tinued into 6 M urea + 0.1% Na dodecylsulfate and 0.14 M β-mercaptoethanol. The desalted and freeze-dried combined protein extracts were subjected to gel filtration on a column of Sephadex G-50 (fine) (Pharmacia, Sweden) equilibrated with 0.05 M ammonium acetate buffer, pH 6.06. The column had dimensions of 1.1 × 102 cm and a volume of 100 ml. The rate of elution was 20 ml/h.

All the fractions were collected and dried, and on subsequent separation in a high-performance liquid chromatograph two radioactive peaks (other peaks were not radioactive) of electrophoretically homogeneous proteins with molecular masses of approximately 25-30 kDa (I) and 10-15 kDa (II) were isolated. The proteins (I) isolated from the nuclei of rabbit and bovine neuron cells and also from those of the cotton plant were lyophilized and used to determine their amino acid compositions. The proteins were hydrolyzed as described in [8]. Amino acid compositions were determined on a Biotronik IC-7000 analyzer (FRG).

LITERATURE CITED

1. W. R. Allen, Trends Biochem. Sci., 3, No. 1, 225 (1978).
2. E. F. Zimmerman, J. Nackey, P. Nelson, and J. Arias, Biochemistry, 8, 2636 (1969).
3. M. Lavel and Y. Mouli, Biochimie, 58, 1137 (1976).
4. K. S. Matinyan and S. R. Umanskii, Biokhimiya, 43, No. 1, 111 (1978).
5. O. Kh. Saitmuratova and O. G. Nemtsova, Dokl. Akad. Nauk UzSSR, No. 12, 43 (1988).
6. O. Kh. Saitmuratova, O. G. Nemtsova, and V. B. Leont'ev, Khim. Prir. Soedin., No. 4, 565 (1987).
7. O. Kh. Saitmuratova, D. Akhmedova, and G. Alimkhodzhaeva, Khim. Prir. Soedin., No. 3, 791 (1983).
8. O. Kh. Saitmuratova, V. B. Leont'ev, D. Akhmedova, and G. Rashidkhodzhaeva, Uzb. Biol. Zh., No. 4, 62 (1980).

CHLORINATION OF LIGNIN AT LOW TEMPERATURES

S. V. Demidov, S. I. Kuzina,
L. A. Serous, Yu. A. Filonenko,
I. P. Kim, and A. I. Mikhailov

UDC 678.01

A high reactivity of the olefinic double bonds of the lignin (Lg) macromolecule in the reaction with molecular chlorine has been found which has permitted the chlorination of Lg to be carried out under cryogenic conditions at 150-250 K. The process takes place by a radical-chain mechanism with a length of the kinetic chain of approximately 1000 units. The bulk of the chlorine is consumed in the olefinic chains of the Lg. Other reaction products besides chlorolignin are hydrogen chloride and phenoxyl radicals, which have singlet ESR spectrum (ΔH = 1.0-1.2 mT) and are retained in the chlorinated samples of Lg to ~250 K.

The reaction of chlorine with lignin (Lg) presents considerable interest for the study of the mechanism of the bleaching process that is widely used in the pulp and paper industry. Lg chlorination processes are usually considered at temperatures above 270 K. Under these conditions ionic reactions of chlorine with the aromatic ring [1] are usually discussed; a free-radical mechanism has been regarded as unlikely. However, Lg is also a polyolefin with a high content of double bonds in the side chains, and therefore it is possible to expect the appearance of features characteristic for the radical-chain chlorination of olefins.

In order to separate the different stages of the process and to reveal the relative reactivities of the functional groups of the macromolecule, in the present work we have investigated the low-temperature (150-250 K) chlorination of hydrolysis lignin by molecular chlorine.

Institute of Chemical Physics, Academy of Sciences of the USSR, Chernogolovka, Moscow Province. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 671-676, September-October, 1990. Original article submitted November 15, 1989; revision submitted March 25, 1990.

