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Application of Inductively Coupled Plasma Mass Spectrometry for Multielement Analysis in Small Sample Amounts of Thyroid Tissue from Chernobyl Area

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ABSTRACT

As a result of the Chernobyl nuclear power plant accident in 1986, thyroid pathologies occurred among children in some regions of Belarus. Besides the irradiation of children's thyroids by radioactive iodine and caesium nuclides, toxic elements from fallout are a direct risk to health. Inductively coupled plasma quadrupole-based mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA) were used for multielement determination in small amounts (1-10 mg) of human thyroid tissue samples. The accuracy of the applied analytical technique for small biological sample amounts was checked using NIST Standard Reference Material oyster tissue (SRM 1566b). Almost all essential elements as well as a number of toxic elements such as Cd, Pb, Hg, U etc. were determined in a multitude of human thyroid tissues by quadrupole-based ICP-MS using micronebulization. In general, the thyroid tissue affected by pathology is characterized by higher calcium content. Some other elements, among them Sr, Zn, Fe, Mn, V, As, Cr, Ni, Pb, U, Ba, Sb, were also accumulated in such tissue. The results obtained will be used as initial material for further specific studies of the role of particular elements in thyroid pathology development.

Key words: inductively coupled plasma mass spectrometry / instrumental neutron activation analysis / multielement analysis / thyroid tissue / trace elements

INTRODUCTION

The greatest health problem after the accident at Chernobyl nuclear power plant (NPP) is thyroid pathologies among children. Studies of thyroid pathologies have been carried out for a number of years[1, 2]. However, the results obtained are insufficient to explain such a high morbidity in some regions of Belarus and to estimate its dynamics in the following years.

Taking into account the fact that the estimations of the risk of the thyroid pathology have been made on the basis of experimentally measured irradiation doses of children's thyroids by radioactive iodine, considering all the iodine nuclides (^{131}I, ^{129}I etc.) set free in the accident, as well as the external irradiation by radioactive caesium nuclides (^{137}Cs and ^{134}Cs), it becomes obvious that the explanation of this phenomenon should be sought in other mechanisms.

A possible reason can be found in ecological factors, connected with iodine deficiency and the presence of endemic goitre that has affected the trace element exchange within the thyroids.

Considerable differences in the content of trace elements in environmental components, such as foodstuffs, organs and tissues, have been demonstrated by previous studies for people living in the regions of Belarus where goitre is endemic[3]. Fallout due to the Chernobyl NPP accident, containing

complex mixtures of radioactive nuclides, as well as stable elements harmful to health (e.g., Pb, Cd, Hg, U), have caused the ecological situation to deteriorate significantly.

Deficiency, excess and imbalance of trace elements can also be the reason for pathologies. Thus, accumulation of some elements (in particular heavy metals), which possess mutagenic and carcinogenic properties, in the organism of a human being is a direct risk to health. They can promote thyroid disease initiated by radioactive iodine.

To study the contents of vital and toxic elements and radionuclides in thyroid tissue highly sensitive and microscale analytical procedures are required that combine high sensitivity with the possibility of simultaneous multielement determination in thyroid tissues, but only small amounts of sample materials (up to 1 mg dried tissue) are available. The measurements of such small samples can also be important if only a small volume of thyroid sample from a human being is obtained by a puncture.

Among the available trace analytical techniques the powerful inductively coupled plasma mass spectrometry (ICP-MS) seems most suitable as a very sensitive multielemental analytical method with a wide coverage permitting the multielement determination of major, minor and trace element concentrations and their isotope abundances in different types of samples[4-6]. The lowest detection limits were measured in the sub - pg l^{-1} concentration ranges as demonstrated for different long-lived radionuclides (e.g., ^{239}Pu, ^{237}Np, ^{230}Th or ^{241}Am) in aqueous solutions by double-focusing sector field ICP-MS and ultrasonic nebulization for solution introduction in the inductively coupled plasma[7]. In the more frequently used quadrupole-based ICP-MS (ICP-QMS) the detection limits are some orders of magnitude higher due to lower sensitivity and higher background. The detection limits were determined by ICP-QMS on aqueous solutions using ultrasonic nebulization up to 10 pg l^{-1} [8]. The excellent element sensitivity of ICP-MS permits ultratrace analysis in biological samples (after chemical digestion) down to the ng g^{-1} concentration range and lower[9].

