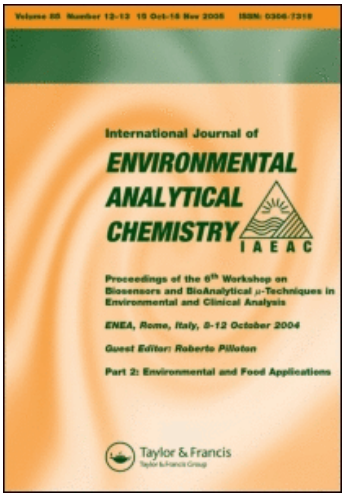

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EXTRACTION AND DETERMINATION OF TRACE AMOUNTS OF RHENIUM IN PLANTS

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Three procedures for extraction of rhenium from plant material are developed with a view of its subsequent quantitative determination according to a catalytic *N*, *N*-Dimethyldithiooxamide (DMDTO) spectrophotometric method. They include: (i) laboratory variant through ashing the plant material and extraction of Re from the ash with hot NaOH solution; (ii) field test – Re is coextracted with the chlorophyll from the plant by hot ethanol. Ethanol extract is further evaporated. The chlorophyll is destructed either thermally or with HCl. Re is then extracted with NaOH solution; (iii) direct extraction of Re from the chlorophyll with HCl, alkalization of the extract with NaOH, oxidation of organic substances with H_2O_2 under heating. The developed procedures allow the subsequent determination of Re content in plants using catalytic DMDTO spectrophotometric method in a wide concentration interval from 3 to $n \times 10^3$ ng g^{-1} Re. They are applied to analysis of plant samples collected in the vicinity of ore mining and in unpolluted areas in Bulgaria.

Keywords: Rhenium; Extraction; Determination; Plants

INTRODUCTION

It is known that rhenium is easily accumulated in green leaves and plants, it amounts in them exceeding many times its occurrence in the earth's crust (7×10^{-8}%) [1,2]. This fact has been used to reassess data about rhenium distribution in the biosphere. According to the existing classification [3] of biological objects with respect to their possible ability to accumulate chemical elements one can distinguish 4 types of accumulation: (1) unlimited (provides high degree of concentration exceeding local background or natural distribution of the element by a factor of 300); (2) practically unlimited (provides element concentrations exceeding local background or natural distribution of the element by a factor of 100); (3) limited (accumulated amounts exceed natural abundance by a factor of 10); and (4) limited to the background

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(accumulated amounts are close to natural abundance or to background values). Most of the elements are accumulated according to a limited mechanism. 70% of plants accumulate Zn, Mo, Au, Bi, Rb, and As according to unlimited and practically unlimited mechanism [2]. Rhenium belongs to the elements that are freely accumulated in plants – its amounts in studied plants exceed its occurrence in the earth's crust by a factor of 1000 or tens thousand.

Our previous investigations on dynamics of Re bioaccumulation in plants indicated that Re is rapidly extracted from soils and waters [4]. The survey of literature data reveals that rhenium in plants has been determined after ashing at about 400°C, additional concentration through precipitation with thiosulfate and finally using emission spectroscopy with arc excitation [5]. The procedure, however, suffers of a number of drawbacks: (i) there exist no data about losses of rhenium in the course of ashing (it is very likely that a volatile Re_2O_7 is formed); (ii) the sensitivity of emission spectroscopic method is insufficient for direct determination of nanogram amounts of Re. In this case a preconcentration is necessary, the latter complicating the analysis.

The aim of the present study is to develop reliable procedures for complete extraction of nanogram bioaccumulated amounts of Re from green plants and its subsequent catalytic determination by the highly sensitive and selective spectrophotometric method with *N*, *N*-Dimethyldithiooxamide (DMDTO) [6]. The developed procedures for determination of rhenium content in plants can be applied in search of new rhenium containing deposits (field tests). They help us as well to elucidate the mechanism of Re accumulation in green plants [9].