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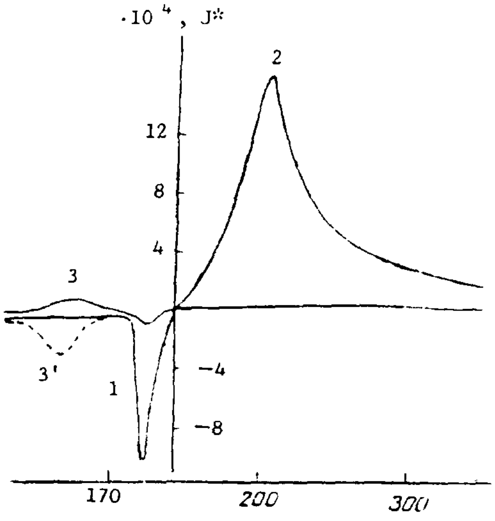


Fig. 1

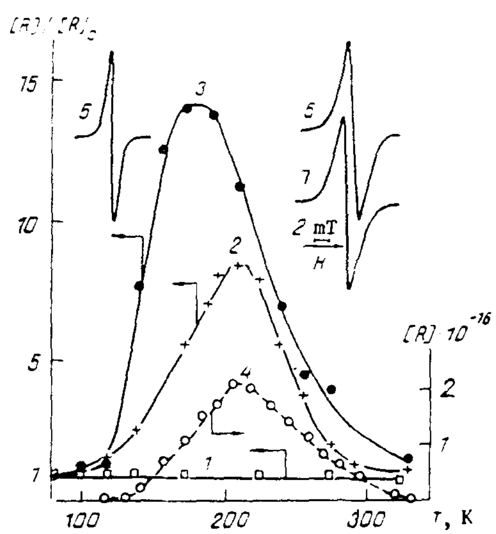


Fig. 2

Fig. 1. Calorimetric curves of the heating of the chlorine—lignin system (weight ratio 1:1): 1) melting of the chlorine; 2) evolution of heat from the lignin chlorination reaction; 3) curves of the sorption of the reaction products after reheating of the Cl_2—Lg system; 3') peak of the melting of HCl. *Unclear in Russian original — ed.

Fig. 2. Change in the concentration (1-3) and form of the ESR spectra (5, 6) of the free radicals in the low-temperature chlorination of lignin: weight ratio of chlorine to lignin: 1) 0:1; 2) 1:2; 3) 1:1; 5) ESR spectrum of the initial lignin; 6) spectrum of lignin chlorinated at 190 K; 4, 7) change in the concentration of free radicals in the low-temperature chlorination of phenol, and their ESR spectrum at 190 K, respectively.

With a slow rise in the temperature of the Lg + Cl_2 system above 77 K, on the calorimetric curve immediately above the exothermic peak of the melting of the chlorine (Fig. 1, curve 1) an evolution of heat connected with the spontaneous chlorination of the Lg in the temperature range of 170-220 K was observed (Fig. 1, curve 2). The effective activation energy of this reaction, E, ≈ 27 kJ/mole, and the observed evolution of heat, q, ≈35 kJ per 1 mole of chlorine consumed. In the course of the reaction on the heating of the Lg + Cl_2 system a sharp increase took place in the concentration of paramagnetic centers (PMCs) to $|R| \simeq (5\text{-}7)\cdot 10^{18}$ spins/g, which was 10-15 times greater than the background level of PMCs in the initial Lg ($|R|_0 \simeq 4.5\cdot 10^{17}$ spins/g) observed in the same temperature range. Figure 2 gives curves of the change in the concentration of PMCs in Lg at various ratios of chlorine in the system (curves 2 and 3). In the process of a further rise in the temperature from 220 to 250 K, the chlorination reaction slowed down (according to calorimetric results) while the concentration of PMCs fell practically to the background level (Fig. 2, curve 1).

The background ESR spectrum in the initial Lg consisted of an isotropic single line of Lorentzian form having g = 2.003 and the width $\Delta H \simeq 0.5$ mT (Fig. 2, spectrum 5). The narrowness of the symmetrical single line, the g-factor close to the purely spin value of the g-factor, and the observance of Curie's law for the temperature dependence of the intensity of the signal permitted the assumption that this spectrum was connected with radicals in which the unpaired electron was delocalized over a system of conjugated double bonds in sections of the macromolecule having a system of conjugation.