The aim of the present work is to develop and apply a microanalytical technique using a quadrupole-based ICP-MS for studies of element distribution of the minor and trace elemental concentrations in human thyroid samples obtained during operations on people living in the Chernobyl area.

EXPERIMENTAL

Instrumentation of ICP-MS

A Perkin Elmer ELAN 6000 quadrupole-based mass spectrometer (Perkin Elmer Sciex, Ontario, Canada) was used to measure concentrations of minor and trace elements in aqueous solution of the digested thyroid tissues. The mass resolution (m/ Δm) of the ICP-QMS was 300.

For solution introduction in the inductively coupled plasma a microconcentric MicroMist nebulizer (Model MicroMist AR30-1F02) with a minicyclonic spray chamber (both from Glass Expansion, Pty. Ltd., Camberwell, Victoria, Australia) with solution uptake rates of 160 and 320 $\mu l\ min^{-1}$ were used. The nebulizer gas flow rate was controlled using an original ELAN 6000 mass flow controller (MKS Instruments). Solution aspiration was performed with a peristaltic pump (Perimax 12, Spetec GmbH, Erding, Germany). The experimental parameters of the mass spectrometric measurements are summarized in Table 1.

Samples

The thyroid tissues were obtained from operations on persons in hospitals in Minsk, Belarus. After molecular, cellular and biological studies the thyroid samples were subjected to trace element analysis. For accuracy control during sample digestion and measurement 20 mg samples of NIST Standard Reference Material 1566b Oyster Tissue were used.

Reagents

The subboiled nitric acids and 30% hydrogen peroxide were used, both of supragrade purity from Merck (Darmstadt, Germany). Single element and multielement standard stock solutions for the

calibration procedures were obtained from Merck (Darmstadt, Germany) and from the National Institute of Standards and Technology (NIST). For all dilutions deionized Milli-Q water (18 MΩ) was obtained from a Millipore Milli-Q-Plus water purifier.

Table 1. Experimental parameters used for Elan 6000 ICP-QMS

Rf power (W)	1350	
Cylindrical lens potential (V) (automatic setting)	10.2	
Dwell time (ms)	500	
Mass range	23-238 u	
Scanning mode	peak hopping	
Optimization	maximum $^{137}Ba^{+}$ intensity	
Detection system dead time (ns)	53	
Coolant gas flow rate (l min^{-1})	13.5	
Auxiliary gas flow rate (l min^{-1})	0.7	
Nebulizer type	MicroMist	
Spray chamber	Mini-cyclonic	
Solution uptake rate (ml min^{-1})	0.32	0.16
Nebulizer gas flow rate (l min^{-1})	0.76	0.81
No. of replicates	10	8
Measurement time (min)	4.0	3.2

Sample preparation

To analyse such a small amount of biological tissue a microwave-induced digestion procedure using NIST Standard Reference Material oyster tissue (SRM 1566b) was developed. 20 mg of SRM samples were digested by microwave induction in a mixture consisting of 0.5 ml concentrated subboiled nitric acid and 0.2 ml 30% hydrogen peroxide. In the same way human thyroid samples were digested. The biological samples were digested in small vessels (6 ml and 3 ml) in a microwave oven (Microwave Activated Reaction System Mars 5, CEM Corporation, USA).

Measurement procedure

The optimization of the experimental parameters (see Table 1) was performed using the maximum ion intensity of $^{137}Ba^{+}$. ^{103}Rh was used as an internal standard element for quantitative ICP-MS measurements. The ICP-MS was flushed with a 2% HNO_3 solution for 10 min between aliquot measurements especially to reduce the memory effect of iodine. NIST SRM 1566b was used for quality control during routine measurements of thyroid samples. This oyster tissue reference sample was digested and measured together with all the nine thyroid samples in one digestion cycle. In this way, three independent measurements of the SRMs mentioned above, including independent sample digestions, were done.

