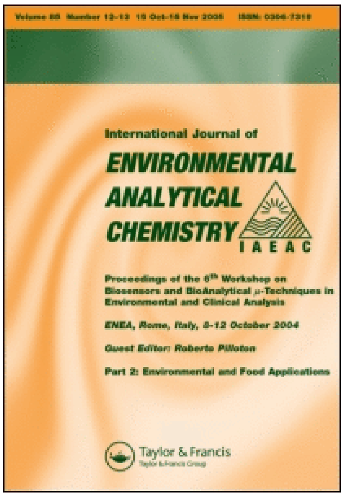

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Determination of Gold and Platinum Traces in Biological Materials as a Part of a Multi-Element Radiochemical Activation Analysis System†

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For the analysis of human tissues for traces of gold and platinum—being used as constituents of therapeutic agents—a radiochemical neutron activation method has been developed. The radiochemical separation involves the selective removal of radioactive gold—formed by the reaction $^{197}Au(n,\gamma)^{198}Au$ and the reaction $^{198}Pt(n,\gamma)^{199}Pt \rightarrow {}^{199}Au$—as small metallic nuggets. The determination of gold and platinum is carried out as a part of an automated multi-element radiochemical separation scheme, allowing the determination of about 20 additional trace elements,

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and thus giving the possibility to study interelement relations. The analytical characteristics of the determination are evaluated. Gold and platinum levels measured in Bowen's Kale, NBS Bovine Liver and NBS Orchard Leaves are presented. Values are shown for gold, platinum, and 20 other trace elements in various healthy and cancerous tissues from patients treated with *cis*-platin. (Cis-Diamminedichloroplatinum II).

KEY WORDS: Gold (traces); platinum (traces); multi-trace-element determination; trace elements in human tissues; radiochemical neutron activation analysis.

INTRODUCTION

Gold and platinum compounds are used in the treatment of certain human disorders, viz. of rheumatic arthritis and of particular types of cancer respectively. For a better understanding of the pharmacological action and of the side-effects, knowledge is required of the residual concentrations of both metals in various tissues of the human body. Moreover, the administration of gold and platinum compounds may influence the levels of other trace elements in the human body. For instance, in the case of *cis*-platin injected in patients suffering from malignant tumours, a change in the levels of some trace elements in plasma has been observed.[1] The study of interelement relations between Au or Pt on one hand and various other trace elements of clinical interest on the other hand calls for a multi-element determination with adequate sensitivity.

Radiochemical neutron activation analysis has good prospects for a sensitive determination of both metals, when based on the following two nuclear reactions: $^{197}\text{Au}(n,\gamma)^{198}\text{Au}$ ($T_{1/2}=2.7$ day; $E_\gamma=411.8$ keV) and $^{198}\text{Pt}(n,\gamma)^{199}\text{Pt}$ ($T_{1/2}=30.8$ min)$\rightarrow^{199}\text{Au}$ ($T_{1/2}=3.15$ day; $E_\gamma=158.3$ and 208.1 keV).

This study involves the development of a radiochemical step for the determination of Au and Pt in biological materials. This step has been inserted in an already existing automated radiochemical separation scheme for the determination of about 20 trace elements in biological materials.

EXPERIMENTAL

In the radiochemical separation scheme,[2-6] being routinely used at the Interuniversity Reactor Institute in Delft, radioactive gold ends up in both ion exchange resin columns as a rather tightly bound element. However, the recovery of gold is not always quantitative, while also the distribution of the gold over both resin columns is not a constant ratio. Moreover, sometimes appreciable interferences in the gamma spectrum are present, e.g. a gamma peak of 160 keV from ^{47}Sc being the daughter radionuclide of ^{47}Ca ($T_{1/2}=4.7$ day). These factors are the reasons that a sensitive and reliable measurement of the induced ^{198}Au and ^{199}Au activities cannot be performed with the present radiochemical separation scheme.

