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MULTI-ELEMENT ANALYSIS OF BIOLOGICAL MATERIAL BY RNAA USING HPLC WITH ON-LINE YIELD DETERMINATION

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The idea to use high performance liquid chromatography with continuous UV detection for multi-element radiochemical separation and simultaneous multi-element "on-line" yield determination is introduced. The validity of the concept was evaluated by demonstrating the constancy of the ratio of the signals for a main isotope (^{60}Co) and a carrier (^{59}Co) as a function of the elemental mass processed in the separation. The suitability of the method for practical purposes is demonstrated by its application to the RNAA determination of cobalt in biological materials. It is concluded that the method, although not yet completely developed, bears the potential of improving both accuracy and precision of multi-element RNAA.

INTRODUCTION

As compared to other trace element analytical techniques, neutron activation analysis (NAA) in its instrumental mode benefits from superior accuracy. When chemistry is involved, the so-called isotope specific techniques, isotopic dilution mass spectrometry (IDMS) and neutron activation analysis with radiochemical separation (RNAA) are the methods of choice.¹ While IDMS demands addition of a spike to establish the efficiency of the chemical separation, in RNAA spiking is optional (addition of carrier is not). Carrier added for yield determination in RNAA is referred to as spike here. RNAA results obliged to be as accurate as possible, such as for certification purposes, cannot be founded on the assumption of a high and constant yield. Thus, a yield determination for every

element in each sample is mandatory. This means that apart from γ -ray spectrometry, a second element analytical technique is required. In classical RNAA, this problem is solved by using gravimetry or spectrometry for each individual element.² Alternatively, spiking with one or two radiotracers is also applied.³ At present, we are not aware of any publication on multi-element analysis by RNAA with multi-element yield determination. For various reasons both reactivation or addition of tracers and established existing multi-element techniques such as ICPAES, ICPMS or XRF are of limited or no use for yield assessment. Furthermore, considering the demand for highest obtainable accuracy, both single and multi-element yield determination add a third analytical step apart from separation and γ -ray spectrometry, inducing a risk of additional analytical errors.

Our investigations focus on the possibility to combine a multi-element separation step and a multi-element yield determination step, thus serving the need for high accuracy, using the potential multi-element capability of RNAA and without the inclusion of a third analytical technique. Reverse phase high performance liquid chromatography of metal-ligand complexes with continuous UV monitoring appeared to be a promising alternative to meet the criteria of "on-line" yield determination. As a feasibility study, the diethyldithiocarbamate complexes of transition metals were separated on a semi-preparative C-18 column. For its advantageous chemical and nuclear properties, Co (as ^{60}Co for tracer experiments and NAA and as ^{59}Co for addition as spike) was chosen as the target element.

Details on the chromatographic conditions and optimization of the analytical procedure via radiotracer experiments are presented elsewhere,⁴ while this paper reports on the validation of the "on-line" yield concept using radiotracers and RNAA.

EXPERIMENTAL

Chemicals and equipment

Individual standard solutions of Zn(II), Cu(II) and Co(II) salts were prepared by dilution of Tritisol standard solutions with Milli-Q water. The ligand stock solution was 50mM sodium diethyldithiocarbamate. H_2SO_4 , HNO_3 , H_2O_2 , HClO_4 , used for decomposition and equilibration, and CH_3COOH used as a buffer, were of analytical grade. A 50 mM acetate buffer solution at pH 6.0 along with methanol served as mobile phase. Chromatography was performed on a modular system comprising a LKB 2150 HPLC pump with ceramic lined pump heads, a LKB SuperRac fraction collector, a Rheodyne 7125 titanium injector with 1.0 mL loop, a Waters 484 Tunable Absorbance Detector and a Model 1020 Personal Integrator. The column used was a Nova-Pak C18 HR stainless steel semi-preparative one.

Tracer experiments

- Experimental design

Tracer experiments have been performed to evaluate the suitability of "on-line" yield determination without the burden of analytical errors due to irradiation. The radiotracer ^{60}Co served as the analyte, while ^{59}Co acted as the spike necessary to correct the analytical signal of ^{60}Co . The

basic assumption which has to be tested is the constancy of the measured ratio of signals from both isotopes, independent of the mass of Co processed (i.e. no effects due to, e.g., non-linearity in the detection or due to differences in oxidation state of the two isotopes). To perform this test, solutions were prepared containing a constant $^{60}\text{Co}/^{59}\text{Co}$ isotopic ratio, of which variable aliquots were introduced into the analytical procedure so as to create different masses of cobalt. This experimental design may simulate the situation of yield losses in the radiochemical separation step. In order to mimic real elemental analysis as close as possible, additions were done to erlenmeyer flasks, containing about 200 mg NIST SRM 1577A "Bovine Liver". Subsequently, a procedure including destruction, pH adjustment, extraction and chromatography was followed. This procedure resulted in a CoDDTC effluent fraction, from which the $^{59}\text{CoDDTC}$ signal was determined by continuous UV spectrometry at 254 nm and the ^{60}Co signal was obtained by "off-line" gamma-ray spectrometry. A total of 16 different additions were performed, with cobalt masses ranging from 60 upto 100 μg . The individual ^{60}Co signals were corrected by their corresponding UV signals, with the UV signal of 100 μg Co chosen as an arbitrary reference value.

