

Infrared spectroscopy of copper-poisoned rat liver

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We presented the study of components in the liver of rats poisoned with the copper by infrared (IR) absorption spectra. Spectroscopic analysis of copper poisoned rat livers had been performed by the infrared absorption technique with frequency of 3000–4000 cm⁻¹. The spectral investigation allowed to trace the changes in the rat liver samples. We determined significant changes in lipids layer distortion between control and experimental samples at 1800–1000 cm⁻¹ frequency. Furthermore, we also registered definite changes for the CH layer distortion in the band of 3000–2500 cm⁻¹.

Keywords: Copper; Intoxication; Infrared spectroscopy; Liver; Rats

Introduction

Infrared spectroscopy is the study of the interaction between matter and electromagnetic radiation in the infrared region. This type of spectroscopy is used to study the vibrations of molecules; typically, the energy of molecular vibrations is the same as that of infrared light. Because of this, infrared spectroscopy can be used to determine molecular characteristics, such as bond length, bond angles, vibrational frequencies, and, indirectly, bond strengths. In addition, infrared spectroscopy is tremendously useful in determining the presence of certain characteristic groups in large molecules (Dumas et al., 2003; Stuart, 1997). Infrared spectroscopy can detect and monitor characteristic changes in molecular composition and structure. Spectroscopic techniques have been extensively studied as a potential diagnostic method that can provide information about both the chemical and morphological structure of tissue. The electromagnetic spectrum consists of radiation with very high energy and frequency and short wavelength (such as gamma and X-rays) and radiation with very low energy and frequency and long wavelength (such as radio waves). Visible light falls approximately in the middle of this spectrum. Infrared radiation has a lower energy than red light in the visible spectrum. Thus, infrared light lies in the energy range of about 0.5–9.5 kcal (2–40 kJ). The energy of most molecular vibrations also within this range. Molecular spectroscopy is very complicated because molecules contain two or more atoms. In fact, the more atoms there are in the molecule, the more complicated the interpretation of the spectrum can be. However, it is possible to simplify the interpretation of a molecular spectrum based on the types of movement of molecules and the energies required for these movements. Infrared spectroscopy is one of the standard working methods in semiconductor production, process control in chemical engineering, and environmental monitoring. It is relatively inexpensive, rapid, very sensitive, and able to provide quick and efficient information on chemical reactions detecting the characteristic vibrations of molecules.

The goal of our work was to characterize the changes in the copper poisoned rat livers.

Materials and Methods

The study was conducted on white male rats of the same age, weighing 180–200 g, kept under standard conditions of vivarium, with free access to food and water. We formed two groups of animals: an intact (control) and the experimental, where second animals were administered the solution of copper sulfate at a dose of 3 mg/kg. Intoxication was performed within 14 days, and then the rats were killed by decapitation using short-term ether anesthesia and the livers were removed. All working activity was carried out in accordance with European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. Spectroscopic studies were performed by Nicolet 380 FT-IR spectrophotometer (Thermo Scientific, USA). All spectra were obtained using the attachment on light reflection from the sample; the working wavelength range was 650–4000 cm⁻¹. These absorption spectra were processed by specially developed software. Each absorption band characterized the position in the spectrum, i.e., precisely defined maximum absorption frequency and intensity - width and height. Quantitative content of different types of secondary structure were calculated by expanding the total absorption spectra of the individual components and determined the percentage ratio for the area of each component relative to the total area under the curve. We also identified the component spectra in the class of Gaussian and Lorentzian curves by algorithm derivation.

Results and Discussion

The IR absorbance spectra in all investigated samples were within the range of 650-4000 cm^{-1} (Figure 1).

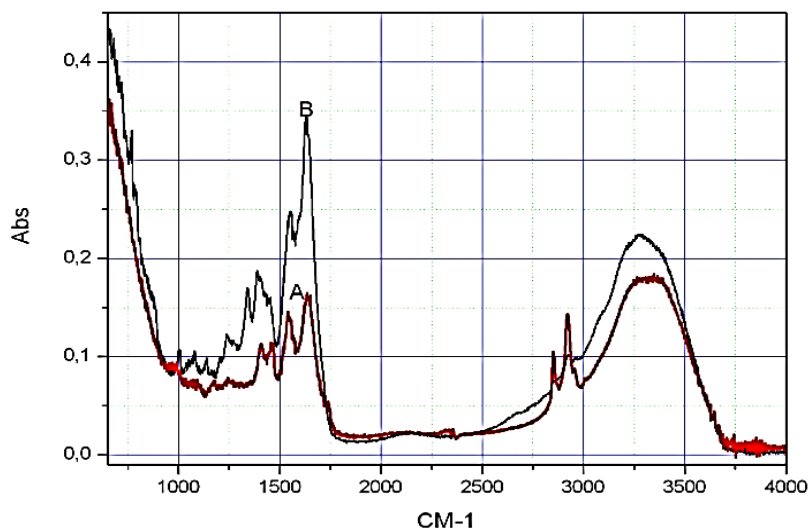


Figure 1. Experimental IR absorbance spectra of control rat livers in control group (A) and in group intoxicated with the copper sulfate (B).