It has been suggested earlier[5] to explore for the determination of gold the possibility of removing this element as metallic gold. This approach has been followed and evaluated in this study, leading to a modification of the radiochemical separation scheme. The modification consists of the following steps: Prior to destruction a known amount of inactive gold is added as a carrier. After destruction and distillation of the volatile elements, the radioactive gold is recovered as a couple of spongy nuggets. The chemical yield of the recovery is easily determined via weighing. Before counting with a Ge(Li) detector, the nuggets are dissolved in aqua regia to ensure a reproducible counting geometry.

A description of the entire analytical procedure is given (Figure 1). For further details, the reader is referred to the earlier publications.[2–6]

Preparation of samples and standards

Necessary precautions are employed prior to sample irradiation to minimize the blank effect and contamination (e.g. handling in a low dust level room, use of plastic utensils). The irradiation vials (outer diameter 9 mm, length 7 cm, internal volume 2.5 cm^3) are made of synthetic quartz (Suprasil, Heraeus Quarzschmelze, Hanau, FRG). The vials are thoroughly cleaned before use by etching in hydrofluoric acid, leaching in aqua regia, and washed with doubly distilled water.

The biological reference materials are analyzed as received and 250 mg aliquots are taken for analysis. The dry weight is determined in separate aliquots; Bowen's Kale and NBS Orchard Leaves are dried in an oven at 105°C for 24 hours, while NBS Bovine Liver is

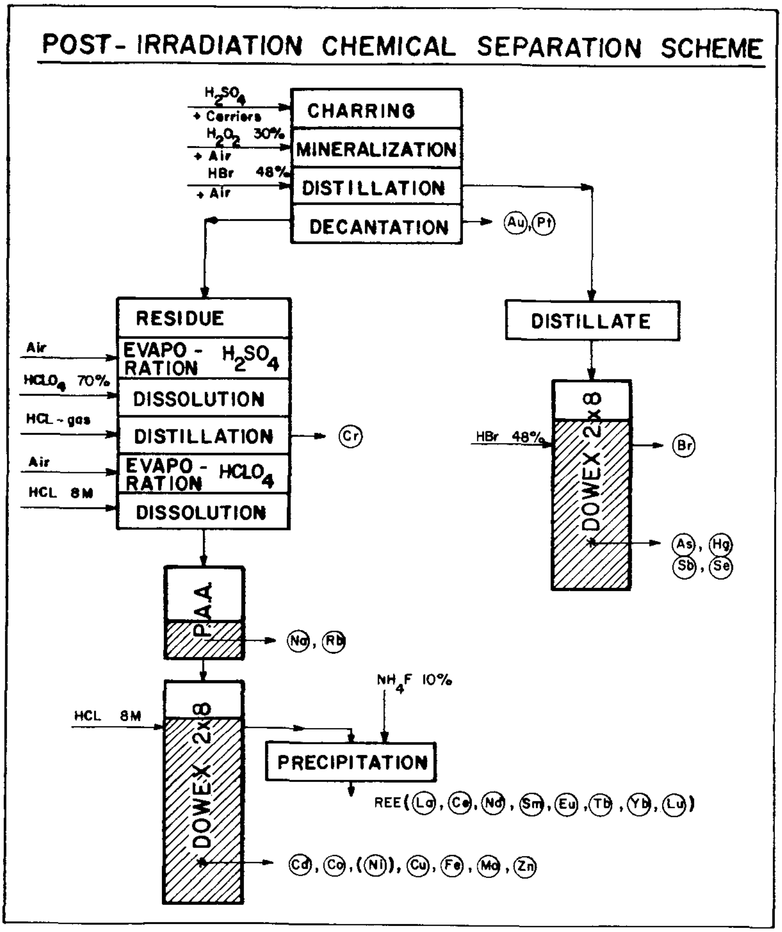
POST- IRRADIATION CHEMICAL SEPARATION SCHEME
H2SO4 + Carriers
CHARRING
H2O2 30% + Air
MINERALIZATION
HBr 48% + Air
DISTILLATION
DECANTATION
Au, Pt
RESIDUE
Air
EVAPO - RATION H2SO4
HClO4 70%
DISSOLUTION
HCL - gas
DISTILLATION
Cr
Air
EVAPO - RATION HClO4
HCL 8M
DISSOLUTION
P.A.A.
Na, Rb
HCL 8M
DOWEX 2x8
NH4F 10%
PRECIPITATION
REE (La, Ce, Nd, Sm, Eu, Tb, Yb, Lu)
Cd, Co, (Ni), Cu, Fe, Mo, Zn
DISTILLATE
HBr 48%
DOWEX 2x8
Br
As, Hg
Sb, Se

