A RAPID AND SENSITIVE PROCEDURE FOR DETERMINATION OF COPPER IN RUNNING WATER, URINE AND PLASMA BY ESR

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原　報

A RAPID AND SENSITIVE PROCEDURE FOR DETERMINATION OF COPPER IN RUNNING WATER, URINE AND PLASMA BY ESR

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水道水・尿・血漿中微量銅のESRによる迅速定量法

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Summary

The ESR was applied to quantitation of Cu^{++} in running water, urine and plasma. Diethyldithiocarbamate (DDC) reacted with Cu^{++} in a wide pH range from 1 to 10. The Cu^{++}-DDC complex could be extracted with many organic solvents at these pHs. Among organic solvents tested, 1-octanol was the most suitable solvent, which enabled condensation of Cu^{++} by 50 times with one extraction. By this method, we could measure Cu^{++} in 1.5 ml of either running water or urine and in 5 μl of plasma within 5 min. Bilirubin and anticoagulants such as EDTA, oxalic acid, succinic acid and heparin did not inhibit the reaction of Cu^{++} with DDC. Signals of other transition metals were not observed in the ESR spectrum of Cu^{++}, though most transition metals reacted with DDC. The Cu^{++}-DDC complex in 1-octanol was quite stable and was not decomposed for 3 days under room light at room temperature. Data of Cu^{++} levels in actual samples were also presented.

Key words: Copper; ESR; Diethyldithiocarbamate; Octanol; Urine ; Plasma

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Introduction

The concentrations of metals are usually measured by colorimetry and atomic absorption spectrophotometry. These methods, however, are not suitable for sensitive determination of Cu^{++}. For example, the absorbance at 480 nm of a copper complex with bathocuproine disulfonic acid is not so high($\varepsilon = 1.3 \times 10^4$) [1,2], and the atomic absorption at 324.7 nm specific to copper is also not high [3]; both methods require as much as 2 ml of plasma or serum for measurements of copper. Therefore, much more sensitive and specific methods are needed for measurements of much smaller amounts of Cu^{++} present in running water and urine.

The ESR method has been used in the present study, because Cu^{++} is paramagnetic, while Cu^{+} is diamagnetic. To maintain Cu^{++} stable, we have used a complex of Cu^{++} with diethyldithiocarbamate (DDC), which was originally used for colorimetric determinations of metals [4], and used later for condensation of metals in atomic absorption spectrometry. The optimal pH range and solvent composition have been also examined to increase sensitivity and specificity of the present Cu^{++} assay method.

Experimental

Materials

Small plastic tubes with caps, usually used for PCR and tolerable for pH range from 1 to 10 as well as for centrifugation at 13,000 rpm, were obtained from Eppendorf, Netheler-Hinz-GmbH, Hamburg, Germany; quartz hematocrit capillaries from Drummond Scientific Co., Broomall, PA., USA. $Cu(NO_3)_2$ in 0.1 N HNO_3 for atomic absorption was used for the standard Cu^{++} solution. All common chemicals used were of atomic absorption grade or biochemical grade from Wako Pure Chemical Ind., Ltd., Osaka. The putty for sealing the hematocrit capillary was obtained from Oxford Labware, St. Louis, MO, USA. The ultra-pure water having specific conductivity of 5.5×10^{-8} S cm^{-1} was used. All glassware and plastics were soaked in conc. HNO_3 and 0.3 N HNO_3, respectively, and rinsed with the ultra-pure water.

ESR conditions

The ESR measurements were made on a JEOL JES-FE2XG ESR spectrometer (Tokyo). The ESR conditions were: ESR spectrometer setting, 330 mT; sweep range, 100 mT; sweep time, 8 min; response time, 1 s; microwave power, 6 mW; and a modulation width, 2 mT. The peak height at the highest magnetic field of 335 mT was used for the quantitation of copper. It took about 30 s for the measurement.