Disturbing molecular ion interferences (e.g., $^{40}Ca^{16}O^{1}H^{+}$, $^{42}Ca^{16}O^{+}$, $^{40}Ca^{18}O^{1}H^{+}$, $^{44}Ca^{16}O^{+}$) on atomic ions of analyte ions (e.g., $^{57}Fe^{+}$, $^{58}Ni^{+}$, $^{59}Co^{+}$, $^{60}Ni^{+}$) in ICP mass spectra were considered by correction of experimental values using measured molecular ion intensities in synthetic matrix-matched solutions under the given experimental conditions.

Instrumental neutron activation analysis (INAA)

Sample irradiations were carried out at the FRJ-2 reactor. The use of the k_0 method of INAA as applied in the Central Department for Analytical Chemistry of Research Centre Juelich [10] allowed the accuracy to be improved and did not require the use of standards thus opening up the possibility of absolute measurement.

The irradiation of the samples with neutrons was carried out in the In-Core Irradiation System

(central channel) of the FRJ-2 reactor with the density of the thermal neutron flux being $\Phi = 8?10^{13}$ $cm^{-2}s^{-1}$ for 1 hour For the measurement of the induced γ-activity and γ-spectrum processing, a Genie-PC Spectroscopy System (Model S400) with coaxial semi-conductor Ge-detectors from Canberra Packard was used. Every sample was measured twice after "cooling" times of 1 day and 7 days.

METHOD

Optimization of the mass spectrometric method for the determination of element concentrations in small amounts of biological samples

The optimization of experimental parameters for the analysis of digested biological samples was performed with respect to the maximum sensitivity of $^{137}Ba^{+}$ as described above. The results of the optimization procedure on standard solutions using a microconcentric MicroMist nebulizer for two different solution uptake rates (160 and 320 µl min^{-1}) are summarized in Table 2. The sensitivities in ICP-QMS were observed in the range of 25-106 MHz/ppm for the solution uptake rates of 0.32 mL/min and of 22 – 90 MHz/ppm for 0.16 mL/min. The amount of each analyte for different solution uptake rates comprises 12.8 ng (10µg/L of analyte x 4 min x 0.32 mL/min) and 4.8 ng (10µg/L x 3 min x 0.16 mL/min), respectively.

Due to the multielement capability and the good sensitivity of ICP-MS the analytical method developed allows the determination of the concentrations of most essential and toxic elements.

Table 2. Sensitivities (MHz/ ppm) for some elements determined at different solution uptake rates.

Analyte	Mass (u)	Solution uptake rate (ml min^{-1})	
		0.32	0.16
Na	23	25.5	22.5
K	39	106.4	90.1
Mn	55	40.2	33.8
Sr	88	56.0	46.6
Rh	103	55.3	45.6
Ce	140	50.7	39.2
U	238	60.0	43.1

Detection limits of elements

The detection limits in biological samples were determined from the mass spectrum of the (matrix-matched) blank solution using the 3σ criterion (the detection limit is given by $m_b+3\sigma_b$, where m_b is the mean value of the blank measurements and σ_b the standard deviation of five independent measurements of the blank value) considering the measured ion intensities in standard reference material using the known element concentrations (Table 3). These detection limits in biological samples were affected by blank values of ICP-MS and chemicals and disturbing interferences of molecular ions on atomic ions of analyte. The detection limit of iodine using conventional pneumatic nebulizers (such as Meinhard, cross-flow or microconcentric nebulizer) is poor due to the loss of volatile iodine during the sample introduction and relatively high first ionization potential. In order to improve the sensitivity for determining iodine a special apparatus for introducing elemental iodine via the gas phase into the Ar plasma of ICP was proposed in our lab[10]. The sensitivity for iodine determination was improved by a factor of 30 –70 compared to the conventional Meinhard nebulizer and the detection limits were decreased to 100 pg g^{-1} (for ^{129} I) in aqueous solutions. This analytical technique of iodine determination

is combined with a loss in multielement capability. Because the iodine concentration in thyroid samples is in the $\mu g\ g^{-1}$ range we used the commercial solution introduction (pneumatic nebulization) in order to allow the simultaneous multielement determination of minor and trace elements by ICP-QMS.