FIGURE 1.

dried over P_2O_5 at room temperature for one week. For the analysis of the human tissues, aliquots of about 1 gram are taken from the larger hospital samples received, using a titanium knife. After transfer into quartz irradiation vials, the samples are freeze-dried for 48 hours and then reweighed.

As standards 0.5 ml aliquots of a freshly prepared 4 M hydrochloric acid–4 M nitric acid mixture is used, containing all elements of interest, except Au and Pt, which are contained in a second standard. This standard is prepared by dissolving the pure metals in aqua regia and pipetting 0.5 ml of the liquid containing 50 ng of Au and 20 μg of Pt.

Irradiation

Irradiations are carried out in the high-flux facility of the nuclear reactor of the Interuniversity Reactor Institute at Delft, in which the thermal neutron flux amounts to 10^{13} cm^{-2} s^{-1}. The irradiation time is limited to 12 hours since under this condition no losses of irradiated material occur due to internal pressure build-up when opening the vials. Each irradiation batch consists of five sealed vials with samples (including sometimes empty vials as blanks), two sealed vials with multi-element standards and two pieces of zinc metal which serve as flux monitors. The flux monitors are used to compare the results of the ratio of the net photo peak counting rate of the individual elements in the liquid standard to the net counting rate of the zinc monitor (element-to-zinc ratio) of the various runs as a control on the experimental conditions over a given period.

Radiochemical separations

The irradiated samples are allowed to cool for 2–3 days, resulting in a substantial reduction of the activity level, mainly due to ^{24}Na. Before opening the vials, the outside surface contamination is removed by boiling in aqua regia for 20 minutes and by rinsing with running water. The vial is then cooled in liquid nitrogen, wrapped in paper tissue, and loosely packed in an open polyethylene bag. In a pneumatic press the packed vial is lightly broken. The sample, quartz splinters, and paper tissue are transferred from the bag to the destruction/distillation vessel.

The radiochemical separations are performed in semi-automated devices, enabling the simultaneous processing of six samples. The modified radiochemical procedure—schematically shown in Figure 1—is as follows.

Prior to destruction, carrier solution is added containing 20 mg of Au and 50 μg of all remaining elements of interest, except for Pt and the lanthanides. The samples are charred with 15 ml of concentrated sulfuric acid at 200–250°C (the time required depends on the type of sample) and mineralized by oxidation with a 30 percent hydrogen peroxide solution added at a rate of 0.5 ml/min.

The volatile elements are removed via distillation at 220°C with concentrated hydrobromic acid (47 percent), added at a rate of 1 ml/min. The distillate is passed over a 10 cm Dowex 2*8 ion-exchange resin column (4 gram) previously equilibrated with concentrated hydrobromic acid. The elements As, Sb, Se and Hg are absorbed on the column. The column is washed three times with 20 ml concentrated hydrobromic acid. In contrary to earlier publications[2,5,6] the elements absorbed are not eluted selectively, but counted as one group on the ion-exchange resin material.[3] Prior to counting the resin is homogenized by adding 10 ml paraffin oil, shaking and centrifuging the resin to the bottom.

To the hot residue 15 ml of H_2O is added to promote the formation of metallic gold, deposited as a couple of spongy nuggets on the bottom of the destruction/distillation vessel. After cooling, the sulfuric acid medium is decanted, leaving the nuggets and quartz splinters in the vessel. The nuggets are then collected by a pair of tweezers, washed three times with water in a beaker placed in an ultrasonic bath, washed with acetone, dried and weighed for the chemical yield determination (generally between 70–90 percent). Finally, the nuggets are transferred to a counting vial and dissolved in 1 ml aqua regia.

