J Wood Sci (1998) 44:106–110

ORIGINAL ARTICLE

Takanori Imai · Hideyuki Goto · Hiroyuki Matsumura
Seiichi Yasuda

Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique VII: Double radiolabeling of xylan and pectin in magnolia (*Magnolia kobus* DC) and comparison of their behaviors during kraft pulping by radiotracer technique*

Received: June 24, 1997 / Accepted: September 9, 1997

Abstract *myo*-Inositol-[2-^{3}H] and D-glucuronic acid-[6-^{14}C] were administered simultaneously to a growing stem of magnolia (*Magnolia kobus* DC) to label xylan and pectin, respectively, in the cell wall. Determination of the radioactivity of nitrobenzene oxidation products and sulfuric acid hydrolysates of the newly formed xylem indicated that xylan and pectin were labeled with ^{3}H and ^{14}C, respectively. The doubly labeled wood tissue was treated to kraft pulping, and the radioactivity of the pulping black liquor and treated wood tissue were determined at various stages of the pulping to compare the dissolving behavior between pectin and xylan during the pulping. The results showed that pectin was not dissolved as easily as xylan and was not redeposited on pulp fiber at the late stage of the pulping.

Key words Xylan · Pectin · Double radiolabeling · Radiotracer technique · Kraft pulping

Introduction

Noncellulose polysaccharides remaining in pulps influence not only pulp yields but also their physical and chemical properties.[1] Kraft pulping is the most widely used pulping method, and many papers have reported the behaviors of cell wall polysaccharides during the pulping.[2–7] In those papers sulfuric acid hydrolysates of pulp and pulping black liquor were determined quantitatively, or precipitates obtained by adding alcohol to the acidified black liquors were examined quantitatively and qualitatively. A part of the carbohydrate is supposed to be examined using hydrolysis and precipitation, but it is doubtful whether the quantitative and qualitative changes of the hydrolysates and precipitates represent the behaviors of cell wall polysaccharides.

The radiotracer method is effective not only for detecting components in cell walls but also for tracing their topochemical reactions.[8–10] We have been developing methods for the selective radiolabeling of polysaccharides in tree cell walls by employing suitable precursors radiolabeled at specific positions and by regulating the metabolism of the precursors with enzyme inhibitors.[11–15] The labeling methods were applied for visualizing the depositions and distributions of polysaccharides in cell walls by microautoradiography.[13,14]

Recently, the wood meal in which xylan was radiolabeled selectively was treated to kraft pulping, and dissolution and redeposition of xylan during the pulping were demonstrated clearly by a radiotracer method.[16] It was shown that the selective labeling method is useful for studies on the behavior of polysaccharides during kraft pulping.

Pectin is one of the important noncellulose polysaccharides constituting plant cell walls. The distribution, role, and reaction of pectin have been studied using young tissues, such as hypocotyl or root tip. In tree cell wall, however, pectin has not been well investigated because it is difficult to isolate and analyze it.

A pectin radiolabeling method has been developed to study pectin in tree cell wall.[12] The deposition and distribution of pectin during tree cell wall formation were visualized by microautoradiography.[13] The radiolabeling method will be available for studies on behavior of pectin in various reactions, such as chemical analyses or pulping.

Yields and physical properties of mitsumata pulps prepared by sodium hydroxide or ammonium oxalate treatment of bast fiber are affected by the remaining pectin in the pulp.[17] Pectin dissolved during alkaline peroxide bleaching of Norway spruce mechanical pulp formed a complex with cationic paper-making additives, and the complex facilitated the removal of lipophilic extractives, so-called pitch or resin, by air flotation.[18–20] It is important to study the

T. Imai (✉) · H. Goto · H. Matsumura · S. Yasuda
School of Agricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
Tel. +81-52-789-4161; Fax +81-52-789-4012
e-mail: takaimai@agr.nagoya-u.ac.jp

*Part of this report was presented at the 40th Lignin Symposium at Tsukuba, October 1995

behavior of pectin during pulping, and the radiotracer method provides information we are not able to obtain by other common and traditional methods.

Behavior of xylan during kraft pulping has been well investigated, and the behavior of pectin could be contrasted well with that of xylan to study the reaction of pectin in wood cell wall. If xylan and pectin are respectively radiolabeled in cell wall, the doubly labeled cell wall becomes the most available portion for a comparative study.

