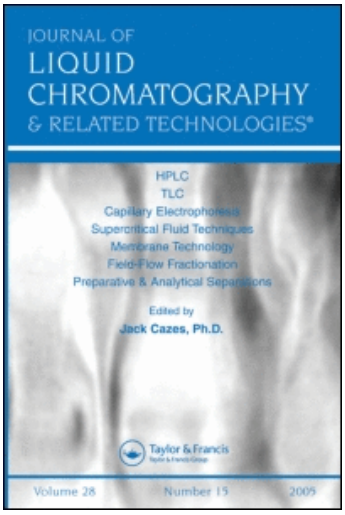

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Atomic Absorption Spectrophotometry as a Chromatography Detector for Copper-Amino Acid Complexes in Human Serum

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ATOMIC ABSORPTION SPECTROPHOTOMETRY AS A CHROMATOGRAPHY DETECTOR FOR COPPER-AMINO ACID COMPLEXES IN HUMAN SERUM

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ABSTRACT

In this paper an automatic sampling device is used for the electrothermal atomic absorption detection of copper-amino acid complexes in human serum after separation on an ion exchange chromatographic column. Adsorption chromatography on a silica gel column is used to separate the major naturally occurring copper amino-acids, copper-histidine and copper-glutamine, with subsequent detection by electrothermal atomic absorption.

INTRODUCTION

Compared to amino acids, information available on metal-amino acids, their possible physiological roles and analytical methods for their determination is very scant. Possible reasons for lack of data in this field include: the instability of these complexes during extraction from a biological matrix, and the low concentration of these complexes in the biological matrix. This latter demands a method with very low detection limits and a high degree of specificity.

A combination of separational techniques, for example, high pressure liquid chromatography, solvent extraction and ion exchange chromatography are now being utilized to study metal amino acids. Of all the metal-amino acid complexes, copper-amino acids have been studied in the greatest detail because of their well known biological significance.

Hallman, Pervin and Watt (1) have computed the equilibrium distribution in human blood plasma. Some aspects of extraction of copper-(II) complexes with amino acids (glycine, alanine, asparagine, lysine, valine and amino-butyric acid) have been studied by Karczynski (2). The effects of various copper amino acid complexes on the growth and survival of chicks have also been reported (3).

Separation of copper-amino acids has been attempted by various techniques. Glycine and alanine were isolated from certain synthetic copper-amino acid complexes in 75.2-99.0% yield by passing an aqueous solution of the mixture over the H-form of an ion exchanger (4).

Thin-layer chromatographic separation on Kiesulguhr plates of copper-(II) complexes with certain α-amino acids was achieved in aqueous butanol-propanol (5,6). The authors of these reports could not confirm the presence of mixed complexes, i.e. having two different amino acid ligands. Earlier, Sarkar and Kruck (7), also

using thin-layer chromatography on silica gel and using 60% (v/v) acetone in water as the solvent, were able to separate copper-histidine, copper-threonine and copper-glutamine and confirm the presence of histidine-copper-threonine in normal human serum.

In the present work, atomic absorption spectroscopy is used as a detector for ion exchange and adsorption chromatography studies of copper-amino acids. Because atomic absorption is metal specific, it yields relatively simple chromatograms which contain peaks only of the compounds of the metal of interest. Thus in complex systems such as human serum this approach could lead to a method which is fundamentally much simpler than any procedure presently employed.

The compounds copper-EDTA and copper-trien have been included with the copper-amino acid complexes since EDTA and trein are commonly administered chelating agents for patients suffering from elevated copper levels.

EXPERIMENTAL

A Perkin-Elmer Model 603 atomic absorption spectrophotometer was used with a Perkin-Elmer Model 56 recorder and a deuterium arc background correction system. An electrothermal atomizer, the HGA 2100, was used together with a Perkin-Elmer AS-1 auto sampling system. The equipment is shown in Fig. 1.

