SPECTROCHEMICAL COBALT, MOLYBDENUM AND COPPER DETERMINATIONS IN PLANTS

by

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I. INTRODUCTION

Spectrochemical analysis is one of the few possible methods for determining trace elements in plant samples, and in other biological material, soils and soil extracts, etc. In this paper the elements considered are restricted to Co, Mo and Cu, which elements are present in oven dry plant material for about $5 \cdot 10^{-5}$, $2 \cdot 10^{-4}$ and $10^{-3}\%$ respectively. The analysis method dealt with may also be used for the other enumerated kinds of samples and as a general method regarding elements and samples. It has been extensively treated elsewhere[1]. In many aspects the pioneering work of MITCHELL c.s. was followed [2, 12, 13, 14]. Additional and differing aspects will be treated below.

II. LIGHT SOURCE, OPTICS AND METHOD OF INTENSITY MEASUREMENT

1. Light source

After a suitable pretreatment (see III) the content of the concerned elements in the sample amounts to about $10^{-2}\%$ (except for a case touched in III. 4).

It has been checked that only the carbon arc cathode layer [3, 4] and the so-called "high voltage a.c. arc*" [5, 6] — which essentially is a cathode layer arc over its whole length — possess a sufficient sensitivity for an accurate trace element determination in the content range concerned. This sufficient sensitivity arises from the favourable line to background ratio in the spectrum of the cathode layer light. In the experiments for the carbon arc cathode layer the sample was introduced in the hollow, lower electrode as a dry powder; for the high voltage arc the sample was present as a thin film of salts after evaporation of a solution on the top of the flat lower electrode.

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* It had better be called "small electrode distance arc".

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As the result of the orientation the carbon arc cathode layer has been preferred for its simpler design, while it is also less dangerous in handling. For details of the arc arrangement literature[1,2] may be consulted.

2. Spectral apparatus

As a spectral apparatus a Hilger Large Quartz spectrograph was applied. Because of its high resolving power the favourable line to background ratio of the carbon arc cathode layer is well retained. A high resolving power is desirable because many iron spectral lines are present, coming from some percents of iron in the pretreated sample (see III).

A prism spectrograph is more preferable than a grating spectrograph for its great luminosity. In a provisional experiment the estimative luminosity ratio of a large prism and a 2 m grating spectrograph appeared to be ca. 50 (the surfaces of the side wall of the prism and of the grating were 50 and 20 square cm respectively).

A quantitative analysis has been carried out of the influence of temperature on the adjustment of a large size Littrow type spectrograph. It showed that the only significant change in adjustment is caused by two effects in the quartz prism. Firstly, the refractive index is dependent on temperature and, secondly, the refracting angle decreases at increasing temperature because of the anisotropy of quartz. These effects result in a shift of the spectral line of ca. 0.005 cm/°C. The theoretical line halfwidth on the plate amounts to ca. 0.003 cm. Comparison of both values shows that the effect is troublesome for photographic work with very long exposures and for series of photoelectric measurements, unless the temperature is kept constant.
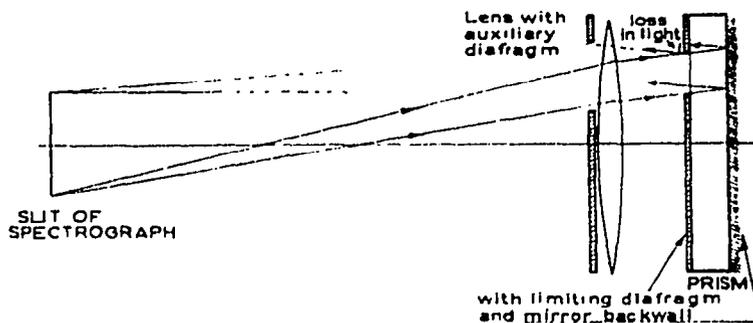
3. Optics

To separate the cathode-layer light from the light emitted by the rest of the discharge the arc was larger imaged on a diaphragm. To have a short light path we have erected that diaphragm inside the spectrograph. In this way the required homogeneous slit illumination (see II. 4) is obtained simultaneously.

