

Study of Cellulose Sulfates by X-ray Photoelectron Spectroscopy

A. S. Romanchenko^a, A. V. Levdansky^a, V. A. Levdansky^{a, b}, and B. N. Kuznetsov^{a, b, 1}

^a*Institute of Chemistry and Chemical Technology, Siberian Branch, Russian Academy of Sciences, Akademgorodok 50/24, Krasnoyarsk, 660036 Russia*

^b*Siberian Federal University, pr. Svobodnyi 79, Krasnoyarsk, 660041 Russia*

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Abstract—Samples of microcrystalline cellulose (MCC) have been studied by X-ray photoelectron spectroscopy before and after sulfation. It has been found that the reaction of MCC with chlorosulfonic acid in dioxane results in the incorporation of sulfate groups into the structure of cellulose. The content of sulfur in sulfated MCC varies from 4.0 to 9.7 at %, sulfur being in the form of sulfate. The sulfur content on the surface and in the bulk of MCC sulfate samples has been compared. The difference in the shape of 1s lines of carbon in the spectra of initial and sulfated MCC samples indicates that the electron state of a fraction of the carbon atoms undergoes changes during sulfation.

Keywords: microcrystalline cellulose, sulfation, chlorosulfonic acid, dioxane, cellulose sulfates, electron spectroscopy for chemical analysis

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INTRODUCTION

Cellulose sulfates are substances of plant origin that hold much promise for practical implementation. They can be used as blood coagulants, immunomodulators, antiviral preparations, and sorbents of toxic metals [1, 2].

At present, the main anticoagulant used in clinical practice is the polysaccharide heparin isolated from the raw materials of animal origin. However, heparin can be infected with pathogens and viruses. Therefore, the development of simple methods for the synthesis and physicochemical examination of CSs as an alternative to heparin is a problem of current interest.

The methods for obtaining CSs of high DS from cotton, MCC cellulose and their properties are described in [3]. The sulfation of MCC by sulfuric acid in the presence of *n*-propanol and the application of aminosulfonic acid resulted in products of a low degree of etherification, which only partially dissolve in water. The authors were able to achieve a high DS using a SO₃–pyridine complex at reaction temperatures of 80–90°C. Further increase in the reaction temperature led, along with an increase in DS, to a fall in reduced viscosity (η_{red}) for CS solutions, indicating the destruction of the polymer. Among the shortcomings of the method are the complexity of the isolation of the target product and the use of toxic pyridine. The

authors of [4] performed the MCC sulfation by a ClSO₃H–dimethylformamide complex under different conditions, which gave products with different DS. The DS values were in the range between 0.6 and 1.7 and increased with the concentration of the sulfating agent. The average molecular weight varied in the range of 12–27 kDa. The resulting CS sodium salt was to be dialyzed and was characterized by a wide distribution of molecular weights. In most products, a polydispersity due to the hydrolysis of the cellulose main chain in acidic medium was observed. The data of IR and NMR spectroscopies and of elemental analysis indicated that the sulfation predominantly occurs in the C₆ position, partially in the C₂ position, and very rarely at C₃. Studies of the anticoagulant activity showed good prospects for the development of novel preparations based on CS sodium salt.

We developed a new method of MCC sulfation under mild conditions with the use of chlorosulfonic acid in dioxane [5]. The reaction of sulfation of MCC with chlorosulfonic acid in dioxane begins in a two-phase system and ends in a single-phase system with the almost complete sulfation of OH groups at C₆ atoms of glucopyranose units of MCC. The structure and properties of initial MCC and its sulfated derivatives were studied by elemental analysis and the methods of IR and ¹³C NMR spectroscopy. It was shown that the resulting CSs are anticoagulants of direct action, since they increase the time of human plasma clotting *in vitro* in coagulologic tests [6, 7].

It is known that the anticoagulant activity of sulfates of polysaccharides depends on many factors: the

Abbreviations: CS, cellulose sulfate; DS, degree of substitution; MCC, microcrystalline cellulose; XPS, X-ray photoelectron spectroscopy.

¹Corresponding author: phone: (391) 249-48-94; e-mail: bnk@icct.ru, innm@icct.ru.