The IR spectra of intact rat livers differed from the IR liver spectra in the experimental group and had specific absorption bands. In the studied range of frequencies the detected absorption bands had the peaks at wavenumbers of 1616 cm^{-1} , 1632 cm^{-1} , 1650 cm^{-1} , 1659 cm^{-1} , 1667 cm^{-1} , 1677 cm^{-1} , 1688 cm^{-1} , 1696 cm^{-1} , 2823 cm^{-1} , and 2911 cm^{-1} . Our results proved the presence of three different contributions: the first one was related to OH vibrations of tightly bonded H_2O molecules, the second one was strictly connected to the OH groups due to metabolic processes and the last one, at the highest frequency, was strongly distorted by asymmetric bonds due to OH vibrations belonging to H_2O molecules. It is well known that the hepatic alteration by chemical agents (in particular copper) can irreversibly damage the activity of enzymes, which are devoted to biotransformation processes (Plaa et al., 1994). In the frequency range from 1680 to 1670 cm^{-1} absorption band is the result of fluctuations in which change bond length of the carbonyl group. With these irregular fluctuations related areas in the structure of proteins. In this range, the spectrum recorded absorption bands with maxima at 1690 and 1696 cm^{-1} . In the range of 1670-1688 cm^{-1} the absorption components registered in the liver are caused by the presence of reverse bands in certain segments of the polypeptide chains (Backmann et al., 1996; Byler et al., 1986; Carpenter et al., 1989; Kong et al., 2007). So, all forms of secondary structures were available in the spatial structure of intact rat liver components - α -helix, β -fold parallel and antiparallel type, reverse turns and irregular or amorphous areas. Absorption bands of certain structures were partially overlapped. We registered that the liver proteins had β -type spatial configuration structures with a certain number of α -helical and disordered segments (Sasic et al., 2005; Surewicz et al., 1988; Susi et al., 1987; Wolpert et al., 2006). Below we presented the IR spectra in range of 1590-1710 cm^{-1} (Figure 2).

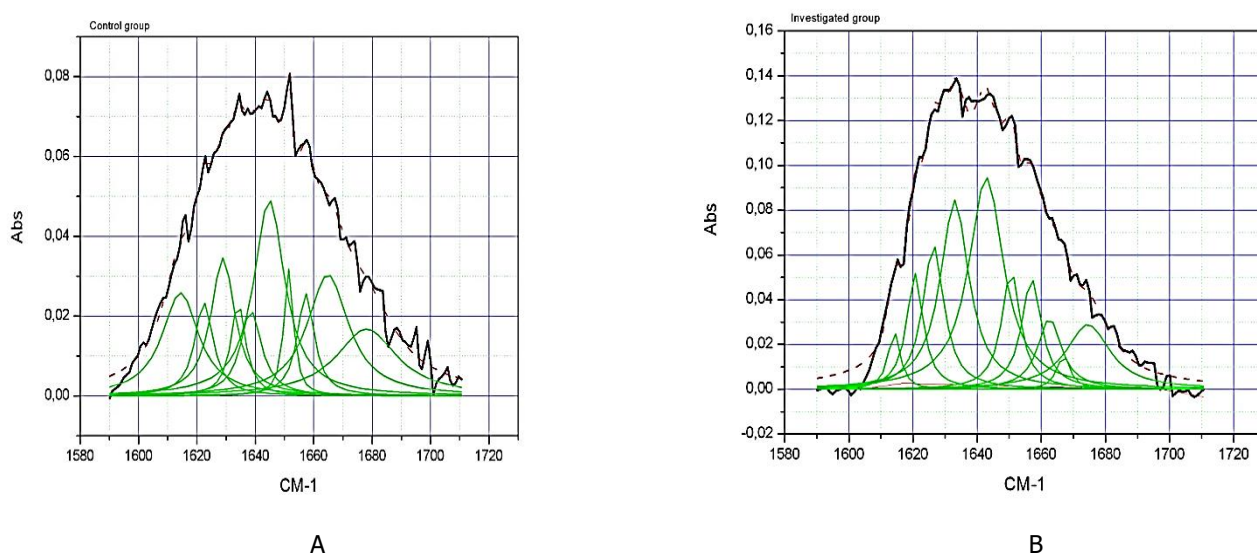


Figure 2. IR absorbance spectra in the control group (A) and experimental group (B) with Gaussian and Lorentz distributions.

For more in-depth qualitative and quantitative analysis of spectrograms law we applied the normal Gaussian distribution and the distribution of Lorentz where each complex curve with many overlapping lines can be decomposed into the sum of the individual curves. We also statistically proved the difference between the spectral characteristics of intact animal livers and livers intoxicated by the copper.

Conclusion

Infrared spectroscopy could be successfully used as a tool to study the changes occurring in rat liver after copper intoxication. We performed the infrared analysis on rat liver samples in a range of 650–4000 cm⁻¹. Our work characterized the damage caused by copper intoxication to the rat liver. We supposed that the IR absorption technique provided reliable and reproducible spectra, which can be used to separate normal and inquired specimens. We also believed that IR spectroscopy could enhance a quantitative analysis with fast characterization, good reproducibility, and possibility of examining unfixed samples with reduced dimensions. Our studies of intact rat liver IR spectra indicated the presence of various spectral characteristics. Moreover, we determined the increase or decrease in the integrated intensity along with the shift of absorption band maximums, which could be used in studies of heavy metal influences on the animal tissues.

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