It must be noted that a homogeneous slit illumination does not always give the wanted homogeneous spectral line on the plate*. The homogeneous line is only obtained when the light of the separated layer entering the slit is nowhere in the spectrograph prevented in reaching the plate**. For this purpose it was thought preferable to erect in the used Littrow type spectrograph the previously

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* A spectral line is called homogeneous when the (maximum) photometric density of the photographed line is independent of the place in the longitudinal direction of the line.
** An inevitable loss in light as a consequence of reflection at the quartz surfaces is left out of account.

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Fig. 1. Showing an exaggeration of the inevitable (minimum) loss in light when the diaphragm is erected just in front of the prism.

mentioned diaphragm just before the prism side wall and not before the collimator lens. An auxiliary diaphragm may be present on the lens to avoid unwanted reflection light; its opening, however, must be so large that it does not limit the light path.

When all mentioned points are observed a small inevitable inhomogeneity remains in the line on the plate. It arises from the fact that in a Littrow type spectrograph the light passes in its forward and backward way the diaphragm limiting the light path. The diaphragm cannot coïncide with the mirror behind the prism and therefore prevents a part of the light, reflected by the mirror, from reaching the plate, as it is illustrated in Fig. 1.

The deviation from homogeneity can be calculated and measured. When the height of the opening in the diaphragm is 1 cm, the deviation amounts to about 3% when this limiting diaphragm is erected in front of the prism. The deviation increases when the mentioned diaphragm is more removed from the mirror back wall of the prism, e.g. it amounts to about 10% when the diaphragm is erected in front of the collimator lens.

It is desirable to have the homogeneity of the spectral line checked at intervals, since it may be disturbed by small disarrangements in the optics and the slit of the spectrograph.

When a spectral region of larger extent (e.g. 1000 A) is observed, an achromatic lens is required for the focussing of the source on the diaphragm. This image must be very sharp, otherwise errors far beyond the mean error of the method (see IV. 3) are introduced, at any rate in the case of a very inhomogeneous light source as a cathode layer*. It is not necessary to place this imaging lens just in front of the spectrograph slit. This is even undesirable when the lens shows small irregularities, as a quartz-fluorite achromatic lens often does.

4. Plate calibration

It was convenient to start the research with the photographic plate as a detector. A photoelectric measurement method is in preparation now[7].

In agreement with MITCHELL c.s.[2] the blackening curve separation method[8,9] has been chosen for the determination of relative intensities in the spectrum.

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* Quantitative data: page 59 of the first reference.

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This method was preferred in our case to the method of the precalibrated iron lines[10] and the line width method[11]. Other methods were not to be considered.

The choice of the method of the blackening curve separation implies the requirement of a homogeneous illumination of the slit, in front of which a rotating step sector has been placed.

In the plate choice a great number of considerations play their part (*e.g.* desirable three minutes exposure time given by complete evaporation of the sample, luminosity of spectrograph after diaphragmation, trace element content range to be considered, etc.). It was a lucky chance that all the requirements could be met by application of a usual plate (*e.g.* Gevaert Normal or Ilford Special Rapid).

III. CHEMICAL PRETREATMENT

1. Scopes of pretreatment

For biological samples it is necessary in most cases to apply a pretreatment to obtain the material in a suitable state for spectrography (to avoid foaming and spluttering at arc striking etc.). At the same time concentration of the trace elements and standardisation of the sample may be reached.

2. Earlier pretreatment for Co- and Mo-determinations

At first a pretreatment was applied as it is described in literature [2,12,13]. In short this pretreatment consists of: dry ashing of the sample (mostly 10 grams) for 12 hours at 450° C, *e.g.* in a muffle furnace — exclusion of silica inclusions by soda melt — dissolving the melt in dilute acid — precipitation of trace elements, iron and aluminium (added as a carrier) by supply of chemicals, *e.g.* oxyquinoline — filtration of the precipitate, followed by dry ashing. The sample consists then of Al_2O_3, Fe_2O_3 (some percents) and trace elements (mostly about 0.01%).