Table 1. Characteristics of MCC samples

Sample	Degree of polymerization (DP)	Degree of crystallinity (DC)	Content of residual lignin, %	Content of hemicelluloses, %
Wheat straw MCC	200	0.66	0.1	5.5
Asp wood MCC	210	0.71	0.1	5.3
Industrial MCC	249	0.75	Not detected	5.2

degree of sulfation, the position of sulfate groups, the molecular weight of a polysaccharide, and others [8]. To establish the structure–biological activity relationship, a detailed physicochemical study of samples of MCC sulfates is needed.

In the present work, the composition and structure of sulfated derivatives obtained by the novel method, the treatment of MCC from wheat straw and asp wood with chlorosulfonic acid in dioxane, were studied for the first time using the method of XPS.

EXPERIMENTAL

MCC samples were obtained by an ecologically safe method involving the oxidative delignification of wheat straw and asp wood by hydrogen peroxide in acetic acid–water in the presence of a sulfuric acid catalyst [9, 10]. In addition, MCC Vivapur® Type 101 (JRS Pharma; Weissenborn, Germany) was used. Some characteristics of MCC samples are given in Table 1.

The sulfation of MCC was carried out with chlorosulfonic acid in a three-necked 100-mL flask equipped with a stirrer, a thermometer, and a dropping funnel. One gram of MCC preliminarily dried to a constant weight at a temperature of 103–105°C was charged into the flask, 1,4-dioxane (30 mL) was added, and the mixture was stirred at room temperature for 4 h. Then, chlorosulfonic acid was added dropwise under cooling and intensive stirring, and the mixture was stirred at 20–50°C for three to four hours. Thereafter the reaction mixture was poured under stirring into a glass containing 100 mL of 50% aqueous ethanol solution and 3% sodium hydroxide. The resulting CS sodium salt was separated by filtration, washed on a filter with 96% ethanol, and dried in a vacuum.

The content of sulfur in CS was determined using a modified method [11] by combustion in an oxygen flow followed by the absorption of combustion products by hydrogen peroxide and titration with alkali in the presence of the indicator methyl red.

The DS in CS samples was determined by the equation

$$DS = \frac{162S_{\text{mass}}}{3200 - 102S_{\text{mass}}}$$

where S_{mass} is the content of sulfur (wt %). The atomic content of sulfur S_{at} was calculated without taking hydrogen atoms into account by equation

$$S_{\text{at}} = \frac{N_S}{N} \times 100\%,$$

where N_S is the number of sulfur atoms, and N is the total number of atoms in an elementary unit of a polymer. It was assumed that the structure of an elementary unit of the polymer corresponds to the formula $[C_6H_7O_2(OH)_{3-DS}(OSO_3Na)_{DS}]_n$.

XPS spectra were taken using samples of CS sodium salt in the form of powder and films. Transparent films of sulfated MCC were obtained by the evaporation of 6–7% aqueous solutions on a smooth glass support. Spectra were measured on a SPECS spectrometer (SPECS GmbH, Germany) in ultrahigh vacuum at a pressure no higher than 1×10^{-9} mbar. For performing an experiment, powder or a film was applied to a carbon ribbon glued to a molybdenum support. The source of X-ray radiation was an X-ray tube with a magnesium anode (MgK_{α} , $E = 1253.6$ eV). The radiation power was 180 W, and the tube voltage was 12.5 kV. The electrostatic charging of samples was eliminated using irradiation with slow electrons. The analysis of photoelectrons energies was carried out using a hemispherical energy analyzer PHOIBOS 1500 MCD9; the operation of the analyzer was controlled by the program SpecsLab2. Wide scans were recorded with a step of 0.5 eV and a pass energy of 20 eV. Then, narrow scans for each element: O, C, S, as well as Na, Si, and Ca with a pass energy of 8 eV and a step of 0.05 eV were recorded. The spectra were processed using the program CasaXPS 3.0, and the decomposition was performed using the Gaussian–Lorentz components.

The relative content of elements (except for H and He) in the zone of analysis (the depth of analysis 2–3 nm) was determined from the integral intensities of XPS lines, corrected for the corresponding atomic sensitivity factors.

RESULTS AND DISCUSSION

Survey spectra and atomic composition. Survey XPS spectra of samples of wheat straw, asp wood, and industrial MCC are shown in Figs. 1–3.