Procedure

For measurements of Cu^{++} in urine or running water, 1.5 ml of the sample solution containing Cu^{++}, 15 mg of DDC Na and 30 μl of 1-octanol were placed in a small tube and mixed for 30 s using a vortex mixer. The mixture was centrifuged at 13,000 rpm for 2 min. A 5 - μl aliquot of the upper 1-octanol layer was drawn into a hematocrit capillary and both sides of the capillary were sealed with putty. It was inserted into a quartz ESR cell to place it into the ESR cavity, and the peak hight at 335 mT was measured. The total time required for each assay was within 5 min.

For measurements of Cu^{++} in plasma, 5 μl of plasma, 0.75 μl of 1 N HNO_3, 0.75 μl of 100 mg/ml DDC Na and 6 μl of 1-octanol were mixed using the vortex mixer, and the following procedure was the same as that for urine or running water.

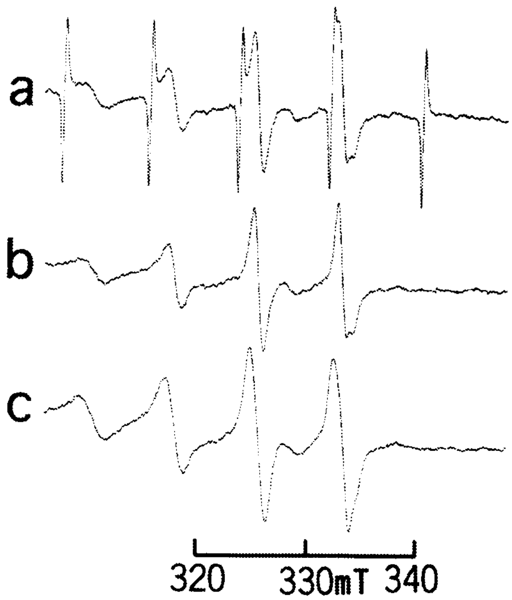

Fig. 1 . Determination of g value and hyperfine splitting of the Cu^{++}-DDC complex in 1-octanol. a is the ESR spectrum of the Cu^{++}-DDC complex produced from 5 μl of the standard Cu^{++} dissolved in the ultra-pure water at 2 ppm and extracted with 1-octanol, and measured together with an Mn^{++} signal to determine g value (the center of the signal) and hyperfine splitting (splitting between the lines). b and c are ESR spectra of the complex from 5 μl of Cu^{++} standard solution at 2 ppm measured with modulation width of 1 mT and 2 mT, respectively. Gain setting was 2.5×10^3.

Results and discussion

Optimization of conditions

Suitable conditions for ESR measurements were examined using Cu^{++} standard solutions. As shown in Fig. 1 a, Cu^{++}-DDC in 1-octanol shows a characteristic 4-line ESR spectrum with hyperfine splitting with an interval of 8 mT and with a g-value of 2.047. Thus the peak height at 335 mT was used for Cu^{++} quantitation.

As shown in Fig. 2, the Cu^{++}-DDC complex was made at several pHs ranging from 0 to 13.6 by dropping appropriate amounts of either 1 N HNO_3 or 1 N NaOH, and was extracted with 1-octanol completely. The complex showed good stability for a wide pH range. The complex extracted at pH 0, however, decreased to 90 % after 1 h and disappeared after 24 h. Cu^{++} precipitated in aqueous solution at pHs higher than 10. Since the suitable pH range was so wide that pH adjustment was not required in most cases for running water and urine. To liberate Cu^{++} from bound proteins in plasma, the pH of the solution had to be set below 5 [5]. HNO_3 was used to acidify plasma, because HNO_3 can change Cu^+ to Cu^{++}. The final pH values were 3.3 and 1.5 after addition of 0.1 and 0.15 ml of 1 N HNO_3 to 1 ml of plasma, respectively.