In this study we examined whether xylan and pectin were radiolabeled in cell wall by feeding two radiolabeled precursors simultaneously to tree. The difference between xylan and pectin during kraft pulping was then determined using the doubly labeled wood tissue.

Materials and methods

Plant material and administration of precursor

A 3-year-old magnolia (*Magnolia kobus* DC) was grown in a pot, and a small V-shaped groove (2 × 4 mm) was made with a razor blade in a circumferential direction on the stem. Mixture of D-glucuronic acid-[6-^{14}C] (0.74 MBq, synthesized according to the method of Schaffer and Isbell[21]) and *myo*-inositol-[2-^{3}H] (1.85 MBq) (ARC, St. Louis, MO, USA) was added dropwise to the groove in June, and the plant was allowed to grow for 1 month. Small sticks (3 × 3 × 25 mm) including newly formed xylem were cut from the wood tissue near the groove and extracted with benzene-ethanol (2:1 v/v) and hot water successively. Another part of the newly formed xylem was milled to pass a 40-mesh filter, and extractive free wood meal was prepared.

Nitrobenzene oxidation and sulfuric acid hydrolysis

A part of the extractive free wood meal (30 mg) was hydrolyzed with sulfuric acid, and the hydrolysate was submitted to high-performance liquid chromatographic (HPLC) analysis according to the method of Imai and Terashima.[11] The fractions containing xylose and glucose were collected separately. Another part of the wood meal (10 mg) was subjected to alkaline nitrobenzene oxidation followed by preparative HPLC according to the method of He and Terashima.[22] The fractions containing vanillin and syringaldehyde were collected separately.

Kraft pulping

Six stainless steel bombs containing the radiolabeled wood stick (150 mg each) were prepared, and the wood stick was treated to kraft pulping. The pulping conditions were as follows: effective alkali 20%, sulfidity 30%, liquor/wood ratio 5:1. Temperature was raised from 25°C (room temperature) to 170°C in 90 min and held at 170°C for the following 60 min. Pulping was stopped at six stages: at 20, 40, 60, 90, 120, and 150 min after the start of pulping. Pulping black liquor and treated wood tissue were separated at each stage. Part of the black liquor was infiltrated onto filter paper immediately, and the wood tissue was washed with distilled water until the washings became neutral. The filter papers and the wood tissues were dried in a desiccator.

Radioassay

The radioactivities of the fractions separated by HPLC were determined by a liquid scintillation counter (Beckman model 3801) after adding Bray's cocktail. The extractive free wood meal, the filter papers on which pulping black liquor infiltrated, and the kraft-treated wood tissue were subjected to combustion by a sample oxidizer to give $^{14}CO_2$ and $^{3}H_2O$. Their radioactivities were determined by scintillation counting.

Results and discussion

Double radiolabeling of xylan and pectin in cell wall

We have reported previously that when *myo*-inositol-[2-^{3}H] was administered to a growing stem of magnolia, xylan was radiolabeled almost selectively in cell wall.[14] Chemical analyses of the labeled tissue showed that a large amount of radioactivity was found in xylose obtained by sulfuric acid hydrolysis, and some of the activity was detected in glucose derived from cellulose; no radioactivity was detected in lignin degradation products, namely vanillin and syringaldehyde, obtained by nitrobenzene oxidation. Microautoradiography of the differentiating xylem showed that radioactivity was incorporated mainly into the cell walls during secondary wall formation. Thus selective radiolabeling of xylan was demonstrated by both chemical and autoradiographic studies. Pectin was radiolabeled selectively in mitsumata and magnolia by the feeding of D-glucuronic acid-[6-^{14}C].[12,13] A large amount of radioactivity was found only in galacturonic acid derived from pectin by acid hydrolysis, and no radioactivity was detected in degradation products derived from other cell wall components. With microautoradiography, radioactivity was incorporated into the cell walls during the earliest stage of xylem differentiation. Thus selective radiolabeling of pectin was achieved in the cell wall. It was expected that the cell wall in which xylan and pectin were labeled with ^{3}H and ^{14}C, respectively, could be prepared by feeding *myo*-inositol-[2-^{3}H] and D-glucuronic acid-[6-^{14}C] simultaneously.