In the process of chlorination, the width of the singlet in Lg increased to ΔH = 1.0-1.2 mT (Fig. 2, spectrum 6), which may indicate the appearance of new PMC also having a singlet ESR spectrum but with a greater width. Such new radical particles may apparently have been phenoxyl radicals that were formed in the reaction of molecular chlorine with the phenolic fragments of the lignin and had a singlet ESR spectrum in the solid phase (because of the unresolvability of the hyperfine structure). To confirm the possibility of the reaction of the phenolic groups of Lg with chlorine (with the formation of PhO·) at such low temperatures, the chlorination of phenol and of 4-tert-butylphenol (which may be considered as low-molecular-mass analogs of the phenolic structures of Lg) was carried out specially, and in the same temperature region (150-250 K) the appearance of free radicals was observed (Fig. 1, curve 4).

It is known that, under the action of various oxidants, phenols and their derivatives form phenoxyl radicals [1]. It is natural to consider that the action on phenols of such an oxidant as molecular chlorine at low temperatures will also cause the formation of phenoxyl radicals, the singlet spectrum of which agrees in its parameters with the spectrum of chlorinated Lg (Fig. 2, spectrum 7). An indirect confirmation of the phenoxyl nature of the radicals in chlorinated Lg was the behavior of the ESR signals with an increase in the power of the ultra-high-frequency field: the curve of the saturation of the ESR signal of the chlorolignin was close to the curve of the saturation of the ESR spectra of the phenoxy radicals in Ionol or 4-tert-butylphenol and differed from the behavior of the signal of the initial lignin by its smaller tendency to saturation. Consequently, it may be considered that the main radical particles that are stabilized in the low-temperature chlorinaton of Lg are the phenoxyl radicals that are formed in the reaction of chlorine with the phenol groups and also, apparently, as the result of the inhibition by phenols of active intermediate radicals in the alkyl chains of the Lg.

In the chlorination of Lg, the consumption of chlorine, determined gravimetrically, amounted to approximately 1 g of Cl_2 per 1 g of Lg. Here, half the chlorine went to the formation of HCl, the concentration of which was determined calorimetrically from the melting point (Fig. 1, curve 3) and from the IR spectra. The remainder of the chlorine added to the Lg molecules. According to the results of elementary analysis, the chlorolignin molecule contained ~30% of chlorine. After chlorination, the concentration of double bonds in the chlorolignin had decreased 100-fold as compared with the initial material. The number of double bonds was determined on a ADS-4M double-bond analyzer. The working of the instrument is based on the principle of predominant oxidation by ozone of isolated double bonds in an organic substance. It is known that the rate constant of the reaction of ozone with olefins ($K \simeq 10^5$ liters/mole·sec) is 4-6 orders of magnitude greater than the corresponding constants with aromatic hydrocarbons and approximately 8 orders of magnitude greater than the rate constants of the interaction of ozone with primary C—H bonds in hydrocarbons [2]. An estimate of the maximum amount of chlorine capable of adding to the isolated double bonds of lignin, determined with the aid of the ADS-4M, gave a value of 1.2 g of Cl_2 per 1 g of Lg. The correspondence of this value to the results obtained gravimetrically shows that practically the whole of the chlorine was consumed in the chlorination of the double bonds of the olefinic chains of the Lg. The lightening of the color of a sample of Lg observed in this process (from dark brown to yellow) may also be connected with the decrease in the number of conjugated double bonds, which are the cause of the dark coloration of the substance.

Usually, the chlorination of hydrocarbons in the gas phase initiated by radiation or heat takes place by a radical-chain mechanism. In the present work we are considering the chlorination of a solid Lg matrix by liquid chlorine in which the reaction takes place at low temperatures spontaneously without external initiation. On the basis of the experimental results obtained from the ESR spectra, the initiation stage could be represented as a direct reaction of the chlorine with the hydroxy group of a phenolic fragment to form a phenoxyl radical, HCl, and atomic chlorine. However, the direct chlorination with the splitting out of a hydrogen atom from the OH group of a phenol is an endothermic process (formally for the gas phase the enthalpy of the reaction, $\Delta Q \simeq +163$ kJ/mole). In the condensed state, however, the O—H bond can apparently be greatly weakened through heterolytic effects leading to a situation in which, in the limit, the reaction of the molecular chlorine takes place not with the OH group but with the phenolate ion as a reaction in which an electron is transferred to Cl_2:

$$\mathrm{PhO^-} + \mathrm{Cl_2} \rightarrow \mathrm{PhO^{\cdot}} + \mathrm{Cl_2^-}. \qquad (1)$$