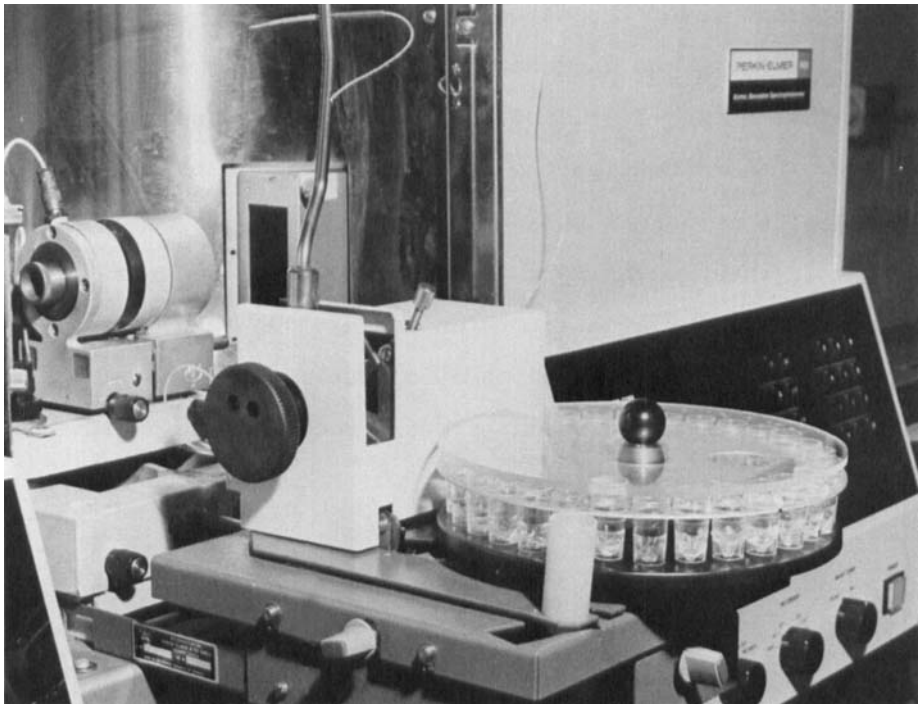

FIG. I.

A PERKIN ELMER MODEL 603 ATOMIC ABSORPTION SPECTROPHOTOMETER EQUIPPED WITH A PE HGA-2100 GRAPHITE FURNACE AND A PE AS-1 AUTO SAMPLING SYSTEM.

The pH determinations were carried out with Orion Research analyzer Model 801.

Ion Exchange Chromatographic separation of copper-amino acid complexes

Ten-ml burettes were used as chromatographic columns. Bio-Rex 70, a weakly acidic cation exchange region, in

the sodium form (Bio Rad Labs), was soaked overnight in water and then packed into a column as a slurry in water. The resin length was adjusted to be about 8.0 cm when equilibrated in water (the column effluent shows a pH of 6-8).

A mixture of copper-EDTA, copper-trien and copper-amino acid solutions (25-µl) was applied to the column in water. The column effluent was collected in plastic sampling cups (capacity about 1.5 ml) which were then

TABLE 1

Atomic Absorption Operating Conditions

Slit:	4 (0.7 nm)
Integration time:	0.5 seconds
Expansion:	As indicated in the figures
Wavelength:	324.7 nm (copper)

Furnace Operation

	Temperature	Time (seconds)
Drying:	100°C	20
Charring:	700°C	30
Atomizing:	2500°C	10

Recorder Operating Conditions

Range:	1 volt
chart speed:	20 mm min^{-1}

placed in the automatic sampling device to be analyzed by electrothermal atomic absorption. The same procedure was used for the separation of copper-amino acid complexes from human serum (35-ml applied on the column).

Copper-EDTA comes off with a water rinse. The solvent was then changed to 2M ammonium nitrate, which elutes copper-trien, followed by 4M nitric acid which elutes the copper-amino acid complex.