EXPERIMENTAL

Reagents and Solutions

A stock standard solution of rhenium ($1000\,\mu g\,mL^{-1}$) was prepared by dissolving 0.1553 g of $KReO_4$ in 100 mL of distilled water. Working solutions ($1\,\mu g\,mL^{-1}$ Re) were prepared by diluting 0.01-mL aliquots of the stock solution with 10 mL water.

The solution of DMDTO ($0.04\,mol\,L^{-1}$) was prepared by dissolving 0.0593 g of the reagent in 10 mL of $10\,mol\,L^{-1}$ NaOH. The use of fresh solutions is recommended.

The solution of tin(II) chloride in $10\,mol\,L^{-1}$ NaOH was prepared by dissolving 0.542 g of $SnCl_2 \cdot 2H_2O$ in 0.3–0.8 mL of distilled water, followed by the addition of about 8 mL of $10\,mol\,L^{-1}$ NaOH under constant stirring until a clear, colorless solution was obtained, finally diluting to 10 mL with $10\,mol\,L^{-1}$ NaOH. The solution was prepared fresh daily.

Sodium hydroxide solution (32%, $d = 1.35$, E. Merck, Darmstadt, Germany) was used throughout.

Ethanol (96% v/v) analytical grade, hydrogen peroxide (30% v/v) analytical grade, hydrochloric acid (37% v/v) analytical grade, ammonia (25% v/v) analytical grade.

Instruments

A Beckman, Model DK 2A UV–Vis, near infrared, double-beam spectophotometer was used throughout (Beckman Instruments, Fulerton, CA USA). For the ashing procedure a crucible furnace equipped with an ESPA thermostatic controller (Bulgaria) was used.

Quartz glasses, quartz cells (1 cm), Teflon® spot plate (for the semiquantitative test) were used. A centrifuge Janetzki, T 32 A (Poland), 4000 rpm was used.

Procedures

Preparation of Plant Samples with Known Re Content

Three separate portions (3 g) of row leaves of geranium, acacia or other plant are immersed in three glasses containing 25 mL distilled water spiked with 1000 μg Re ($KReO_4$ solution, $C_{Re} = 40\,\mu g\,Re\,mL^{-1}$). The leaves are allowed to stand for varying periods of time, e.g. 1,2,3, or more days depending on the desired amount of Re to be accumulated. The leaves are removed from the solution and stored for further experiments. The remaining aqueous phase is brought to 25 mL with distilled water and analyzed for Re content. The difference between the initial concentration and the final one corresponds to the rhenium amount accumulated in the leaves. Parallel analysis of as treated leaves confirm this. The prepared leaves were further used as 'standard reference material' in optimization of the operating parameters of determination of rhenium.

Destruction of Plant Samples Through Ashing

Weigh 1–7 g of row green leaves and cut them into small pieces. Transfer the pieces into a quartz glass and put the latter into a furnace at 450–470°C (the ashing continues until the ash becomes pale gray). The ash is transferred on a quantitative wide pore filter and rhenium is extracted with 5 portions (2 mL each) of hot $1\,mol\,L^{-1}$ NaOH. The combined filtrates are brought to 10 mL with $1\,mol\,L^{-1}$ NaOH. An aliquot of the resulting solution is analyzed using the DMDTO method [6].

Acid Extraction of Rhenium from Green Leaves

0.5–1.0 g of finely ground green leaves are introduced into a Teflon® centrifuge tube. Then 0.17 mL of $12\,mol\,L^{-1}$ HCl and 4.83 mL of distilled H_2O are added. The mixture is centrifuged for 5 min at 4000 rpm. The acid centrifugate is filtered through a wide pore filter, and the remaining leaves are rinsed with three 3-mL portions of water. The combined filtrate and washings are alkalized with 1 mL of $10\,mol\,L^{-1}$ NaOH, 0.5-mL portion of 30% H_2O_2 is added and the solution is heated on hot plate until organic constituents are completely decolorized. If necessary the procedure is repeated until a complete decolorization is achieved. The solution is brought to boiling until peroxide is completely decomposed (until the evolution of bubbles ceased). Then the solution is transferred into 10-mL calibrated cylinder and diluted to volume with water. An aliquot of the resulting solution is analyzed for Re content by the catalytic spectrophotometric method.