The decanted sulfuric acid solution is quantitatively collected in another destruction/distillation vessel and evaporated to dryness at 400°C. To the residue 15 ml of 70 percent perchloric acid solution and 40 mg of chromium (as $CrCl_3$) are added. At a temperature of 200 ± 5°C dry hydrogen chloride gas is passed through the perchloric acid medium at a rate of 90 ml/min for 10 min. The chromium is distilled over as chromyl chloride and is then collected in a receptacle with 5 ml of a 10 percent hydroxylammonium chloride solution to reduce the hexavalent chromium to a less volatile state.

The perchloric acid medium is evaporated at 250°C and the residue is taken up in 20 ml 8 M hydrochloric acid. This solution is first passed over a 5 cm Polyantimonic Acid column (Applied Research, Brussels, Belgium) (2 gram) to absorb the elements Na and Rb. The effluent is passed over a 10 cm Dowex 2*8 column (4 gram), equilibrated previously with 8 M hydrochloric acid. The elements Cd, Co, Ni (as ^{58}Co), Cu, Fe, Mo and Zn are absorbed on the column. The column is washed with 20 ml 8 M hydrochloric acid. Also here the elements are not selectively eluted from the column as described earlier,[2, 5, 6] but counted as one group on the ion exchange resin material[3] after homogenization (see above).

To the hydrochloric acid effluent of the Dowex 2*8 column 10 mg of La carrier, 2 gram of the di-sodium salt of EDTA (in order to complex Ca) and 50 ml of a 5 M sodium hydroxide solution are added. Under heating sodium hydroxide pellets are further added until the La(lanthanides)hydroxide precipitate is formed. The precipitate is centrifuged, the upper liquid decanted, the precipitate is washed with water, centrifuged again, and finally dissolved in 10 ml 0.2 M hydrochloric acid. Then 10 ml of a 10 percent ammoniumfluoride solution is added and the La(lanthanides)F_3 precipitate formed is filtered over a 25 mm diameter micropore filter by suction.

Counting and data handling

The following fractions are counted: (i) Au and Pt in the geometry of 1 ml solution; (ii) As, Hg, Se and Sb in the geometry of 4 gram of Dowex 2*8 resin; (iii) Cr in the geometry of 20 ml solution; (iv) Cd, Co, Ni (as ^{58}Co), Cu, Fe, Mo and Zn in the geometry of 4 gram of Dowex 2*8 resin; (v) La, Ce, Nd, Sm, Eu, Tb, Yb and Lu in the geometry of a flat filter paper; and (vi) Rb in the geometry of 2 gram of Polyantimonic Acid.

All fractions, except Cr, are counted with a 60 cm^3 active volume Ge(Li) semiconductor (FWHM: 1.9 keV at 1,332 keV), built into a 10 cm thick lead shield and coupled via a CAMAC interface to a DEC PDP 11/70 computer. For the measurement of Cr a 3″ × 3″ NaI(Tl) detector with a 1.25″ diameter well is used. The counting times vary from 1,000 to 10,000 s, depending on the activity to be measured. Changing of the fractions to be counted is performed by an automated 20 positon sample changer.

The identification of the photo peaks in the gamma spectra, the measurement of the net peak areas and the calculation of the element concentrations are performed by the computer programme IICPXM.[7, 8]

RESULTS AND DISCUSSION

Analytical evaluation of the determination of Au and Pt

The radionuclide ^{198}Au—formed by the ^{197}Au$(n, \gamma)^{198}$Au reaction—has a large neutron capture cross-section (2.6 10^{-20} m^2), leading to a not negligible formation of ^{199}Au via the reaction ^{198}Au$(n, \gamma)^{199}$Au. The ^{199}Au produced via this route—being an interference in the determination of Pt via the reaction sequence ^{198}Pt$(n, \gamma)^{199}$Pt$\rightarrow^{199}$Au—increases not only with the amount of Au but also with the irradiation time and the square of the neutron flux. Under standard working conditions—irradiation in a neutron flux of 10^{13} cm^{-2} s^{-1} for 12 hours, 4 days of decay, and counting on a 60 cm^3 Ge(Li) detector for 3 hours—per ng of Au an amount of ^{199}Au is produced equivalent to 2.4 ± 0.3 ng of Pt (average of four determinations and standard deviation). For this "apparent Pt level" a correction is applied when necessary.