Adsorption chromatography of copper-amino acid complexes

Silica gel (100-200 mesh, ASTM D1319-61T, grade 923, Fisher Scientific Co.), prepared as a slurry in an acetone-water mixture (60-40, v/v, pH 7-8), was packed in the column as evenly as possible to a length of 24 cm. A flow rate of 0.40 ml min^{-1} was maintained throughout the separation. A mixture of copper-histidine and copper-glutamine (0.25 ml of the mixture) was applied to the column using a specially modified Mohr pipette. The buret was then topped up with the acetone-water solvent, and maintaining a flow rate of 0.40 ml min^{-1}, one-ml fractions were collected in the plastic cups. These were subsequently placed in the automatic sampler for electrothermal atomic absorption.

Copper-histidine and copper-glutamine are chosen for this separation since these two complexes, together with copper-threonine, constitute the major copper-amino acid complexes in serum (8).

RESULTS

Fig. 2 shows the separation of copper-EDTA, copper-trien and copper-histidine by ion exchange chromatography with subsequent detection by electrothermal atomic absorption.

The peak showing the copper-amino acid group from normal human serum is superimposed over the standards in Fig. 3. Identification of this peak is based on the similarity of R_f value compared to standards separated

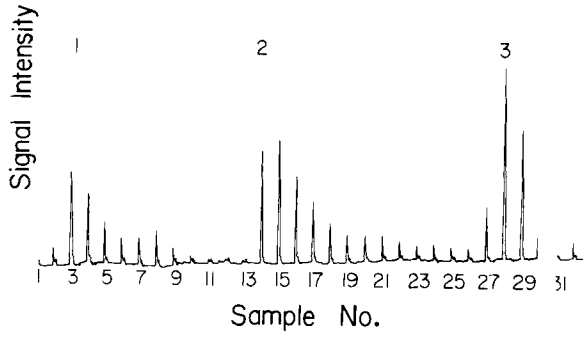

FIG. II:

THE SEPARATION OF COPPER-EDTA (PEAK 1), COPPER-TRIEN (PEAK 2) AND COPPER-HISTIDINE (PEAK 3) ON A WEAK ACID CATION EXCHANGE COLUMN USING DETECTION BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROPHOTOMETRY.

Sample size: 25-µl

Expansion: 0.1

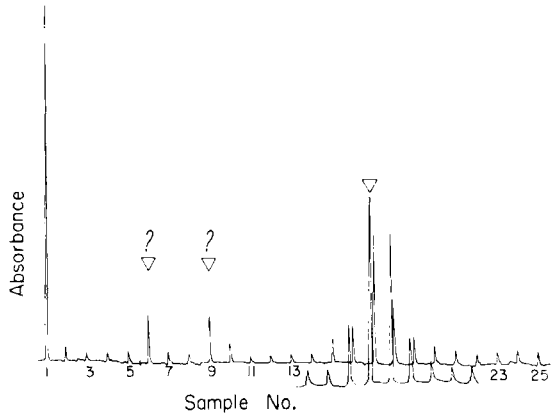

FIG. III

THE SEPARATION OF THE GROUP COPPER-AMINO ACID COMPLEXES FROM HUMAN SERUM ON A WEAK ACID CATION EXCHANGE COLUMN USING DETECTION BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROPHOTOMETRY.

Sample size: 35-ml

Expansion: 10

under the same chromatographic conditions. Retention times were computed by using the area under the curve in real time. Agreement between the serum amino acid peak and the standard, referring to Fig. 3, has a standard deviation of less than 5%.

Quantitatively it was determined that the copper-amino acid group constitutes about 0.6% of the total serum copper. This value compares well with the

acceptable range of 0.4-1.0% (12). Fractions 6 and 9 (Fig. 3) contain copper; this is certain since the atomic absorption detector is metal-specific. These absorptions signify the presence of two additional copper complexes in the serum. The nature of these compounds will be the subject of further studies.

Fig. 4 shows the separation of copper-histidine and copper-glutamine on a silica gel column with subsequent detection by electrothermal atomic absorption.

