# Determination of Copper and Iron in Human Blood Serum by Energy Dispersive X-Ray Analysis

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Summary: Human blood serum has been analyzed by X-ray fluorescence spectroscopy utilizing the effect of background reduction by total reflexion of the incident X-ray beam on an optical flat as sample support. For sample preparation a drop of 10 $\mu$l serum was pipetted onto the support and dried to a thin film. The minimum detectable limit was about 1.5 mmol/l in 1000 s and the precision in the 20 mmol/l range of the metals was 3—5%.

*Bestimmung von Kupfer und Eisen in humanem Blutserum durch energiedispersive Röntgenfluoreszenzanalyse*

Zusammenfassung: Der Metallgehalt in humanem Blutserum wurde mittels energiedispersiver Röntgenfluoreszenzanalyse bestimmt, wobei zur Verminderung des Untergrundes der Effekt der Totalreflexion der anregenden Röntgenstrahlung an einem hochebenen Probenträger aus Quarzglas ausgenutzt wurde. Zur Probenherstellung wurde ein Tropfen von 10 $\mu$l Serum auf den Probenträger pipettiert und zu einem dünnen Film getrocknet. Die Nachweisgrenze lag unter 1,5 mmol/l in 1000 s und die Reproduzierbarkeit bei einem mittleren Metallgehalt von etwa 20 mmol/l betrug 3—5%.

## Introduction

The determination of iron and copper in human blood serum is an increasingly important tool for the diagnosis of certain cancers and hepatic and metabolic disorders. For this determination standardized techniques have been developed which are routinely used in the medical laboratories. The present paper describes a new method that requires no pre-treatment such as deproteinization, is element-specific and allows the measurement of several elements simultaneously. The technique may therefore become a valuable supplement in medical laboratory practice.



Fig. 1. Experimental setup for X-ray fluorescence analysis with totally reflecting sample support.

## Experimental

The measuring equipment was a preliminary experimental setup very similar to those used in previous work (1, 2, 3), but without vacuum. It utilizes the effect of total reflexion at the sample support for background reduction. We use a fine structure X-ray tube with molybdenum anode together with a power supply DEBYEFLEX 2002 from SEIFERT, goniometer with a holder for the sample support (SEIFERT), and slits, diaphragms and filters for collimation and background reduction in the geometric arrangement shown in figure 1.

The incident X-ray beam strikes the surface of the sample support, an optical flat made of SUPRASIL quartz glass with dimensions 50 × 30 × 15 mm, at an angle of about 6 minutes of arc. The sample is deposited in the center of the support surface. Immediately above the sample (about 1 mm) a diaphragm
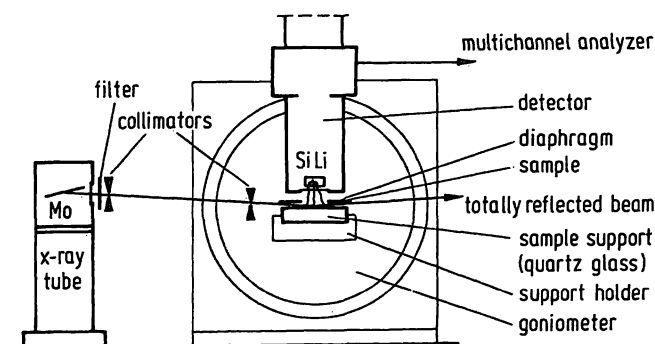
with an inner diameter of 8 mm is attached to the sample holder. The fluorescence radiation is analyzed by a Si(Li) detector located at 10 mm from the sample and connected to standard electronics. A typical serum spectrum is shown in figure 2.

In contrast to the conventional XRF technique (4) the used method needs practically no sample preparation. 10 $\mu$l of serum were pipetted onto the cleaned surface of the glass block and allowed to dry in the air.