The detection limits of ICP-QMS are compared with those for INAA calculated from minimal detectable activities achieved under experimental conditions described[11] (see Tab. 3). For different elements (e.g., Cd, Cu, Hg, Ni, Pb, Rb, Sr, U) significantly lower detection limits were observed in ICP-QMS.

Table 3. Detection limits ($\mu g\ g^{-1}$) in ICP-QMS and INAA on solid thyroid tissue.

Element	ICP-MS	INAA
As	0.002	0.003
Ba	0.03	0.6
Bi	0.004	-
Ca	80	20
Cd	0.0009	0.05
Ce	0.002	0.02
Co	0.007	0.002
Cr	0.3	0.02
Cu	0.1	3
Fe	4	2
Hg	0.007	0.2
I	70	0.01
K	3	0.6
Mg	5	3
Mn	0.07	0.03
Mo	0.006	0.06
Na	1	0.02
Ni	0.1	-
Pb	0.005	-
Rb	0.002	-
Sb	0.0006	0.003
Se	0.04	0.03
Sn	0.04	-
Sr	0.05	0.6
U	0.0002	0.01
V	0.005	0.01
Zn	0.2	0.05

Experimental analysis of certified reference material SRM 1566a Oyster Tissue by ICP-QMS

The accuracy of the ICP-QMS measurements was checked by analysing certified reference material SRM 1566a Oyster Tissue.

In Table 4 the analytical results obtained for 3 independent ICP-QMS measurements, including 3 sample digestions, are compared with the certified values. A good agreement with certified values for the most of the elements was found considering the small sample amount. Inhomogeneities of SRMs, which were found by additional SIMS measurements in our laboratory, could reduce the accuracy and precision of the determination of element concentrations.

Comparison of ICP-QMS results with those of INAA on thyroid samples

Furthermore some thyroid samples were investigated using instrumental neutron activation analysis (INAA) in comparison to ICP-QMS. The advantage of INAA in comparison to other analytical methods which require digested solution is the possibility of analysing the sample without any preliminary chemical treatment, so the contamination problems can be reduced to a minimum. Therefore INAA can be used as a reference method to check the accuracy of ICP-MS.

But the - in comparison to ICP-QMS including sample preparation - more time-consuming and very expensive INAA requires powerful irradiation facilities, a specially equipped laboratory and produces radioactive waste. E.g. the analysis of such a wide range of trace elements in 10 thyroid samples using ICP-MS described above requires approximately 8 hours, including sample digestion, sample and standard solution preparation, instrument optimization, calibration and measurement. The same analysis via INAA requires at least two irradiations in order to determine elements, using short- and long-lived radionuclides, "cooling" the samples and several successive measurements. The final results can often be obtained only after 10 days. Furthermore, a number of elements (Ni, Cu, Sn, Sr, Cd, Hg, Pb) cannot (or only with difficulties) be determined using INAA. Therefore INAA is less suitable for routine multielement analysis in a multitude of biological samples.

The results of trace element measurement in the same thyroid sample using first INAA as a non-destructive method and then ICP-MS after sample digestion are compared in Table 5. However, only a few elements with long-lived radionuclides could be measured with INAA at the FRJ -2 because of the lack of irradiation facilities with high-speed transportation of samples for the analysis of short-lived nuclides.

The element concentrations measured by two independent analytical methods varied by less than 6% except those for Zn (16%) and Se (24%). Such comparison of results obtained with two analytical methods based on different physical principles and under different sample preparation schemes was useful during development of the analytical procedure.