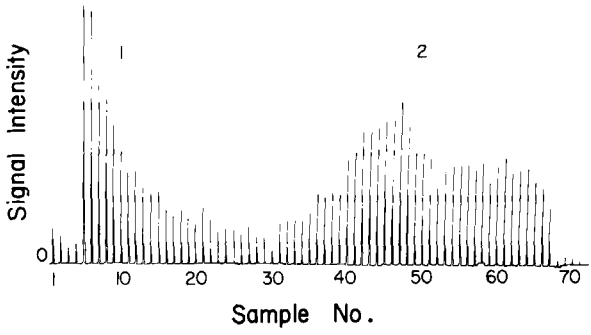

FIG. IV:

THE SEPARATION OF COPPER-HISTIDINE PEAK 1 AND COPPER-GLUTAMINE PEAK 2 FROM AN AQUEOUS MIXTURE ON A SILICA GEL CHROMATOGRAPHY COLUMN USING DETECTION BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROPHOTOMETRY.

Sample size: 0.25-ml

Expansion: 1

Sarkar and Kruck (7) have separated copper-amino acids which included copper-histidine and copper-glutamine, from serum by thin-layer chromatography on silica gel. The amino acid spots were located by spraying the plates first with 2M HCl and then with ninhydrin. Copper was located by spraying with 0.015% zinc dibenzyldithiocarbamate in carbon tetrachloride.

It must be noted that the chromatograms illustrated in the figures appear to have an excessive degree of tailing compared to conventional chromatography. This is an artifact due to the necessary use of an auto sampler in flameless atomic absorption. The latter device gives signal peaks for individial 1 ml aliquots of sample, separated by a 7 mm length of baseline which is the built-in recorder time between samples. To illustrate, with reference to Fig. 2, the Cu-trien peaks represents true peak width of less than 1 min., real time.

CONCLUSION

Ordinary ion exchange chromatography consumes ten times more sample than high pressure liquid chromatography to give an equivalent signal on an atomic absorption instrument. However, ordinary ion exchange chromatography can be used in any laboratory without great expense. The above results show that this simple approach, using atomic absorption as a detector, is

satisfactory for the separation and detection of amino acids in a complex system.

Electrothermal atomic absorption improves the detection limit by almost 100 times, compared to the more conventional flame atomizer. For clinical investigations this is most beneficial since components of interest are usually present at the parts per billion level or less. Also, since the column effluent is not directly interfaced to the instrument using electrothermal atomic absorption, any appropriate column flow rate can be employed. This is very useful when a very slow flow rate is essential to achieve a complete separation of the copper-histidine and copper-glutamine complexes.

Another major advantage of using electrothermal atomic absorption as a detector is that only 10 or 20-μl of the sample solution is essential for analysis, while the rest of the sample solution in the sampling cup is still available for further analysis of compounds of other elements if required.

The simplicity of the proposed system must be emphasized. It is our experience that the proposed approach can be used successfully by relatively unskilled technicians.

Future work will be directed towards achieving the separation of individual copper-amino acid

complexes by high pressure liquid chromatography with subsequent detection by electrothermal atomic absorption. It is hoped that the consistency and reproducibility of the results will be further improved by a modification to allow automatic collection of the column effluent into the sampling cups of the AS-1 auto-sampler. By this modification collection of the column effluent followed by the determination of each fraction by electrothermal atomic absorption will be performed automatically.

Future attempts will be directed towards the separation and determination of other individual copper-amino acid complexes from serum. Work will also be done on employing the metal specific atomic absorption-chromatographic system for the separation and for the detection of other metal-amino complexes or other metal-organic compounds which may be of interest in the serum. In this regard, a study has already been initiated on the mechanism of aluminum transport to the brain. Aluminum deposition in the brain is thought to be a major factor in senility.

ACKNOWLEDGMENTS

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SUMMARY

An automatic sampling device is used for the electrothermal atomic absorption determination of copper-amino acid complexes in human serum after separation on an ion exchange chromatographic column. Adsorption chromatography on a silica gel column has been used to separate the major naturally occurring copper amino-acids, copper-histidine and copper-glutamine, from an aqueous mixture with subsewuent detection by electrothermal atomic absorption.

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