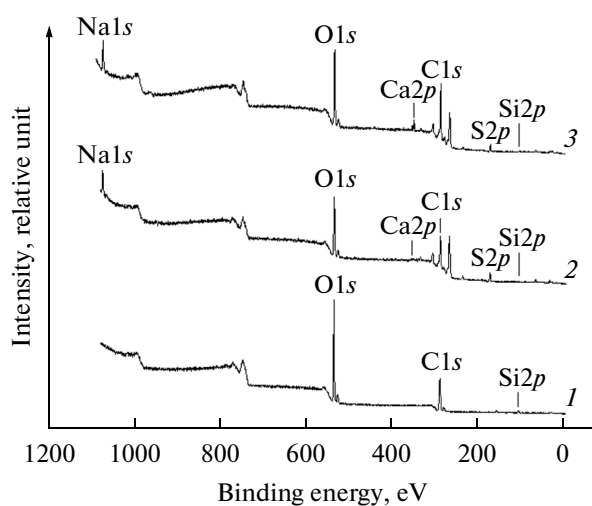


Fig. 1. XPS spectra of initial wheat straw MCC (1) and sulfated wheat straw MCC (2, powder; 3, film).

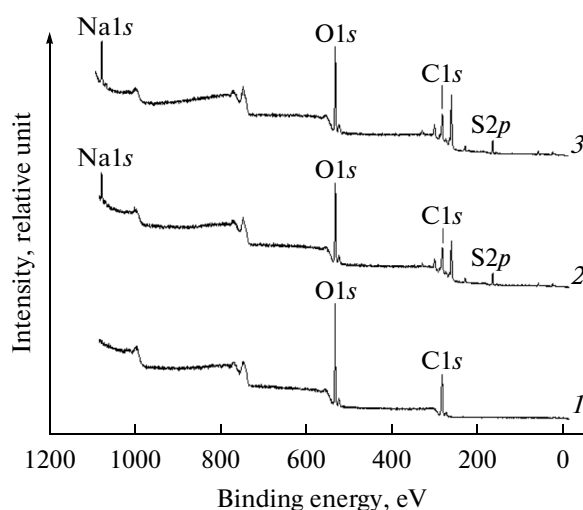


Fig. 2. XPS spectra of initial asp wood MCC (1) and sulfated asp wood MCC (2, powder; 3, film).

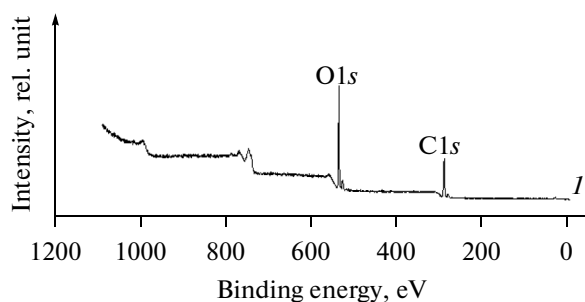


Fig. 3. XPS spectra of industrial MCC (1) and a sample of sulfated industrial MCC (2).

The main lines in the spectra correspond to carbon and oxygen. Typical for the spectrum of wheat straw MCC is the presence of the line of silicon, which is found in small amounts in the initial raw material [12]. The data on the atomic composition of MCC samples, obtained by the analysis of XPS spectra, are given in Table 2.

According to the XPS data, different MCC samples somewhat differ in the content of carbon and oxygen. Wheat straw MCC contains less carbon than asp wood MCC (53.3 and 64.4 at %, respectively) and more oxygen (43.8 and 35.6 at %, respectively). The content of carbon in industrial MCC is 57.3 at %, and that of oxygen is 42.7 at %.