The Cu^{++}-DDC complex was extracted completely with most organic solvents, such as toluene,

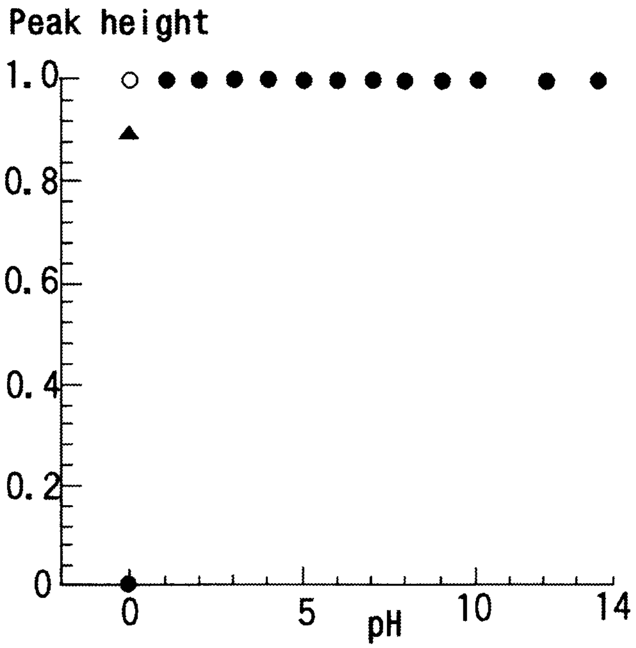

Fig. 2 . Effect of various pHs on the stability of the Cu^{++}-DDC complex in 1-octanol. The Cu^{++}-DDC complex was produced at pH indicated and extracted with 1- octanol. ○, ▲ and ● indicate the amounts of the complex soon after, 1 h after and 24 h after the extraction, respectively. Various pHs were adjusted by dropping appropriate amounts of either 1 N HNO_3 or 1 N NaOH.

Table 1 . Recoveries of the Cu^{++}-DDC complex extracted into various organic solvents as a function of water/solvent volume ratios

Solvent	Recovery % volume ratio of water : solvent		
	1 : 1	10 : 1	100 : 1
2-Butanol	100	0	0
1-Butanol	100	0	0
Cyclohexanol	100	70	0
1-Pentanol	100	80	0
1-Hexanol	100	100	60
1-Octanol	100	100	100
Toluene	100	100	100

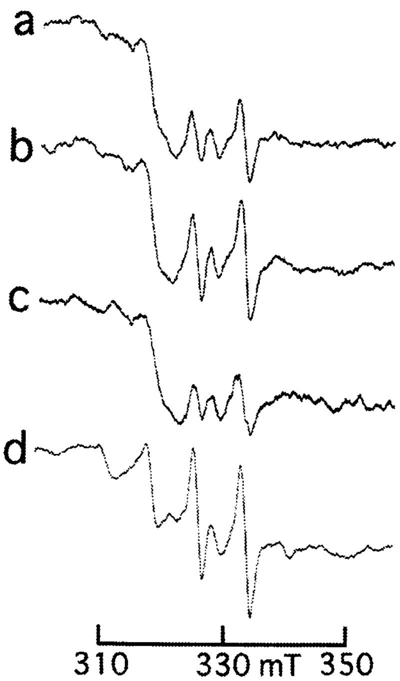

Fig. 3 . ESR spectra of the Cu^{++}-DDC complex in 1-octanol obtained from 1.5 ml of Cu^{++} standard solution at 10 ppb(a), 1.5 ml of running water(b), 1.5 ml ofhuman urine(c) and 5 μl of human plasma(d). Gain setting was 3.2×10^3. The Cu^{++} levels were concentrated 50-fold in a, b and c, and 0.83-fold in d,by the extraction with 1-octanol.

chloroform, pyridine, ethyl acetate and alcohols in a wide pH range. Alcohols were chosen, because they did not dissolve the putty used for sealing the capillary; toluene, chloroform, pyridine and ethyl acetate dissolved the putty. Table 1 indicates the comparison of alcohols suitable for condensation of the complex from dilute aqueous solution. To avoid the interference by salts, the extraction abilities of alcohols were compared without salts in the present work, though the addition of salts facilitates the extraction. 1-Octanol was found to be most suitable to enrich the complex 100-fold with one extraction. In the extraction from running water and urine, however, the volume ratio of water : alcohol = 50 : 1, was adopted, because the manipulation under this condition was easier than that at the ratio of 100 : 1.