In this way, however, we could not succeed in obtaining spectrochemical analysis results, agreeing sufficiently with colorimetric ones for Co and Mo. In the mean the spectrochemical results were about 30% lower.

3. Application of wet ashing

This discrepancy disappeared (see IV. 4) when the first dry ashing and the soda melt were substituted by wet ashing with H_2SO_4, HNO_3, and later on with $HClO_4$. To avoid absorptions at $CaSO_4$, etc. the diluted destruction fluid is boiled for half an hour, whereupon the silica and the occasional calcium sulphate are filtered. The filter is washed two times with hot very diluted sulphuric acid. In the dilute acid filtrate the precipitate is obtained as described above.

The time required for a pretreatment is about the same in both cases (III. 2 and 3).

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4. Pretreatment for Cu-determinations

When one is only interested in the copper content of the sample a dry ashing suffices for reason of the relatively high content in the original sample. After addition of K_2SO_4 as a spectroscopic buffer the sample is ready for spectrography [1,2,14].

The simultaneous determination of Cu in the presence of Co, Mo, etc. is in preparation.

IV. MEASUREMENTS

1. Internal standard

The large fluctuations in the intensity of spectral lines in an arc require application of an internal standard for an accurate deduction of contents in the sample from measured intensities. In orientating experiments concerning Co- and Mo-determinations the results were as follows. When no internal standard is applied and the required contents are deduced from the measured densities of the photographed spectral lines, the accidental error is five times larger than in the case of the application of an internal standard method as also described in this paper (cf. IV. 3); when the contents are deduced from line/background ratios the accidental error is a factor two or three larger.

As analysis lines we used Co 3453.5, Mo 3170.35 and Cu 3274.0 A. According to the literature[2] we first applied as reference lines Fe 3452.0, Fe 3197.0 and Ag 3280.7 A, resp.

Ag 3280.7 is a perfect standard line. The difference in upper level of both elements amounts to 0.1 eV (Cu 3.7, Ag 3.8 eV). This means that at an arc temperature of about 4000° K a temperature variation of 500° causes a variation of only $3\frac{1}{2}\%$ in the relative intensity of these lines*.

Both the iron lines possess an upper level of about 6 eV, while that of the Co- and Mo-line is about 4 eV. Now at 4000° K a variation of 100° K gives a variation of 15% in relative intensities. A wavelength identification of about 80 iron lines on our plates in the region between 3500 and 3100 A showed that only one suitable iron line with an upper level of 4 eV is present, namely 3191.65 A. It may be used in combination with Mo 3170.35.

2. Proportionality between spectral relative intensity and content ratio in the sample

In a standard graph measured relative intensities are plotted against trace element content of the sample at fixed amounts of Fe in the sample. The varying Fe-content of the analysis samples — up to now colorimetrically determined[15] — may be taken in account e.g. with the help of an iron correction curve[1,2].

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* Cf. e.g. form. II. 3 in the first reference. As a consequence a stringent equality between standard and analysis sample is not required at all in such a case; cf. loc.cit. pages 118 and 125.

The procedure of relative intensity measurements includes important corrections for spectral background[1,2] and spectral inhomogeneity (cf. II.3)[1].