This process is exothermic ($\Delta Q \simeq -63$ kJ/mole) and can take place at low temperatures. Consequently, a result of the initiation reaction is the appearance of relatively stable unreactive phenoxyl radicals and the ion-radical particles Cl_2^- (it must be mentioned that the components of Cl_2^- were not observed in the ESR spectra). In frozen alcoholic solution, the Cl_2^- particle is retained to ~140 K [3]; in the solid Lg matrix it obviously appears on the passage of chlorine into the liquid state (~170 K). Since the Lg matrix molecule contains double bonds in alkyl chains, the active particle Cl_2^- can initiate the chain chlorination reaction of the olefinic fragments of the Lg. The stage of chain prolongation in the chlorination of Lg can therefore be represented in a similar way to the halogenation of olefins with the participation of active radicals $R^{\cdot}$ and atomic chlorine [4]:

$$\sim\mathrm{CH_2{-}CH{=}CH}\sim + \mathrm{Cl_2^-} \rightarrow \sim\mathrm{CH_2{-}\dot{C}H{-}CHCl}\sim + \mathrm{Cl^-} \ (\Delta Q \simeq -126 \text{ kJ/mole}), \qquad (2)$$

$$>\!CH + Cl_2^- \rightarrow\ >\!\dot{C} + HCl + Cl^- (\Delta Q \simeq -50 \text{ kJ/mole}), \tag{3}$$

$$\sim CHCl - \dot{C}H - CH_2 \sim + Cl_2 \rightarrow \sim CHCl - CHCl - CH_2 \sim + Cl^{\cdot} \tag{4}$$

$$\sim CH_2 - CH = CH \sim + Cl^{\cdot} \rightarrow \sim \dot{C}H - CH = CH \sim + HCl, \tag{5}$$

$$\sim \dot{C}H - CH = CH \sim + Cl_2 \rightarrow \sim CHCl - CH = CH \sim + Cl^{\cdot} \tag{6}$$

The reaction terminating in the kinetic chains takes place on the recombination of the radicals with one another or with atomic chlorine, or on transfer of the chain to an inactive phenoxyl radical:

$$\begin{gathered} R^{\cdot} + R^{\cdot} \rightarrow R - R \\ R^{\cdot} + Cl^{\cdot} \rightarrow R - Cl \\ Cl^{\cdot} + Cl^{\cdot} \rightarrow Cl_2 \\ R^{\cdot} + PhOH \rightarrow R - H + PhO^{\cdot} \\ Cl^{\cdot} + PhOH \rightarrow PhO^{\cdot} + HCl \end{gathered}$$

Since the concentration of phenol groups in the system is far greater than the concentration of active radicals formed, i.e., $[PheOH] \approx 10^{22}\ g^{-1} \gg [R^{\cdot}]$), then, apparently, it must be considered that in the final account practically all the $R^{\cdot}$ are inhibited by phenols and pass into the phenoxyl radicals observed. Therefore it is possible from the concentration $[PhO\cdot]$ to estimate the maximum concentration of radicals formed in the initiation stage. It is (for curve 3, Fig. 2) $[R^{\cdot}] \approx 7 \cdot 10^{18}$ spins/g; then the consumption of chlorine is $\sim 8 \cdot 10^{21}$ molecules/g and, consequently, the length of the kinetic chain of the low-temperature Lg chlorination process is approximately 1000 units.

It must be mentioned that the paramagnetic absorption in Lg remained, after chlorination, at approximately the level of the background concentrations (Fig. 2, curves 2 and 3). When the samples were stored at room temperature the singlet ESR spectrum of the chlorolignin gradually contracted from $\Delta H \approx 1.0$-1.2 mT to ~ 0.55 mT, which almost coincides with the width of the initial singlet in Lg. Apparently, the initial radicals possess a high stability and are inactive in the low-temperature chlorination process.

Information on the predominant chlorination at low temperatures of the olefinic chains of Lg may be of interest from the point of view of the ecological harmlessness of the cellulose-bleaching process. A specific feature of the chain chlorination of Lg is the formation of fragments of macromolecules containing chlorine atoms on neighboring carbon atoms. In the bleaching of cellulose (which is usually carried out in an alkaline medium), the C—Cl bond is readily hydrolyzed under the action of the OH^- anion and then the process of degradation of the chlorolignin can take place through stage of the formation of polyhydric alcohols and the cleavage of the skeletal C—C bonds with the formation of terminal carbonyl groups and double bonds.