- Results and discussion

A comprehensive description of the procedure of the radio-tracer experiments is presented elsewhere.⁴ Fig. 1 displays the relation between the ^{60}Co counting result and the $^{59}\text{CoDDTC}$ UV signal for 16 different masses of Co, added as a spike. It is clear from the value of the correlation coefficient for linear regression, 0.991, that the measured ratio of

Fig. 1 Linearity study between ^{60}Co activity and UV absorbance signal of ^{59}Co DDTC.
 Δ - Yield corrected ^{60}Co results, $\circ$ - Uncorrected ^{60}Co results.

signals originating from both isotopes remains constant in a mass range of 60 to 100 μg . Correction of the ^{60}Co counting results using the corresponding ^{59}Co DDTC signals leads to a relative standard deviation in the average corrected value of 3.2 %. When this experiment is considered as an attempt to deliberately cause yield losses, correction by the UV signal appears to be an accurate way of compensation for these errors. This means that the concept of "on-line" yield determination works well and application to radiochemical separations of real samples is justified.

Analysis of Co by RNAA

- Experimental design

Having validated the applicability of "on-line" yield determination for cobalt using radiotracers, the analytical aspects of irradiation, complete matrix mineralisation, equilibration of spike with sample, and of radiochemical purity remained to

be investigated. For a pilot experiment, it was decided to analyze different reference materials with a certified or advised cobalt content ranging from 5 upto 210 $\mu\text{g/kg}$. Here again, ^{60}Co is the radionuclide which provides the analytical signal, while ^{59}Co is added as a spike to provide quantitative information on the yield. To minimize additional errors in the ^{60}Co signal due to flux differences, it was decided to irradiate small batches, comprising two samples, one quality control sample (QCS) and one cobalt standard. No flux corrections were applied, the elemental contents were related to the counting result of the standard from the same batch. As a QCS, NIST SRM 1577A "Bovine Liver" was chosen with a certified cobalt content of $210 \pm 50 \mu\text{g/kg}$. From a comparison of UV corrected and uncorrected results for Bovine Liver, and from the possible dependence of the result per batch of the appropriate UV signal, the usefulness of "on-line" yield correction for RNAA in practise can be deduced.

RESULTS AND DISCUSSION

A detailed description of the RNAA procedure will be given elsewhere.⁵ Fig. 2 presents a UV chromatogram recorded for a quality control sample. The peaks of Zn-, Co- and CuDDTTC can be clearly distinguished. The first peak can be attributed to disulfiram, a DDTC dimer. It should be noted that no extra carrier of Zn and Cu are added. The peaks of their respective DDTC complexes are caused by the naturally occurring Zn and Cu in Bovine Liver. This phenomenon demonstrates that the method possesses multi-element potential but is only applicable to elements which occur on the lower and

Fig. 2 Chromatogram of a Bovine liver analysis. 1 - Disulfiram, 2 - Zn, 3 - Co and 4 - Cu.

sub mg/kg level. Also, the Zn and Co peaks are not completely baseline separated. This is due to the forced use of a semi-preparative column, allowing injection of mL amounts of sample, a *conditio sine qua non* in RNAA.⁴ The interference of ⁶⁵Zn in the collected CoDDTC fraction is negligible, as shown in Fig.3, which displays a gamma-ray spectrum of a quality control sample in the region from 1100 to 1200 keV. A practically pure radiochemical prepare has been obtained.

The determination of Co in the QCS resulted in a value of $215 \pm 17 \mu\text{g/kg}$ ($N=9$). This agrees well with the certified value, being $210 \pm 50 \mu\text{g/kg}$. Furthermore, the yield corrected results appeared to be independent of the UV signals. The correlation coefficient for linear regression of the corrected Co- and the UV result is only 0.15, supporting the earlier findings of constancy of the measured isotopic ratio with

Fig. 3 Gamma ray spectrum of NIST 1577A in the region of 1100 - 1200 KeV.

varying ⁵⁹Co mass. The uncorrected result for the QCS was $233 \pm 43 \mu\text{g/kg}$. It is clear from the comparison with the corrected result, that "on-line" yield determination is a beneficial action with respect to both accuracy and precision.

This statement is also valid for the results obtained for Bowens Kale. We found $62.5 \pm 3.8 \mu\text{g/kg}$, while the certified value is $63.2 \pm 10.7 \mu\text{g/kg}$. We also tried the method for BCR CRM 274 Single Cell Protein, BCR CRM 151 Milk Powder and IAEA A-11 Milk Powder. Results for these materials were less satisfactory, about 10 - 20% higher than the certified values. Fig.4 displays the results obtained with the given method compared to certified or advisory values. It is obvious that in the lower $\mu\text{g/kg}$ level a blank problem arises. It is not yet clear whether this is a ⁵⁹Co or a ⁶⁰Co problem.

Fig. 4 Comparison of RNAA results for Co with certified values.

A = NIST SRM 1577A "Bovine Liver" ; B = Bowen's Kale ; C = BCR CRM 274 "Single Cell Protein" ; D = BCR CRM 151 "Milk Powder".

Further research points to aspects of accuracy and precision of trace element analysis rather than the methodological characteristics. Also, since ^{58}Co from the $^{58}\text{Ni}(n,p)^{58}\text{Co}$ reaction is detectable, attention is paid to nickel analysis, using the $^{59}\text{CoDDTC}$ UV signal as a yield monitor.

CONCLUSIONS

It can be concluded from both the tracer experiments and RNAA results that "on-line" yield determination is a fruitful concept. This conclusion is based on the absence of signal ratio effects of analyte- and spiking isotope, and the dramatic improvement of accuracy and precision in the yield-corrected results for Co by RNAA in biological reference materials. The technique bares the potential of multi-element capability without the loss of highest obtainable accuracy.

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