The main application of the X-ray fluorescence analysis with totally reflecting sample support is the determination of trace elements in aqueous solutions or organic extracts with a low content of dissolved matter. In these cases the sample residue on the support after drying is in the range of 0.01 of the original volume and only a few $\mu$m thick. Human blood serum, however,
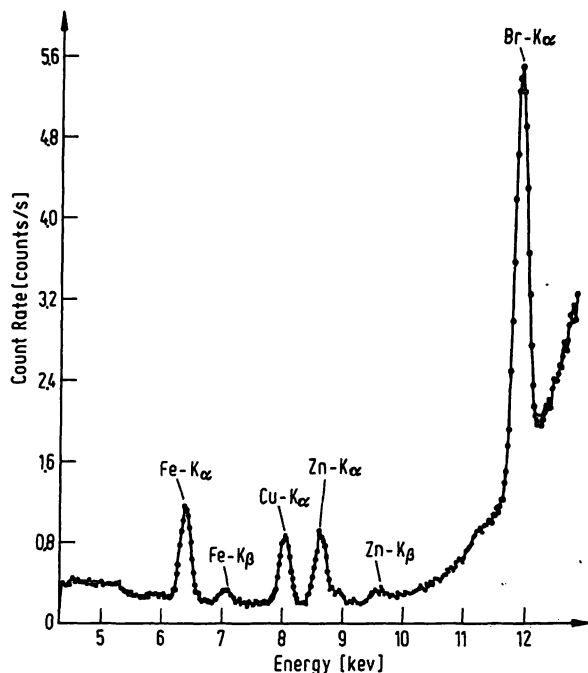
Fig. 2. X-ray fluorescence spectrum of human blood serum, containing 35.3 mmol/l iron, 13.2 mmol/l copper, 13.3 mmol/l zinc, 42.6 mmol/l bromine.

contains about 100 g/l of proteins, lipids and minerals leading to films of 20–50 μm thickness. Taking into account the simplicity of the drying procedure, it cannot be excluded that there were films with humps and marginal ridges up to 200 μm (freeze-drying, e. g., gave much better films). Due to the very small angle of the incident beam we have to face paths of the exciting X-rays up to the mm range in the dried serum which may cause shading effects. It may also happen that certain parts of the sample are not hit by the strongly collimated beam. It is therefore necessary to determine to what extent the sample is actually penetrated by the incident radiation. We found that in this technique, as in conventional XRF, the height of the *Compton* peak is proportional to the sample mass seen by the incident X-rays. Hence, the ratio of metal peak to *Compton* height is proportional to the concentration of metal atoms in the dry substance of the serum and this ratio is independent of film shape and possibly imperfect pipetting. Since the metal concentration of the whole serum is usually requested, the metal peak to *Compton* height ratio was multiplied by the dry fraction. The dry fraction can be easily determined by weighing a small volume of serum (0.5 ml) before and after drying to an accuracy better than 0.5 percent. So all concentrations given in this paper apply to the whole sera.

The calibration was done with commercial control sera which are generally used in medical laboratories for accuracy control. Their iron and copper concentrations are not known to the accuracy desired for our purpose. Nevertheless, in order to avoid systematic errors we did not try to prepare standards by simulating a serum matrix and adding known amounts of metal salts.

## Results

The results of the measurements are given in table 1 and 2. Plots of the calibration of the X-ray spectrometer are shown in Figures 3 and 4, where the solid lines were calculated by least squares fitting. The blank values at 0 mmol/l were caused by the apparatus and could be experimentally confirmed by measuring a metal-free aqueous sodium diethyldithiocarbamate solution with a content of 83 g/l.

Tab. 1. Results of measurements of copper in serum.