Table 2. Elemental composition of initial and sulfated MCC as determined by XPS

Sample	Content, at %					
	C	O	S	Na	Si	Ca
Wheat straw MCC	53.3	43.8	0	0	2.9	0
Asp wood MCC	64.4	35.6	0	0	0	0
Industrial MCC	57.3	42.7	0	0	0	0
Sulfate of wheat straw MCC	53.8	33.3	5.6	5.3	1.4	0.6
Film of wheat straw MCC sulfate	52.4	34.4	4.0	5.1	1.8	2.3
Sulfate of asp wood MCC	46.1	39.5	7.5	6.8	0	0
Film of asp wood MCC sulfate	43.0	38.6	9.7	8.6	0	0
Sulfate of industrial MCC	45.9	39.0	7.9	7.2	0	0

Table 3. Sulfur content in samples of sulfated MCC as indicated by the data of chemical analysis and XPS

Sample	Sulfur content, at %	
	XPS	chemical analysis
Sulfate of wheat straw MCC	5.6	8.5
Film of wheat straw MCC sulfate	4.0	8.0
Sulfate of asp wood MCC	7.5	7.6
Film of asp wood MCC sulfat	9.7	7.7
Sulfate of industrial MCC	7.9	8.2

The sulfation of MCC with chlorosulfonic acid in dioxane and subsequent isolation of CS in the form of the sodium salt leads to the incorporation of sulfate groups into the MCC structure. As a result, lines corresponding to sulfur and sodium appear in the spectra (at 168 and 1070 eV, respectively) (Figs. 1–3). On the surface of samples of sulfated wheat straw MCC, there are also small amounts of silicon, and traces of calcium. The absence of calcium in the surface layer of initial wheat straw MCC is probably due to its uneven distribution in the sample.

According to the analysis of the atomic composition of samples, the sulfur content is 4.0–5.6 at % in the surface layer of sulfated wheat straw MCC, 7.5–9.7 at % (in the surface layer) of sulfated asp wood MCC, and 7.9 at % in sulfated industrial MCC. The ratio of sodium/sulfur atomic concentrations on the surface of samples is close to unity.

Comparison of the data of XPS and chemical analysis. The results of a comparison of the sulfur atomic content in CS, determined by chemical analysis and from XRS spectra, are shown in Table 3.

As it follows from these data, the sulfur content in the bulk of asp wood CS samples and industrial MCC determined by the chemical analysis insignificantly differs from the content on the surface determined by

XPS, indicating a rather even distribution of sulfur atoms in these samples. In sulfates of wheat straw MCC, the sulfur content on the surface is somewhat lower than in the bulk.

XPS S2p lines. XPS S2p lines have a similar shape for all sulfated MCC samples (Figs. 4–6).

The spectrum is decomposed into two components of the doublet, S2p_{3/2} (binding energy 168.6 eV) and S2p_{1/2} (binding energy 169.8 eV), which corresponds to hexavalent sulfur bound to oxygen; in this case, these are OSO₃Na groups. Because there are no other components in the spectra, it is reasonable to assume that, within the layer analyzed (about 2 nm), sulfur is present only in the form of sulfate.

XPS C1s lines of carbon. Differences in the shape of 1s lines of carbon in the spectra of initial and sulfated MCC point to changes in the chemical state of carbon atoms occurring in the process of sulfation. XPS spectra of carbon 1s lines of MCC samples are shown in Figs. 7–9.

The spectra consist of several components with different binding energies, which correspond to different carbon atoms in cellulose. The results of the decomposition of XPS carbon 1s lines, the binding energies of different forms of carbon, and their relative contributions are given in Table 4.

According to the literature data [13], the intense C₁ peak at 284.8 eV belongs to the carbon bound to carbon and hydrogen atoms, i.e., carbon in the sp³-hybridization; probably it is due to hydrocarbon contaminations on the surface of CS. The components with a greater binding energy correspond to carbon chemically bound to oxygen. The peak with a binding energy of 286.4–286.6 eV, designated as C₂, can belong to signals from carbon atoms of either hydroxyl or ether groups of cellulose (–C–O–), and the C₃ peak at 287.9–288.4 eV, to the polyacetal bonds of the cellulose chain (O–C–O–) and the carbon of the carbonyl (–C=O) and carboxyl groups (–O–C=O). The fourth peak with a binding energy of about 290.4 eV corresponds to the Auger line of sodium (Na KLL), which has a complicated structure and is superimposed onto the C1s line.