Figure 1 shows the specific radioactivities of vanillin, syringaldehyde, xylose, and glucose obtained from magnolia xylem administered a mixture of *myo*-inositol-[2-^{3}H] and D-glucuronic acid-[6-^{14}C] and allowed to metabolize for 1 month. Nitrobenzene oxidation gave enough vanillin and syringaldehyde to determine their radioactivities, and incorporation of radioactivity into lignin was estimated by determining the radioactivity in the oxidation products. Incorporation of radioactivity into cell wall polysaccharides

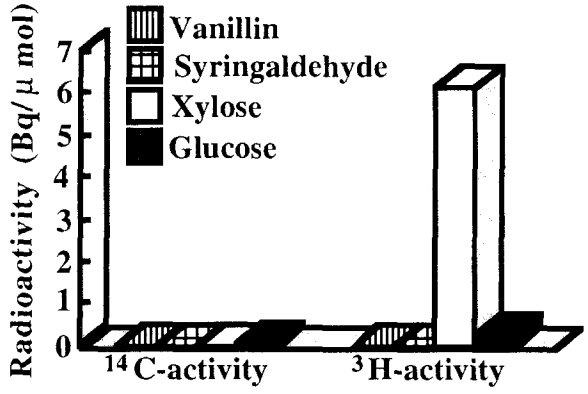

Fig. 1. Incorporation of radioactivity from D-glucuronic acid-[6-^{14}C] and *myo*-inositol-[2-^{3}H] into lignin and polysaccharides as determined by nitrobenzene oxidation and sulfuric acid hydrolysis

was estimated by determining the radioactivity in the sulfuric acid hydrolysate. The hydrolysis of magnolia xylem gave enough xylose and glucose for the radioassay, and only trace amounts of rhamnose, arabinose, mannose, and galactose were found in the hydrolysate by HPLC analysis. A large amount of ^{3}H activity was found mainly in xylose, and some of the activity was detected in glucose; no activity was detected in vanillin and syringaldehyde. These results indicate that ^{3}H activity was incorporated mainly into xylan, as reported previously.[14]

No ^{14}C activity was found in xylose, glucose, vanillin, and syringaldehyde, indicating that radioactivity is not incorporated into hemicellulose (xylan), cellulose, or lignin; in contrast, carbon dioxide obtained by combustion of the xylem tissue was highly radioactive, indicating that the tissue contains considerable radioactive components. ^{14}C activity was considered to be incorporated into pectin as reported previously.[12,13] Radioactivity of galacturonic acid should be determined to estimate the selectivity of pectin labeling, but it is difficult or impossible to isolate galacturonic acid from highly lignified wood tissue.[12,13,23] Consequently, the cell wall in which xylan and pectin were labeled with ^{3}H and ^{14}C, respectively, was prepared. It is expected that the doubly radiolabeled wood tissue has enough selectivity during xylan and pectin labeling to be applied to studies of their behavior during kraft pulping by radiotracer technique.

Behavior of xylan and pectin during kraft pulping

Many investigators have reported the behavior of polysaccharides during kraft pulping after acid hydrolysis or alcohol precipitation.[2-7] Using these methods, however, only some of the carbohydrate is supposed to be examined, and so only limited information is obtained. Recently, the wood meal in which xylan was radiolabeled selectively by feeding *myo*-inositol-[2-^{3}H] to magnolia was treated by kraft pulping. It was possible to obtain information on whole xylan by adding together the radioactivities of the pulping black liquor and the treated wood meal.[16]