The detection limit—taken as three times the standard deviation of the background in the area of the corresponding photo peak—amounts under standard working conditions to 0.002 ng of Au and 0.3 ng of Pt. In the presence of appreciable amounts of Au, the detection limit for Pt may be substantially higher than 0.3 ng, due to the uncertainty in the correction for the ^{199}Au contribution from ^{197}Au.

The precision—expressed as coefficient of variation—was measured for the standards to be about 6 percent for both Au and Pt. This imprecision is caused by (i) neutron flux variations; (ii) errors in the chemical separation including the chemical yield determination; (iii) counting geometry; and (iv) statistical errors in the photo-peak area determination. This last mentioned error source will appreciably grow at decreasing levels of Au and Pt, leading to an increase of the coefficient of variation. Moreover, for a given Pt level, the precision will also decrease with an increasing Au/Pt ratio, because of the already-mentioned uncertainty in the correction for the ^{199}Au contribution from Au.

In the analysis of the three reference materials as well as of various human tissues, the gold nuggets showed only ^{198}Au and ^{199}Au activities, indicating sufficient selectivity of the Au and Pt determination. However, the accuracy of the method developed could not be established, since biological reference materials with low and certified levels of Au and Pt were not available.

The Au and Pt levels determined in three frequently used international reference materials are presented in Table I together with some literature data.[9-11] The table indicates that our data do not substantially deviate from the recent results of Zeisler and Greenberg (National Bureau of Standards, Washington D.C., USA—issuing agency of NBS Bovine Liver and NBS Orchard Leaves).

For the evaluation of the analytical characteristics of the determination of the other trace elements, the reader is referred to earlier publications.[2-6]

TABLE I

Au and Pt abundances (averages and standard deviation) in ng/g (on dry weight) in three biological reference materials

	Au	Pt
NBS Orchard Leaves SRM-1571		
This work ($n=6$)	0.72 ± 0.25	< 1
Nadkarni/Morrison[9]	0.97 ± 0.09	89.2 ± 15.4
Gladney[10]	1.8 ± 1.0	(89–1200)
Zeisler/Greenberg[11]	1.43 ± 0.08	0.2 ± 0.2
NBS Bovine Liver SRM-1577		
This work ($n=6$)	0.083 ± 0.021	< 3
Nadkarni/Morrison[9]	29.9 ± 2.1	
Gladney[10]	10 ± 15	
Zeisler/Greenberg[11]	0.058 ± 0.013	0.070 ± 0.033
Kale of Bowen		
This work ($n=6$)	0.95 ± 0.50	< 1
Nadkarni/Morrison[9]	1.91 ± 0.16	198 ± 8.5

Determination of Au, Pt and some additional trace elements in human tissues

In order to establish the usefulness of the modified system for the determination of Au and Pt together with some 20 additional trace

TABLE II

Trace element abundances (median values) in μg/g (on dry weight) in various types of human tissues