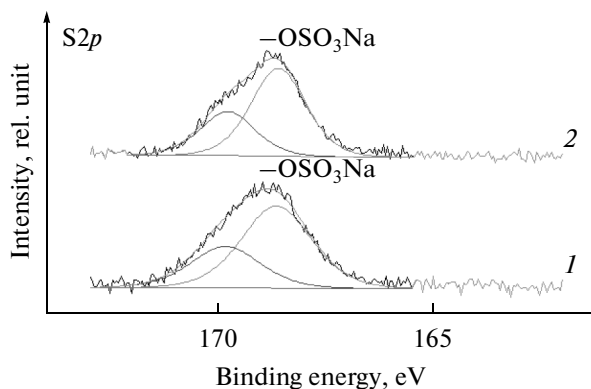


Fig. 4. XPS spectra of sulfur 2p lines of sulfated wheat straw MCC (1, powder; 2, film).

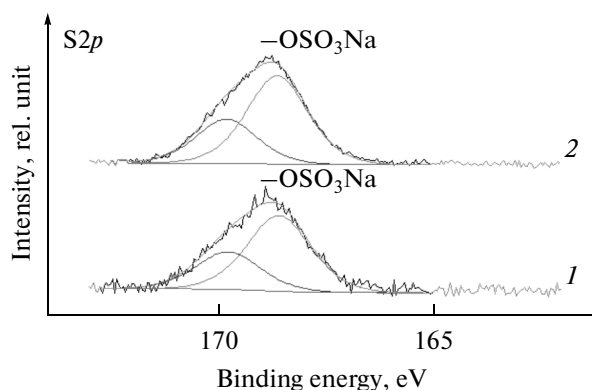


Fig. 5. XPS spectra of sulfur 2*p* lines of sulfated asp wood MCC (1, powder; 2, film).

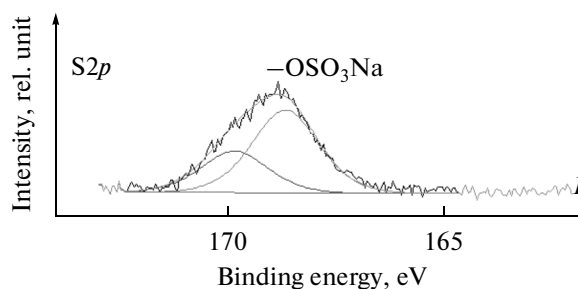


Fig. 6. XPS spectrum of the sulfur 2*p* line of sulfated industrial MCC (powder).

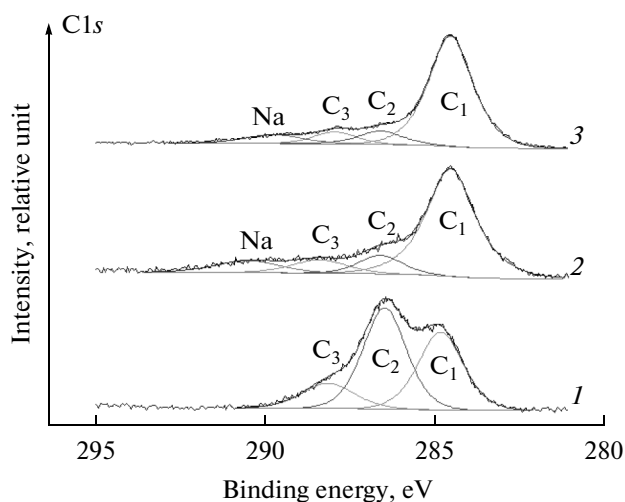


Fig. 7. XPS spectra of carbon 1*s* lines of initial wheat straw MCC (1) and sulfated wheat straw MCC (2, powder; 3, film).

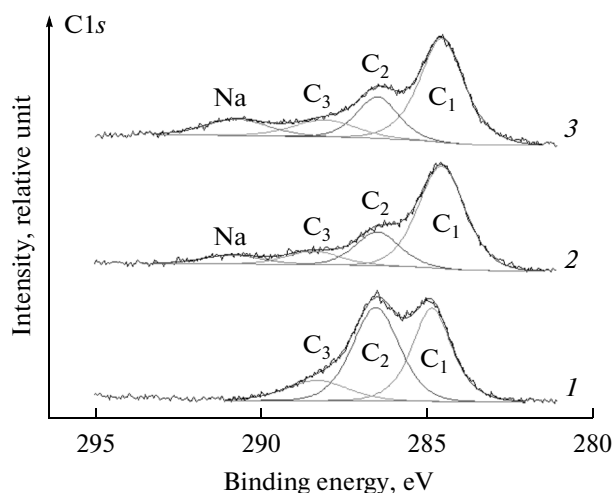


Fig. 8. XPS spectra of carbon 1*s* lines of initial asp wood MCC (1) and sulfated asp wood MCC (2, powder; 3, film).