| Serum name | Mean value[f) (mmol/l Cu) | Acceptable range[f) [g) (mmol/l Cu) | Measured value (mmol/l Cu; $\bar{x} \pm s$[k)) |
|---|---|---|---|
| Individual serum[e) | 12.6 | – | 13.2 ± 0.3 |
| Monitrol II[c) | 16.4 | 14.6 – 18.1 | 15.3 ± 0.3 |
| Precilip[a) | 18.6 | 15.6 – 21.6 | 17.6 ± 0.2 |
| Kontrollogen-L[b) | 19.7 | 17.5 – 21.9 | 18.9 ± 0.5 |
| Monitrol I[c) | 20.5 | 17.9 – 23.0 | 22.3 ± 0.2 |
| Monitrol X[d) | 23.0 | 20.1 – 25.8 | 23.3 ± 0.5 |
| Precinorm-S[a) | 24.7 | 20.9 – 28.5 | 25.2 ± 0.8 |
| Fluinorm-P[b) | 25.5 | 22.7 – 28.3 | 24.2 ± 0.5 |
| Fluinorm-N[b) | 32.3 | 28.3 – 36.2 | 30.4 ± 1.1 |
| Cation-Cal[c) | 34.8 | – | 36.7 ± 2.5 |
| Precipath-S[a) | 78.8 | 71.0 – 86.7 | 78.7 ± 1.6 |
| "R1"[h) | 14.9[i) | 12.7 – 17.8[j) | 13.8 ± 0.3 |
| "R2"[h) | 18.6[i) | 15.3 – 22.8[j) | 16.7 ± 0.3 |

[a) Boehringer Mannheim GmbH
[b) Behringwerke AG/Marburg-Lahn
[c) DADE/Miami Fla. 33152, U.S.A.
[d) Natural mixed serum
[e) One author's serum, analysed by clinical laboratory Dr. Kramer/Geesthacht
[f) Manufacturer's specification
[g) Twice the standard deviation
[h) Measurements of two unknown sera in the course of an intercomparison test (Sept. 1976, 226 laboratories)
[i) Reference value
[j) Acceptable range indicates here the required accuracy to stand the test
[k) at least five determinations over a period of two weeks.

Tab. 2. Results of measurements of iron in serum.

| Serum name | Mean value[f) (mmol/l Fe) | Acceptable range[f) [g) (mmol/l Fe) | Measured value (mmol/l Fe; $\bar{x} \pm s$[k)) |
|---|---|---|---|
| Monitrol II[c) | 12.4 | 10.9 – 13.8 | 13.6 ± 0.5 |
| Kontrollogen-L[b) | 13.3 | 10.4 – 16.1 | 14.9 ± 1.1 |
| Monitrol I[c) | 15.8 | 13.4 – 18.8 | 15.4 ± 1.1 |
| Precinorm-S[a) | 18.1 | 15.4 – 20.8 | 14.5 ± 0.7 |
| Precilip[a) | 23.8 | 20.1 – 27.6 | 25.6 ± 0.5 |
| Fluinorm-N[b) | 27.4 | 25.1 – 29.7 | 24.2 ± 0.5 |
| Fluinorm-P[b) | 30.1 | 27.8 – 32.4 | 29.4 ± 0.7 |
| Cation-Cal[c) | 35.5 | – | 34.0 ± 0.2 |
| "R1"[h) | 32.4[i) | 28.8 – 36.7[j) | 33.8 ± 0.4 |
| "R2"[h) | 19.9[i) | 17.7 – 22.2[j) | 18.8 ± 1.3 |

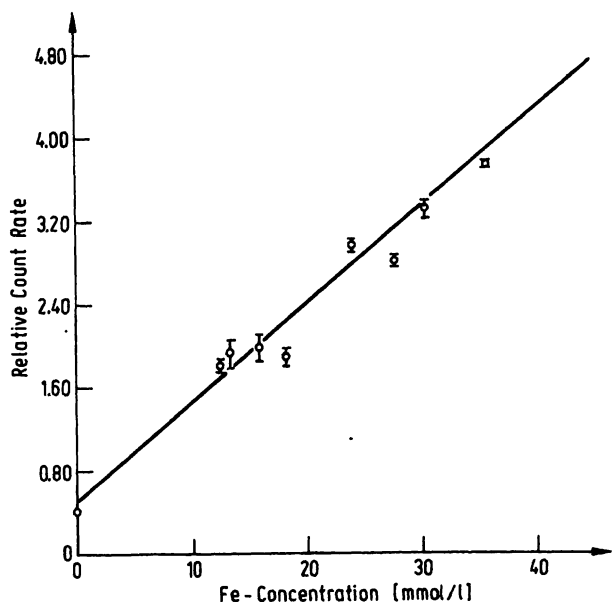[a) – [k) For footnotes see tab. 1.