Among dozens of chelators, DDC was bound to Cu^{++} so strongly that present method could be applied to plasma containing bilirubin or anticoagulants such as EDTA, oxalic acid, succinic acid and heparin; these compounds sometimes result in difficulty for colorimetric assays of Cu^{++} [1,2].

Most metals could form complexes with DDC and were extracted with 1-octanol. Some were diamagnetic and some were paramagnetic. Interfering peaks were not observed in the ESR spectra of Cu^{++} obtained from running water, urine, plasma and solutions of wet-ashed tissues.

Reliability of the method

The precision of the present method was evaluated by 10 repeated analyses for two plasma samples.

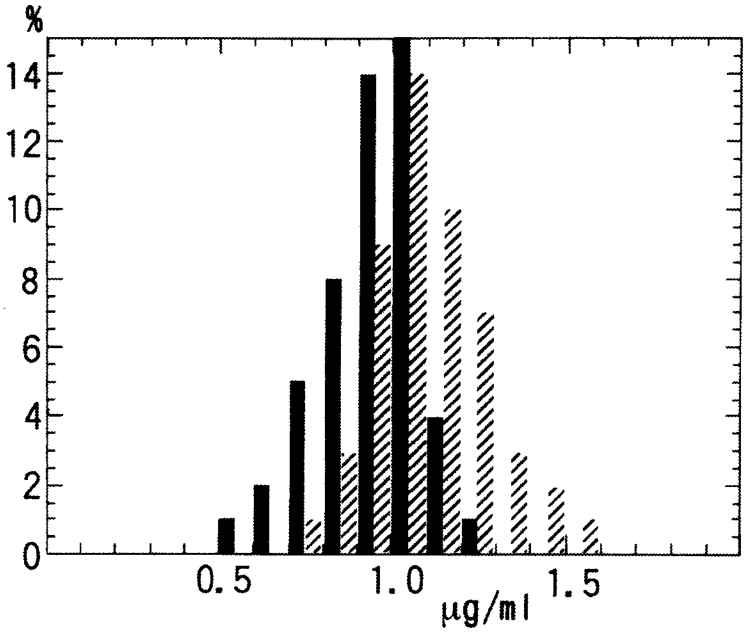

Fig. 4 . Frequency-distribution bar graph of Cu^{++} concentrations in male and female human plasma. Solid bars show data obtained from 50 males aged from 20 to 60, and shadowed bars, those obtained from 50 females of the same age range.

The within-run variation was less than 5 %. The recovery was examined by adding Cu^{++} at 0.5, 1.0, 1.5, 2.0 ppm to plasma with endogenous Cu^{++} content of 1 ppm; the recovery was more than 95 % in any of the four additions.

The Cu^{++}-DDC complex in 1-octanol was quite stable and not affected for 3 days under room light at room temperature.

Measurements of Cu^{++} in real samples

Figure 3 shows ESR spectra of the Cu^{++}-DDC complex in 1-octanol in some of real samples. In this figure, a, b, c and d show the spectra obtained from 1.5 ml of Cu^{++} standard solution at 10 ppb, 1.5 ml of running water, 1.5 ml of human urine and 5 μl of human plasma, respectively. The Cu^{++} levels had been concentrated 50-fold in a, b and c, and 0.83-fold in d, by the extraction with 1-octanol. The Cu^{++} concentrations were calculated to be 14 ppb, 7 ppb and 1.1 ppm for running water, urine and plasma, respectively.

As shown in Fig. 4, Cu^{++} contents in human plasma of healthy 50 males and 50 females(20 - 60 years old for both) were measured. The mean value with SD for males was 0.94 ±0.14 ppm and that for females was 1.10 ±0.17 ppm, which were in good agreement with reported values [1,2,6].

Conclusion

The present method seems very useful for analysis of copper in biological and environmental samples, because of its rapidness and high sensitivity.The present results suggest the possibility for the determination of other transition metals by ESR, if suitable chelators, pHs and organic solvents are found for them.

Acknowledgment

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