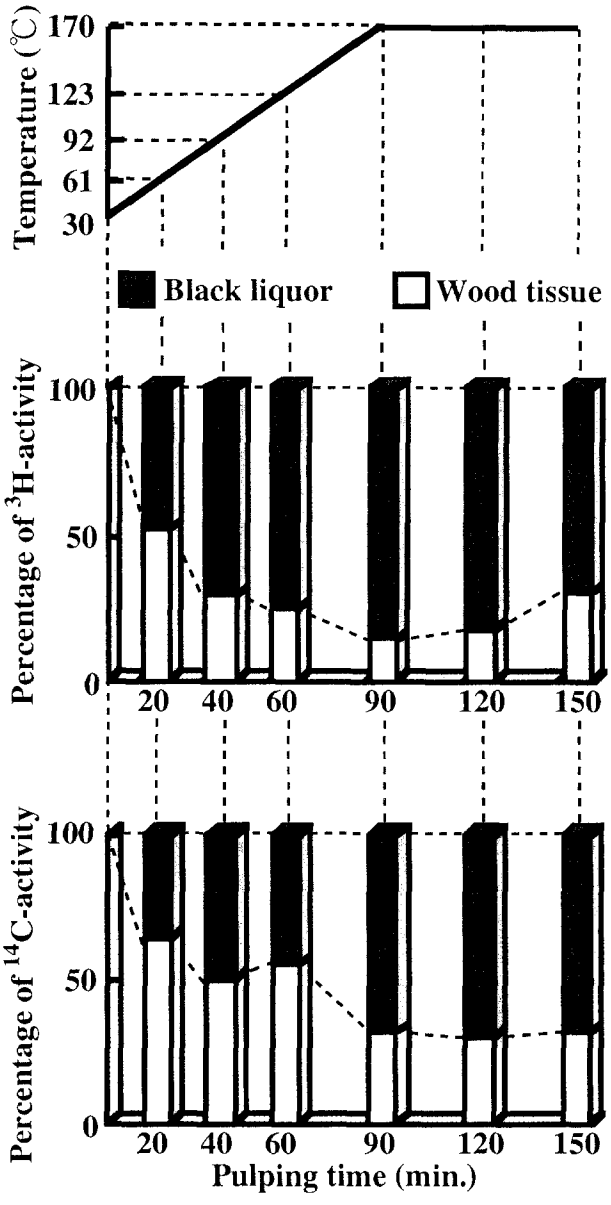

Fig. 2. Pulping temperature and percentages of the radioactivities of pulping black liquor and treated wood tissue at various stages of kraft pulping of the wood tissue in which xylan and pectin were labeled with ^{3}H and ^{14}C, respectively

In this study the wood tissue in which xylan and pectin were labeled with ^{3}H and ^{14}C, respectively, was treated to kraft pulping. The radioactivity of pulping black liquor and of kraft-treated wood tissue was determined at six stages of the pulping, and the percentage of the activity of the black liquor and the wood tissue was calculated. Because most of the ^{3}H activity was incorporated into xylan, the change of percentage with progression of the pulping may represent the approximate behavior of the whole xylan during the pulping. The behavior of pectin during kraft pulping was estimated by the changes in the percentage of ^{14}C activity. Figure 2 shows the percentages of ^{3}H and ^{14}C activities of the black liquor and the treated wood tissue at various stages of kraft pulping.

The change in percentage of ^{3}H activity coincided well with results reported previously.[16] At 20, 40, 60, and 90 min after the start of pulping (pulping temperature 61°, 92°, 123°, and 170°C, respectively), 48%, 71%, 75%, and 85%, respectively, of the activity was distributed in the black liquor, indicating that xylan was dissolved rapidly during the heat-up period, and the dissolution was almost complete before the pulping temperature reached 170°C. After 120 and 150 min (pulping at 170°C for 30 and 60 min, respectively), the radioactivity in the black liquors decreased to 80% and 70%, respectively, indicating that redeposition of xylan on pulp fibers took place rapidly soon after the temperature reached 170°C.

On the other hand, at 20 min after the start of pulping, 36% of the ^{14}C activity was detected in the black liquor, Dissolution of pectin during kraft pulping was demonstrated by the radiotracer method. At 40 and 60 min after the start of pulping, 50% and 42%, respectively, of ^{14}C-activity was distributed in the black liquor, and the percentages were not as large as those for ^{3}H activity. This result indicates that pectin was not dissolved easily as xylan at the earlier stage of pulping. After 30 min the ^{14}C activity of the black liquor increased, and 69% of the activity was distributed in the black liquor after 90 min. This result indicates that the dissolution of pectin progressed preferentially somewhat before the pulping temperature reached 170°C in contrast with the almost complete dissolution of xylan at this stage. After 120 and 150 min, 69% and 70%, respectively, of radioactivities were distributed in the black liquors, and the activity was almost constant. These results indicate that about 70% of pectin was supposed to be dissolved during kraft pulping, and pectin was not redeposited on pulp fiber, in contrast with the rapid redeposition of xylan at the late stage of pulping.