Extraction of Chlorophyll and Rhenium with Ethanol

0.5–1.0 g of finely cut green leaves is placed in a quartz glass and l0-mL portion of ethanol is added. The mixture is heated with an IR lamp to boiling. The green extract containing chlorophyll is decanted and a fresh 10-mL portion of ethanol is added to the

leaves. The mixture is again heated with an IR lamp to boiling. The second extract is added to the first one. The procedure is repeated until the leaves become colorless. The leaves are then rinsed with two successive 5-mL portions of cold ethanol. The combined ethanol extracts are evaporated to dryness (IR lamp). The resulting brown dry residue is ignited in a crucible furnace at 450–470°C. Rhenium is extracted from the ash with five 1-mL portions of hot 1 mol L^{-1} NaOH as described above. A second variant was developed as well based on acid digestion of the dry chlorophyll residue. The procedure is as follows: the dry residue is mixed with 0.1 mL 12 mol L^{-1} HCl followed by 1 mL 10 mol L^{-1} NaOH. Then 0.5 mL H_2O_2 is added and the solution heated on a hot plate until complete decolorization (additional peroxide is added if necessary). The resulting colorless solution is diluted to 10 mL with water. Re is finally determined using the catalytic spectrophotometric method with DMDTO. The described procedure is applicable as a field test. In this case a gas heater is used instead of IR lamp and acid digestion of the chlorophyll is preferable. The semiquantitative determination of Re is further performed according to the expressed spot test with DMDTO [6].

RESULTS AND DISCUSSION

Samples with known Re content were required or samples should be analyzed by independent methods in development of a new method and optimization of operating parameters. Our previous studies on accumulation of rhenium in plants revealed that leaves of geranium grown on 1 kg soil spiked with 1000 μg Re (as $KReO_4$ solution) extract 20 μg Re per g of row leaves for 7 days. Re content was increased to 100 μg/g of row leaves in 3 weeks. Similar experiments have been carried out with green leaves of geranium immersed in aqueous solution of rhenium. It was established that a leaf of green geranium (1-g weight) extracts 55 μg Re from a solution with $C_{Re} = 40$ μg Re mL^{-1} for 7 days, while 110 μg Re per g of row leaves for 12 days [4]. These data were used to prepare plant samples with defined Re content (see Experimental).

Optimum Operating Parameters

Ashing of Plant Sample

The effect of temperature on ashing of leaves were studied in the temperature interval from 310–520°C (see Experimental). The results are listed in Table I. It is seen that the optimum temperature interval providing further complete extraction of Re is 420–460°C. The observed lower extraction of rhenium after ashing in the range 310–410°C could be explained with incomplete ashing of the plant material (the residual ash was dark gray, the latter being an indication for the presence of carbon which is not oxidized to CO_2). The occurrence of a competitive reduction initiated by carbon is also very likely. Lower content of Re observed at temperatures above 520°C can be explained with losses of volatile Re_2O_7.

Experiments were carried out to select the best agent for rhenium extraction from the ash. It is known [7] that perrhenate is quantitatively retained on active carbon under proper conditions and can be further eluted with hot solutions of alkali. It is very

TABLE I Determination of Re in leaves of green geranium (*Pelargonium*) with known Re content at different temperature of ashing ($C_{Re} = 2.50\,\mu g/g$ of row leaves)

Sample	*T°C of ashing*	*C Re in* $\mu g\,g^{-1}$	*% of extraction*
1.	310	0.84	33.6
2.	400–410	1.67	66.8
3.	420–430	2.50	100
4.	430–470	2.50	100
5.	520	2.15	86

likely that organic substances are not completely oxidized to CO_2 and the resulting ash could contain particles of active carbon. Experiments were carried out with plant samples with known Re content (prepared according to the described procedure with leaves of acacia) and different extractants, namely: 1 and $2\,mol\,L^{-1}$ NaOH (hot and cold solutions), 6.5% NH_4OH (cold and hot solution), 96% ethanol and hot distilled water. The plant material was preliminarily ashed at 420–460°C. The results obtained indicate that rhenium is quantitatively extracted (100% recovery) from the ash with hot solutions of 1–$2\,mol\,L^{-1}$ NaOH and 6.5% aqueous solution of NH_4OH (both cold and hot). Hot distilled water and ethanol extract about 50% of rhenium. Extraction with hot $1\,mol\,L^{-1}$ NaOH was applied in all further experiments.

