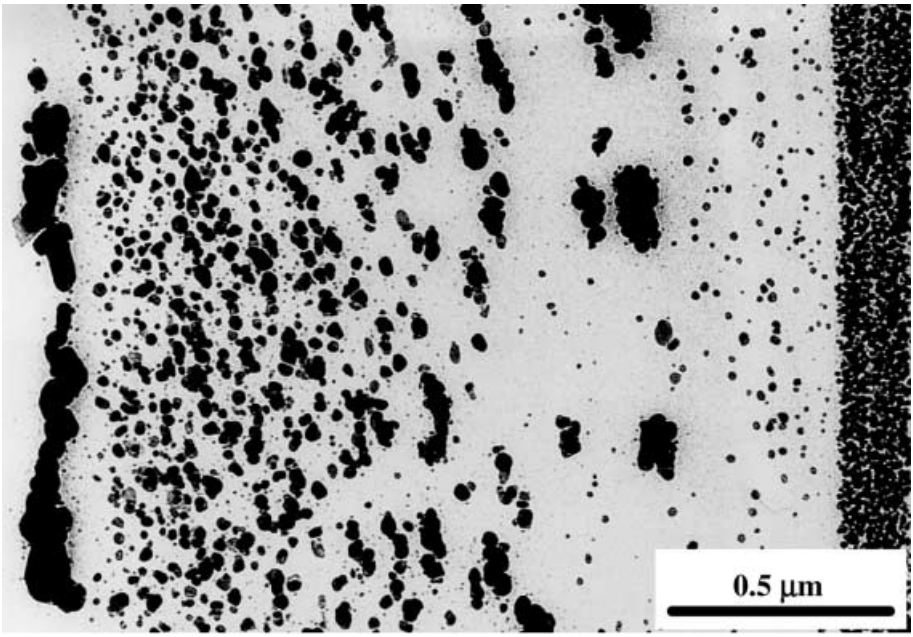

Fig. 11. TEM micrograph showing the silver particle sizes in $AgNO_3$ impregnated high temperature dried Swedish pine

shape. The large particles and the irregular spread is believed to be the result of the presence of damage (microcracks) in the cell wall (Kifetew et al. 1998, Wallström and Lindberg 2000).

The particle sizes (diameters) are around 7–1000 nm (major axis) compared to 2–20 nm for green wood. The larger particles (elliptical shaped) are believed to originate from microcracks where the silver is enriched (Wallström and Lindberg 2000) with fewer particles close to the large ones.

The small amount of silver particles in S_1 and S_3 compared to S_2 is probably due to thickness differences.

Saiki (1973), who investigated silver deposits in the cell walls of *Pinus densiflora*, found that silver deposited from the aqueous solution tends to grow into large grains and therefore does not represent a true picture of the pore systems in the cell wall. The distribution, however, was considered to be closely related to the penetration of the solution into the cell wall.

The authors of the present work believe that the silver particles attached to the glycerate molecules do not give a true picture of capillary pore size. However, the penetration and distribution together with an average value of the distance between the silver particles of 50 nm (measured in the S_2 layer of the impregnated green wood) indicate that the impregnant is distributed at the microfibrilar level and not between the elementary fibrils.

Conclusions

The sizes of the silver particles observed are close to earlier reported wood cell pore sizes. The particles are small and dispersed when wood is impregnated in the green condition. Wood impregnated in the dried condition results in large clusters of particles and an irregular spread. The impregnant is believed to be distributed in the cell wall at the microfibrillar level. The fastest diffusion path into the cell wall is from the lumen over the pit membrane through the compound middle lamella and not from the lumen through the secondary wall layer S_3.

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