The difference in the behavior between xylan and pectin during kraft pulping is revealed clearly in the change in $^{3}H/^{14}C$ values of pulping black liquor and kraft-treated wood tissue with an increase in pulping time. If xylan and pectin behaved similarly during the pulping, at each stage the $^{3}H/^{14}C$ values of the black liquor ($^{3}H/^{14}C_{\text{black liquor}}$) would be nearly equal to that of the treated wood tissue ($^{3}H/^{14}C_{\text{wood tissue}}$), resulting in ($^{3}H/^{14}C_{\text{black liquor}}$)/($^{3}H/^{14}C_{\text{wood tissue}}$) $\fallingdotseq 1$. Figure 3 shows the values of ($^{3}H/^{14}C_{\text{black liquor}}$)/($^{3}H/^{14}C_{\text{wood tissue}}$) at various stages of kraft pulping.

Except after 150 min the values were larger than 1, indicating apparently that more xylan than pectin was dissolved at each stage, and pectin was not dissolved as easily as xylan. During first 60 min especially, the value increased to about 4, indicating again that xylan was dissolved rapidly at the early stage of kraft pulping, whereas pectin was not dissolved easily at this stage. At 30 min later (60–90 min total) the value decreased, indicating that the dissolution of xylan was almost completed at this stage and pectin was dissolved preferentially. After 60 min (90–150 min total) the value decreased. After 150 minutes, redeposition of xylan on pulp fiber took place but that of pectin did not, resulting in a value of <1.

The differences in behavior between xylan and pectin during kraft pulping could be due to heterogeneous distribution of noncellulose polysaccharides in cell walls, that is, xylan mainly in secondary wall and pectin in middle lamella. During kraft pulping, pulping liquor is supposed to penetrate the cell wall from the lumen side. Pulping liquor is rapidly able to reach the part of the cell wall close to lumen, namely the secondary wall; and xylan distributed mainly in that part could be dissolved and move from cell wall to lumen easily, resulting in the rapid elution of xylan from wood. Pectin distributed in the middle lamella, the part of the cell wall far from the lumen, could not move to the lumen as rapidly as xylan. In addition, lignin is highly condensed, and it is present in large amounts in the middle lamella, so middle lamella lignin is not easily removed during kraft pulping. Pectin would be associated intimately with lignin in the middle lamella; hence pectin cannot be dissolved as easily as xylan.

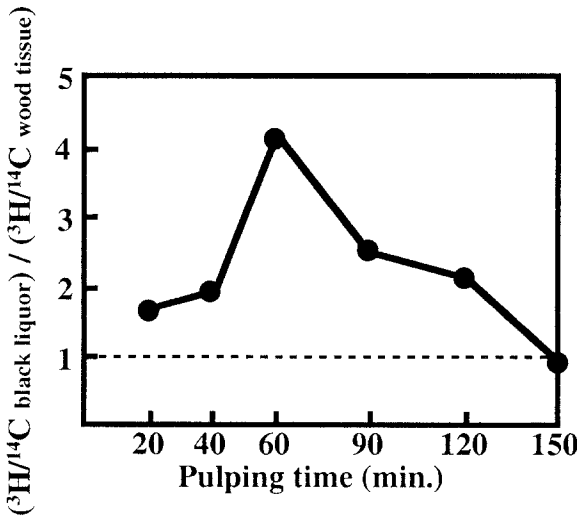

Fig. 3. ($^{3}H/^{14}C_{\text{black liquor}}$)/($^{3}H/^{14}C_{\text{wood tissue}}$) values at various stages of kraft pulping of wood tissue in which xylan and pectin were labeled with ^{3}H and ^{14}C, respectively. $^{3}H/^{14}C_{\text{black liquor}}$, $^{3}H/^{14}C$ value of pulping black liquor; $^{3}H/^{14}C_{\text{wood tissue}}$, $^{3}H/^{14}C$ value of treated wood tissue

Conclusions

Xylan and pectin were labeled with ^{3}H and ^{14}C, respectively, in the cell wall of magnolia by feeding *myo*-inositol-[2-^{3}H] and D-glucuronic acid-]6-^{14}C] simultaneously to a growing stem. The double radiolabeling method was useful for comparative studies on the reaction of polysaccharides in tree cell wall. It was found that pectin was not dissolved as easily as xylan during kraft pulping, and pectin was not redeposited on pulp fiber at the late stage of the pulping.