The amount of the element in question (e.g. Cu, Co), originating from electrodes and chemicals, must be often taken into account. These so-called blank amounts and the correlated relative intensities may be determined with the help of a graph in linear plotting, as it is illustrated in Fig. 2.
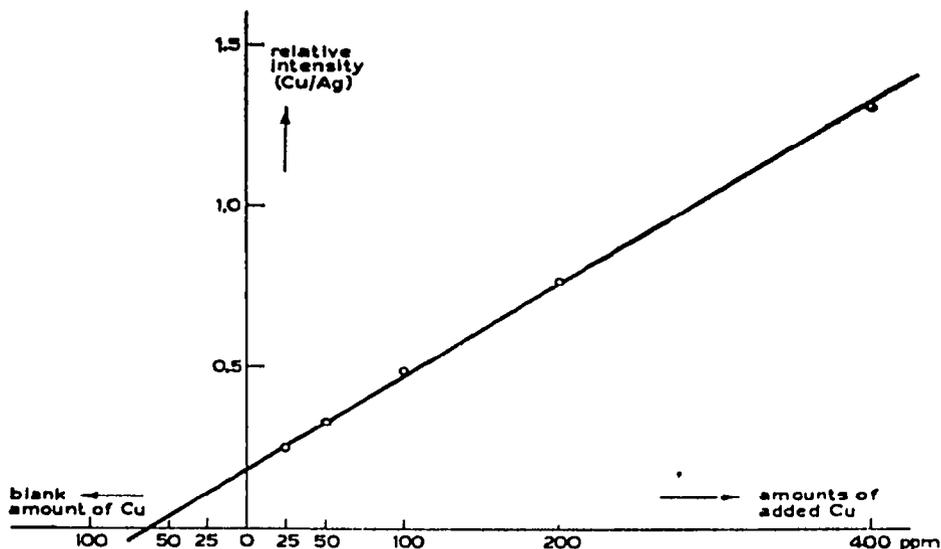

Fig 2

Fig. 2. Linear plotting of relative intensities against content to determine a blank amount of copper.

After application of the required corrections our standard graphs in double logarithmic plotting had approximatively a 45° slope. This means that in the content ranges considered (Co, Mo: 30—1000 ppm; Cu: 25—400 ppm; Fe some percents; Ag: 100—400 ppm) there is proportionality between relative intensities in the arc and content ratio in the sample.

This conclusion was confirmed by a "dilution test". To a sample consisting of Al_2O_3, 5%, Fe_2O_3, 500 ppm Co and Mo Al_2O_3 have been added, the first time half the weight of the sample, the second time the full weight. In that way the trace element standard ratio had not been changed and indeed the measured relative intensities in spectra of the original and diluted samples agreed within the experimental margin*.

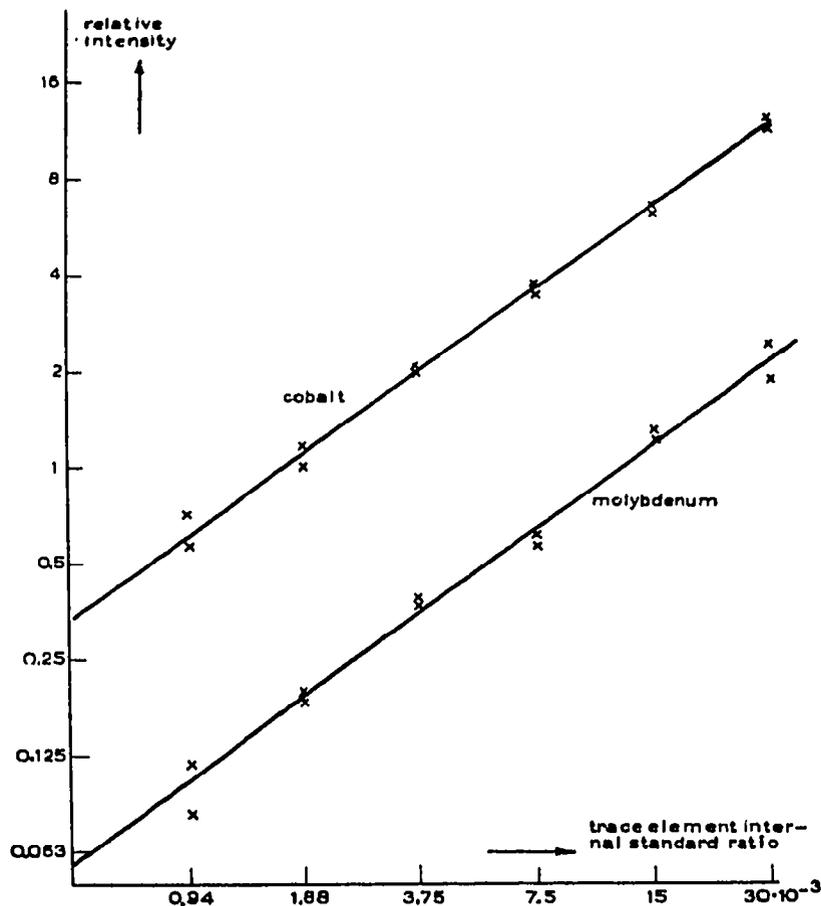* Quantitative results: pages 92 and 102 in first reference.