Since chlorine adds mainly to the double bonds in the alkyl parts of the macromolecule and not to the aromatic ring, then, apparently, after low-temperature chlorination the yield of toxic products, such as chlorophenols, dioxins, etc., in the wastes from the chlorine bleaching of cellulose can be considerably lowered. Furthermore, the considerable bleaching of the chlorolignin samples indicates that low-temperature chlorination may prove to be more effective in increasing the dgree of bleaching of cellulose than chlorine bleaching performed at the ordinary temperatures.

EXPERIMENTAL

In this work we used industrial (Khor factory) hydrolysis lignin in the form of a microdisperse powder which, before an experiment, was treated with water for 10 days and was then dried to the air-dry state. Before chlorination a weighed sample of Lg was placed in a glass tube which was then evacuated and, after being cooled to the temperature of liquid nitrogen (77 K) gaseous chlorine was frozen onto the sample in a Cl_2:Lg ratio of from 5:1 to 1:5. With slow heating of the Cl_2—Lg system the spontaneous Lg chlorination reaction was observed by the method of scanning differential calorimetry [5] from the evolution of heat. To analyze the radical and molecular reaction products we also used the methods of ESR and IR spectroscopies. The ESR spectra were recorded at 77 K on a EPR-21 radiospectrometer at a power of the UHF field of ~10^{-4} W, while the IR spectra were recorded on a UR-20 spectrometer. The concentrations of double bonds were determined with the aid of a ADS-4M double-bond analyzer, the working principle of which is based on the ozonization of isolated double bonds in organic substances.

The authors thank S. S. Chernikov for performing the IR analysis of the lignin chlorination products.

LITERATURE CITED

1. K. V. Sarkanen and C. H. Ludwig, Lignins, Wiley-Interscience, NY. (1971)
2. G. E. Zaikov and S. D. Razumovskii, Ozone and Polymeric Materials [in Russian], Znanie, Moscow (1988), p. 32.
3. D. A. Gordon, S. V. Demidov, and A. I. Mikhailov, Teor. Eksp. Khim., 20, No. 3, 379 (1984).
4. G. B. Sergeev and V. V. Smirnov, The Molecular Halogenation of Olefins [in Russian], Izd. MGU, Moscow (1985), p. 234.
5. I. M. Barkalov and D. P. Kiryukhin, Vysokomol. Soedin., 22A, No. 4, 723 (1980).

INTERACTION OF LIGNOSULFONATE WITH CERTAIN METAL IONS

A. M. Khvan and Kh. A. Abduazimov

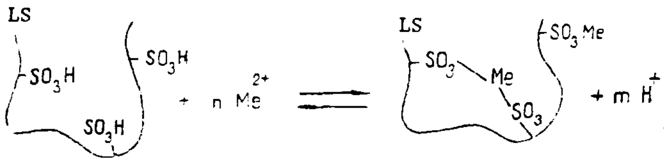
UDC 547.992.3

The reaction of lignosulfonate with the Co^{2+}, Ni^{2+}, and Cu^{2+} ions has been studied by the methods of gel chromatography, potentiometric titration, and UV spectroscopy. Lignosulfonate forms polymer-metal complexes with the metal ions over a wide pH range. In this process the size of the macromolecule scarcely changes, thanks to the reticular structure of the lignosulfonate. The capacity of lignin with respect to Cu^{2+} ions has been determined.

Interest in the investigation of the binding of metal ions by lignosulfonate is due above all to the availability of this material and the wide occurrence of such processes. An interaction of lignosulfonate with a metal is observed, for example, in the wood digestion process. The formation of metal complexes with lignin interferes with the bleaching of cellulose and increases the depth of color of the effluents. Its complex-forming properties also affect the biological activity of biostimulators from lignin. On the other hand, the products of the binding of metals by the lignin macromolecules may exhibit catalytic activity, a high foam-forming capacity, etc. [1].

We have studied the complex-forming capacity of lignosulfonates with the transition-metal ions Ni^{2+}, Co^{2+}, and Cu^{2+}.

The interaction between the lignosulfonate (LS) macromolecules and metal ions (Me) is accompanied by a shift in the acid-base equilibrium in the system according to the scheme shown below.



Institute of Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 676-679, September-October, 1990. Original article submitted December 5, 1989; revision submitted April 2, 1990.