Fig. 3. Calibration line for iron of the X-ray spectrometer obtained with the aid of standard sera.
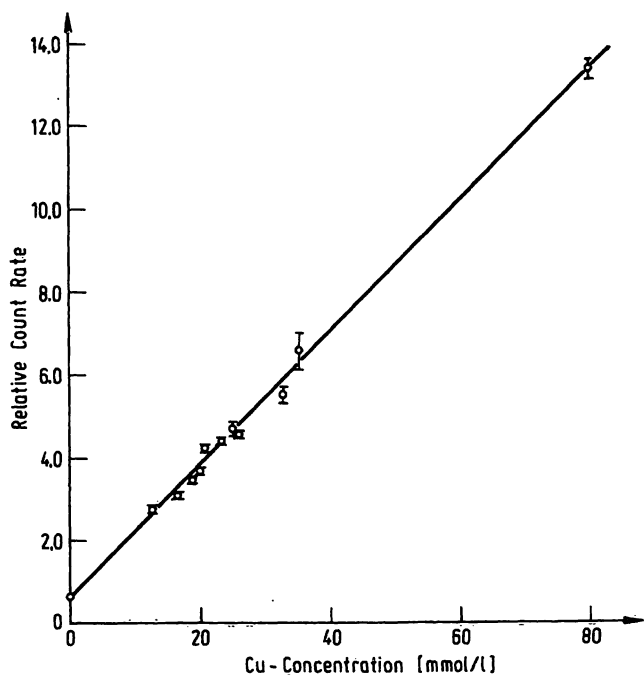


Fig. 4. Calibration line for copper of the X-ray spectrometer obtained with the aid of standard sera.

The reproducibility, i. e. the standard deviation of at least five samples of the same serum, over a period of about 2 weeks was about 3% for copper and 5% for iron using a measuring time of 1000 s.

The accuracy of a calibration with imperfect standards can be estimated by regression calculations. The resulting standard deviation S of a measured concentration c was evaluated as follows (5):

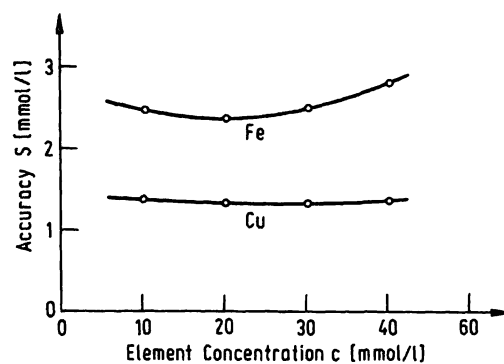$$S^2 = \frac{SQR}{n-2} \cdot (\frac{(c - c_m)^2}{Q} + \frac{1}{n} + 1)$$

Fig. 5. Accuracy of the determination of iron and copper in blood serum based on the standards used in this work.

with

SQR = sum of the squared residuals of the standard concentrations $c_i$

n = number of standards

$c_m$ = mean concentration of the standards

Q = $\Sigma(c_i - c_m)^2$

The above defined standard deviation S for iron and copper with the set of standards used is plotted in figure 5.

The conventional criterion of statistical detectability, where the minimum peak area is three times the square root of the background counts under the peak, would give 0.9 mmol/l for iron and 0.6 mmol/l for copper in 1000 s.

## Conclusion

The measurements were performed with a quite preliminary setup not optimized with respect to some important parameters, e. g., background, collimation and adjusting possibilities. An apparatus with automatic sample changer and mechanical improvements for routine applications is under construction (6).

In the X-ray spectra (fig. 2) of all analyzed sera a zinc peak was also present with an intensity comparable to that of copper. In some cases bromine, mercury and lead were conspicuous. These elements can be determined with practically the same precision and sensitivity as iron and copper in one measurement. It should be pointed out that the essential biological metal zinc is not yet routinely determined in blood serum in the clinical laboratory practice, although there is growing evidence that zinc, too, can be very important in the early diagnosis of certain diseases including cancers. (K.-D. Voigt, personal communication).

## Acknowledgements

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