References

1. Genco JM, Busayasakul N (1990) Hemicellulose retention during kraft pulping. Tappi J 23(4):223–233
2. Sjostrom E (1977) The behavior of wood polysaccharides during alkaline pulping process. Tappi J 60(9):151–154

3. Hamilton JK, Partlow EV, Thompson NS (1958) The behavior of wood hemicelluloses during pulping. I. Examination of the xylose-containing hemicelluloses associated with hardwood and softwood pulps. Tappi J 41(12):803–811
4. Croon I, Enstrom B (1962) The hemicelluloses in sulphate pulps from scots pine. Svensk Papperstidning 65(16):595–599
5. Simonson R (1963) The hemicellulose in the sulfate pulping process. Svensk Papperstidning 66(20):839–845
6. Axelsson S, Croon I, Enstrom B (1962) Dissolution of hemicelluloses during sulphate pulping. Svensk Papperstidning 65(18):693–697
7. Yllner S, Ostberg K, Stockman L (1957) A study of the removal of the constituents of pine wood in the sulphate process using a continuous liquor flow method. Svensk Papperstidning 60(21):795–802
8. Terashima N (1989) Biogenesis and structure of macromolecular lignin in the cell wall of tree xylem as studied by microautoradiography. ASC Symp Ser 399:148–159
9. Fukushima K, Terashima N (1991) Heterogeneity in formation of lignin. XV. Formation and structure of lignin in compression wood of *Pinus thunbergii* studied by microautoradiography. Wood Sci Technol 25:371–381
10. He LF, Terashima N (1990) Formation and structure of lignin in monocotyledons. III. Heterogeneity of sugarcane (*Saccharum officinarum* L.) lignin with respect to the composition of structural units in different morphological regions. J Wood Chem Technol 10(4):435–459
11. Imai T, Terashima N (1990) Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique. I. Selective radio-labeling of polysaccharides in *Magnolia kobus*. Mokuzai Gakkaishi 36:917–922
12. Imai T, Terashima N (1991) Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique. II. Selective radio-labeling of pectic substances in mitsumata (*Edgeworthia papyrifera*). Mokuzai Gakkaishi 37:733–740
13. Imai T, Terashima N (1992) Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique. III. Visualization of the deposition and distribution of galacturonan in the cell wall of magnolia (*Magnolia kobus* DC) xylem by microautoradiography. Mokuzai Gakkaishi 38:475–481
14. Imai T, Terashima N (1992) Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique. IV. Selective radio-labeling of xylan in magnolia (*Magnolia kobus* DC) and visualization of its distribution in differentiating xylem by microautoradiography. Mokuzai Gakkaishi 38:693–699
15. Imai T, Terashima N, Yasuda S (1997) Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique. VI. Selective radio-labeling of mannan in *Ginkgo biloba*. Mokuzai Gakkaishi 43:342–348
16. Imai T, Yasuda S, Terashima N (1997) Determination of the distribution and reaction of polysaccharides in wood cell walls by the isotope tracer technique. V. Behavior of xylan during kraft pulping studied by radiotracer technique. Mokuzai Gakkaishi 43:241–246
17. Sameshima K, Takamura N (1987) The ammonium oxalate pulping of bast fiber. In: Proceedings of 4th international symposium on wood and pulping chemistry 2, Paris, pp 199–201
18. Sundberg K, Thornton J, Ekman R, Holmbom B (1994) Interactions between simple electrolytes and dissolved and colloidal substances in mechanical pulp. Nordic Pulp Paper Res J 9:125–128
19. Sundberg K, Ekman R, Holmbom B (1995) Dissolved air flotation with calcium and polygalacturonic acids. Nordic Pulp Paper Res J 10:201–209
20. Holmbom B (1997) Molecular interactions in wood fibre suspensions. In: Proceedings of 9th international symposium on wood and pulping chemistry, Montreal, pp PL3-1–PL3-6
21. Schaffer R, Isbell HS (1956) Improved synthesis of sodium D-glucuronate-6-^{14}C. J Res Natl Bureau Standards 56:191–195
22. He LF, Terashima N (1989) Formation and structure of lignin in monocotyledon. I. Selective labeling of the structural units of lignin in rice plant (*Oryza sativa*) with ^{3}H and visualization of their distribution in the tissue by microautoradiography. Mokuzai Gakkaishi 35:116–122
23. Westermark U, Hardell HL, Iversen T (1986) The content of protein and pectin in the lignified middle lamella/primary wall from spruce fibers. Holzforschung 40:65–68