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Fig. 3. Calibration curve by the help of which relative intensities can be converted into contents in the sample without application of an iron correction curve. On the abscissa the content ratio Co/Fe or Mo/Fe has been plotted; on the ordinate the line intensity ratio Co 3453.5/Fe 3452.0 or Mo 3170.35/Fe 3197.0.

Consequently for calibration one graph suffices, in which the intensity ratio is plotted against the content ratio of trace and reference element; see Fig. 3. An iron correction curve is superfluous.

3. Spectrographic and spectrochemical reproducibility

When we deal with "spectrography" only the physical part of the determination is meant, starting with electrode filling up to the determination of relative intensities. The term "spectrochemistry" is concerned with the complete analysis starting with plant ashing up to content determination, deduced from measured relative intensities via calibration.

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The spectrographic accuracy* of the relative intensity measurement in a single spectrum for the pairs of lines mentioned sub IV. 1 appeared to be 8%. This result is based on about 100 measurements.

The spectrochemical reproducibility* amounts to 10%. It is a result of amply 100 determinations.

These results are satisfactory for the analysis in question. For further details and a discussion of errors the first reference may be consulted.

4. Comparison of colorimetric and spectrochemical results

In a first experiment Cu has been determined in 50 grass samples in colorimetric and spectrochemical way. The mean of both series of determinations in dry material was 12.8 ppm (12.77) and 12.8 (12.84), resp. It may be concluded that there are no systematical deviations between both methods. The accidental deviations in the couples of results, obtained in the analysis of the same samples in both ways, were in agreement with the accidental error of 10%, for each method separately.

After several series of orientating experiments also for Co and Mo comparable results have been obtained with a smaller number of samples. (10 samples; Co: 0.70 ppm spectrochemical, 0.61 colorimetric; Mo: 1.8 and 1.75, resp.).

The check will be continued for other elements, e.g. Zn.

ACKNOWLEDGEMENTS

SUMMARY

This paper deals with the spectrochemical determination of some trace elements (Cu, Mo and Co), especially in plant samples. Data are given about the choice of light source and spectral apparatus, as well as detailed remarks concerning the optical arrangement.

In the concentrating pretreatment with precipitating chemicals a wet ashing has been proved to give better results than a dry ashing.

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* The standard deviation D is given. For a series of spectra belonging to one sample D is calculated according to the well-known formula: $D = \sqrt{\dfrac{\Sigma(x-\bar{x})^2}{n-1}}$ (x and $\bar{x}$: actual and mean value, n: number of measurements).

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RÉSUMÉ

Cet article concerne l'analyse spectrochimique de traces de cuivre, molybdène et cobalt, en particulier dans les plantes. On traite du choix de la source lumineuse, de l'appareil spectral et de l'arrangement optique.

En ce qui concerne le traitement préalable de concentration, la minéralisation par voie humide est préférable à celle par voie sèche.

ZUSAMMENFASSUNG

Dieser Artikel behandelt die spektrochemische Bestimmung einiger Spurenelemente (Cu, Mo und Co), besonders in Pflanzenproben. Es werden sowohl Angaben gemacht über die Wahl der Lichtquelle und des Spektralapparates, als auch eingehende Bemerkungen, die die optische Anordnung betreffen.

Bei der Vorbehandlung mit Fällungsreagenzien zur Konzentration wurde bewiesen, dass eine Veraschung auf nassem Wege bessere Werte ergab als eine Veraschung auf trockenem Wege.

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