Direct Extraction of Re from Green Vegetation

Direct extraction of rhenium from row leaves using different extracting solutions was studied (see Experimental). The following extracting solutions were investigated: water, sodium hydroxide and hydrochloric acid. No rhenium was detected in water extracts. Alkaline extracts emitted strong foam and had green color due to saponification of the chlorophyll, yielding methanol, phytol and chlorophyllin (a or b) (the latter being magnesium dicarboxylic acids [8]). The extracts did not contain rhenium. When green leaves were treated with concentrated HCl they were partially decolorized and the resulting solution was yellowish. After alkalization the solution turned caramel. It was established that on heating in the presence of concentrated H_2O_2 the solution was gradually decolorized. Re in the solution was determined by the catalytic DMDTO method. The results revealed that this procedure provided complete extraction of Re, the latter being confirmed by parallel analysis of the same sample through ashing. Thus the procedure for analysis of green leaves involved the following steps: rhenium extraction with acid, alkalization of the acid extract, destruction of the organic matter with H_2O_2 under heating, catalytic spectrophotometric determination. The successful extraction of rhenium with hydrochloric acid can be explained as follows: chlorophyll is a complex compound, containing magnesium as a central ion and 4 pyrrole rings bound in a cyclopentane cycle. Magnesium leaves the complex when the latter is treated with acids [8]. The results of the experiments give us grounds to suggest that rhenium is accumulated in the chlorophyll through binding magnesium as $Mg(ReO_4)_2$ [9].

Extraction of Chlorophyll and Re from Green Plants with Ethanol

It is known [10] that chlorophyll is easily extracted from plants with hot ethanol. Our experiments involve extraction of chlorophyll with ethanol under heating from green leaves with known Re content prepared according to the above described procedure.

TABLE II Data of analysis of leaves of green geranium (*Pelargonium*) with known Re content. $C_{Re} = 27.43$ μg Re per g of row leaves

Sample No.	*Pretreatment procedure*	*Found Re, in* μg Re per g *of row leaves, P = 95%*	*RDS*
S-1	Ashing at 450–470°C, extraction with 1 mol L^{-1} NaOH	27.39 ± 0.08 ($n = 6$)	0.29% ($n = 6$)
S-2	Chlorophyll is extracted with ethanol followed by evaporation under IR lamp, ashing of the dry residue at 450°C, extraction with 1 mol L^{-1} NaOH	27.42 ± 0.24 ($n = 4$)	0.55% ($n = 4$)
S-3	Chlorophyll is extracted with ethanol followed by evaporation under IR + HCl + NaOH + conc H_2O_2 + T°C	27.44 ± 0.19 ($n = 4$)	0.44% ($n = 4$)
S-4	Centrifugation with HCl, filtration + NaOH + H_2O_2 + T°C	27.47 ± 0.22 ($n = 4$)	0.52% ($n = 4$)

Our experience in determination of Re in green plants reveals that one can apply two approaches to extraction of Re from chlorophyll in ethanol extracts, namely, ashing of dry chlorophyll residue or its acid digestion. Both approaches have been tested and appeared to be successful. Acid digestion is recommended for field tests, since no sophisticated equipment is necessary, while ashing procedure is suitable for laboratory use.

The results of analyses using different extraction procedures and subsequent catalytic spectrophotometric determination are listed in Table II. Data agree very well and prove that rhenium is preferably accumulated in the chlorophyll.