	Liver ($n=13$)	Liver carc. ($n=4$)	Lung ($n=9$)	Lung carc. ($n=4$)	Cortex ($n=7$)	Medulla ($n=5$)	Kidney carc. ($n=3$)	Heart ($n=5$)	Spleen ($n=3$)	Brain ($n=1$)	Bladder ($n=2$)	Ovarium carc. ($n=1$)	Esophagus carc. ($n=1$)	Mamma carc. ($n=4$)
Au	0.001	0.003	0.008	0.004	0.006	0.002	0.002	0.006	0.002	0.002	0.004	0.001	0.041	0.011
Pt	26.8	38.6	2.39	0.51	1.51	1.35	0.22	2.83	1.77	<0.01	<0.01	<0.01	7.3	<0.01
Cr	0.12	0.04	2.10	0.60	0.13	0.06	0.65	0.13	0.17	0.02	0.22	0.64	0.03	0.07
As	0.017	0.009	0.019	0.011	0.010	0.016	0.009	0.008	0.004	0.009	0.009	0.004	0.011	0.006
Hg	0.73	1.27	1.80	0.03	0.92	0.78	0.47	0.06	0.59	0.03	0.01	0.01	1.17	0.56
Sb	0.025	0.023	0.114	0.036	0.012	0.012	0.008	0.010	0.012	0.014	0.005	0.009	0.016	0.064
Se	1.84	1.76	1.16	1.46	3.70	2.87	1.39	1.06	1.83	1.16	0.36	0.67	1.50	0.99
Rb	12.4	14.7	14.8	13.0	15.3	14.4	12.0	9.1	13.3	11.2	4.2	8.2	15.4	10.4
Cd	5.76	10.60	1.24	0.31	78.0	33.4	0.31	0.28	0.91	0.27	0.23	0.17	0.80	0.60
Co	0.171	0.223	0.284	0.154	0.042	0.056	0.019	0.088	0.075	0.026	0.246	0.560	0.072	0.019
Cu	18.3	15.6	9.2	12.5	12.6	12.4	5.8	12.4	4.4	21.3	21.4	1.4	3.4	3.5
Fe	1052	708	831	526	374	431	455	377	2645	267	101	322	199	80
Mo	3.64	1.35	0.08	0.08	0.06	0.38	0.07	0.15	0.07	0.08	0.03	0.04	0.04	0.07
Zn	392	168	74	76	151	170	113	127	102	74	52	61	126	73
La	0.051	0.022	0.137	0.033	0.008	0.015	0.004	0.004	0.004				0.003	0.007
Ce	0.085	0.036	0.343	0.130	0.020	0.032	0.006	0.005	0.007				0.011	0.015
Nd	0.020	0.029	0.116	0.019	nd	0.024	nd	nd	nd				0.012	0.008
Sm	0.0007	0.0005	0.0054	0.0026	0.0003	0.0004	0.0002	0.0001	0.0002				0.0002	0.0005
Eu	0.0003	0.0009	0.0005	0.0010	nd	nd	nd	nd	nd				nd	nd
Tb	nd	0.0009	0.0024	nd	nd	nd	nd	nd	nd				nd	nd
Yb	nd	0.0004	0.0029	0.0009	0.0003	0.0005	nd	nd	nd				nd	nd
Lu	nd	nd	0.0003	0.0002	nd	nd	nd	nd	nd				nd	nd

nd = not detected (detection limit for Nd = 0.008, Sm = 0.00004, Eu = 0.0001, Tb = 0.0004, Yb = 0.0002, Lu = 0.00005 μg/g.

elements, we have analysed a random selection of tissues obtained from autopsies of cancer patients treated with *cis*-platin. Table II gives a survey of the summary of the results as median values per element per type of tissue.

In all samples analyzed the elements of interest could be determined, except Ni. As found already in earlier studies,[5,12] Ni can only be determined with the radiochemical separation scheme when present in elevated concentrations (>0.1 μg/g).

From a few tissue samples duplicate aliquots were taken for analysis. The results showed sufficient reproducibility (deviations generally between 10–15 percent).

In respect to Pt levels found, it should be noted that these do not only depend on the amount of *cis*-platin administered and the administration scheme, but also on the time lapse between the last administration and moment of death. As can be seen, Pt does not specifically accumulate in cancerous tissue, while the highest values are found in liver and kidney.

Due to the situation that the samples were randomly selected, as well as due to the paucity of trace element values per type of tissue, no attempts have been undertaken to perform interelement correlations.

Conclusively, it may be stated that the modified radiochemical separation scheme has a potential for the simultaneous determination of Au, Pt and a number of trace elements of clinical interest in only a single sample. Also for the determination of Au or Pt alone, the modified scheme—which then only has to be followed through the collection of the gold nuggets—has specific advantages. In particular, it may serve as an independent reference method (e.g. for the determination of Pt via atomic absorption spectrometry), because of the physically and chemically different underlying principles of radiochemical neutron activation analysis.[13]

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