The sulfation of MCC in a chlorosulfonic acid–dioxane system for three to four hours leads to a decrease in the contribution of the C₂ component as well as a shift of the C₁ component to 284.5 eV and a 1.5–2-fold increase in its intensity. This component can belong to carbon in both *sp*³- and *sp*²-hybridization and corresponds to the products of acidic destruc-

tion of cellulose and hydrocarbon contaminations on its surface. The greatest increase in the contribution of the C₁ component is observed in wheat straw MCC, which probably is related to a low content of the crystalline moiety in initial cellulose (degree of crystallinity 0.66). Owing to the dense packing, crystalline regions of a cellulose fiber are less accessible to the

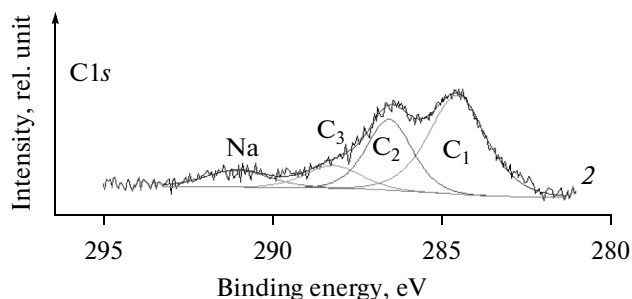
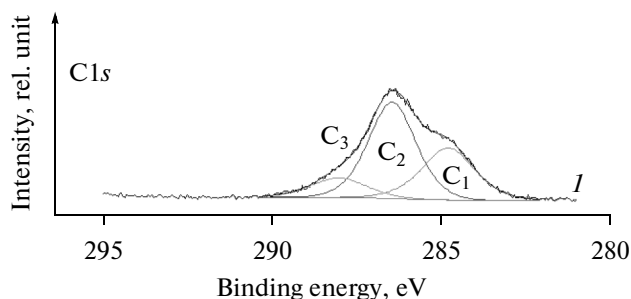


Fig. 9. XPS spectra of carbon 1*s* lines of initial industrial MCC (1) and a sample of sulfated industrial MCC (2).

Table 4. Decomposition of XPS carbon 1s lines

Sample	C ₁		C ₂		C ₃	
	eV	%	eV	%	eV	%
Wheat straw MCC	284.8	38	286.5	48	288.1	14
Asp wood MCC	284.8	41	286.5	46	288.3	13
Industrial MCC	284.8	33	286.5	54	288.0	13
Sulfate of wheat straw MCC	284.5	79	286.6	11	288.3	10
Film of wheat straw MCC sulfate	284.5	82	286.6	10	288.0	8
Sulfate of asp wood MCC	284.5	69	286.4	21	288.3	10
Film of asp wood MCC sulfate	284.5	64	286.4	22	288.0	14
Sulfate of industrial MCC	284.5	56	286.5	32	288.2	12

destructing agent [14], and amorphous regions of cellulose are most prone to degradation in acidic medium [15].

CONCLUSIONS

It was shown by XPS that the reaction of MCC with a sulfating agent results in the incorporation of the sulfate group into the structure of cellulose. The sulfur content is 4.0–5.6 at % in the surface layer of sulfated wheat straw MCC, 7.5–9.7 at % in the surface layer of sulfated asp wood MCC, and 7.9 at % in sulfated industrial MCC.

The spectra of sulfur 2p lines have a similar shape for all samples of sulfated MCC corresponding to the sulfate form. Other forms of sulfur were not detected.

Based on the comparative analysis of sulfur content in the bulk (chemical analysis) and on the surface (XPS), it was concluded that sulfur atoms are uniformly distributed in CSs from asp wood and industrial MCC, and the sulfur content on the surface of wheat straw MCC sulfates is low.

The differences in the shape of carbon 1s lines in the spectra of initial and sulfated MCC point to changes in the chemical state of a part of carbon atoms during sulfation. As a result of the decomposition of carbon 1s spectral lines, three forms of carbon being in the composition of different oxygen-containing groups were recognized.

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