Applications

The developed procedures were applied in analysis of dry leaves collected in areas of ore processing as well in unpolluted areas. Rhenium content in leaves of acacia, collected in 600-m distance from the chimney of the copper works Medet was found to be 2430 ± 268-ng Re g^{-1} (3 replicates). Re content in leaves of acacia collected in the vicinity of the old open mine Asarel was 902 ± 112 ng Re g^{-1} (3 replicates). In leaves of birch collected in the resort 'Byala Cherkva' Rodopi mountain 1650 m above the sea level and in leaves of fir-tree, collected near the hut 'Zdravetz' Rodopi mountain 1350 m above the sea level the rhenium content found was 6.00 ± 0.84 ng Re g^{-1} (3 replicates) and 4.00 ± 0.77 ng Re g^{-1} respectively (3 replicates).

CONCLUSIONS

A rapid method is developed for determination of nanogram rhenium contents in plants. Laboratory standard plant samples are prepared according to original

procedure. Three procedures for pretreatment of the plant material are developed and the optimum operating conditions established: (a) a rapid laboratory analysis – through ashing the plant material and subsequent leaching of rhenium with hot sodium hydroxide solution; (b) field-analysis of plants – through extraction of chlorophyll with hot ethanol followed by its decomposition (thermal or by acid) and dissolution of rhenium with hydroxide in combination with subsequent spot test reaction with DMDTO; (c) extraction of rhenium through degradation of the chlorophyll with hydrochloric acid. It has been established that rhenium is preferably accumulated in chlorophyll. It is very likely that in weak acidic medium chlorophyll is partially decomposed yielding free magnesium ions that are associated with perrhenate atoms forming highly soluble $Mg(ReO_4)_2$. The developed procedures can be applied to biogeochemical indication of new rhenium-containing deposits. They can as well be a basis to development of a cheap and simple biotechnological scheme of extraction of Re from plants grown in areas of ore processing or mining. In this case conventional pyrometallurgy is not efficient. The developed procedures are applied in the analysis of plant material from different regions in Bulgaria.

References

[1] S.K. Kalinin, M.A. Asanov, K.D. Sulejmanov and E.E. Fain, *Vestn.AN Kaz.SSR*, **N10**, 48–51 (1986).
[2] S.K. Kalinin, E.E. Fain and E.Kh. Kim, *Izv. AN Kaz.SSR, ser.Geologicheskaya*, **N1**, 49–53 (1989).
[3] A.L. Kovalevskii, *Biogeochemical Search of Ore Deposits* (in Russian). Moscow (1984).
[4] Ognyan D. Bozhkov and L.V. Borisova, in International Conference-Modern Management of Mine Producing, Geology and Environment Protection, SGEM 2001, pp. 411–415. Balkan Press JSC, Sofia, Varna, Bulgaria (3–9 June 2001).
[5] E.Kh. Kim, Z.I. Numerova, I.N. Vitushkina, I.N. Galkina and E.E. Fain, *Izv. AN Kaz.SSR, ser. Geologicheskaya*, **N5**, 73–75 (1982).
[6] O. Bozhkov and L.V. Borisova, *Anal. Communications*, **33**, 133–135 (1996).
[7] K.B. Lebedev and T.Sh. Tyurekhodzhaeva, In: E.M. Savitskii (Ed.), *Rhenium* (in Russian), pp. 55–60. Nauka, Moscow (1964).
[8] D. Ivanov, *Organic Chemistry* (in Bulgarian), 4th. Edn., pp. 657–658. Publishing house "Nauka i izkustvo", Sofia (1957).
[9] O. Bozhkov and C. Tzvetkova, Solubility Phenomena Abstract Book, 10th International Symposium and Solubility Phenomena, St.St.Constantine and Helen Resort, Varna, Bulgaria, P 15 (21st–26th July 2002).
[10] K. Popov, B. Kitanov, Iv. Gantchev and A. Kotzev, In: Il. Yakov (Ed.), *Botanics* (in Bulgarian) Ist Edn., p. 33. Publishing house "Narodna Prosveta", Sofia (1956).