Table 4. Comparison of trace element concentrations ($\mu g\ g^{-1}$) measured by ICP-QMS and certified values on NIST SRM 1566a Oyster Tissue

Element	Measured value	Certified value
Ag	1.66±0.11	1.68±0.15
As	12.1±1.9	14.0±1.2
Ca	1900±200	1960±190
Cd	3.8±0.4	4.15±0.38
Ce	0.33±0.09	0.4
Co	0.44±0.09	0.57±0.11
Cr	1.8±0.3	1.43±0.46
Cu	64±4	66.3±4.3
Fe	550±60	539±15
Hg	0.054±0.003	0.0642±0.0067
I	4.4±2.4	4.46±0.42
K	7800±900	7900±470
Mg	1100±100	1180±170
Mn	12±1	12.3±1.5
Na	4300±400	4170±130
Ni	2.0±0.5	2.25±0.44

Pb	0.35±0.04	0.371±0.014
Rb	3.1±0.2	3.0
Sb	0.009±0.003	0.01
Se	1.8±0.5	2.21±0.24
Sn	2.2±0.6	3.0
Sr	11±1	11.1±1.0
U	0.14±0.02	0.132±0.012
V	4.9±0.5	4.68±0.15
Zn	840±30	830±57

RESULTS

The minor and trace elements have been measured in a multitude of human thyroid samples. The measured concentrations of trace elements differ from sample to sample to a considerable extent. As was shown in[11] the thyroid tissue affected by pathology is characterized by a higher calcium content in comparison to normal tissue. Approximately the same calcium concentration range was also found in pathologically changed thyroids in this work, which points to the fact that in such tissue sclerotic processes take place even including calcinosis. One of the reasons for these processes could be the influence of biogeochemical features of the regions affected with radioiodine during the Chernobyl NPP accident, such as a deficiency of iodine in food and water.

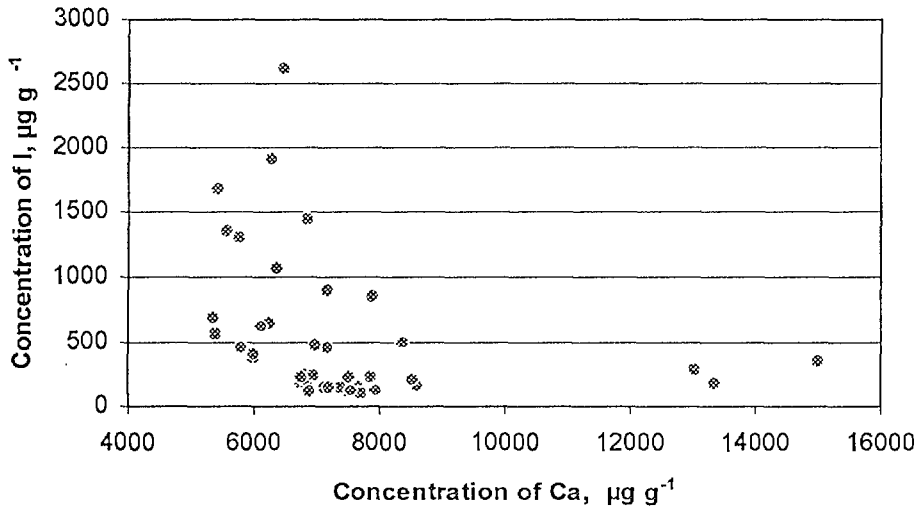

Fig.1. Dependence of iodine concentration on calcium concentration in analysed human thyroid tissues.

As is seen from Fig. 1, thyroids containing less iodine are characterized by a higher concentration of calcium. The follicular cells of thyroid in the regions where goitre is endemic underwent a maximum effect of radioactive isotopes of iodine. At small irradiation doses a full recovery of the affected sections would occur. However, because of the non-uniform spatial distribution of radioiodine there could be places with rather high local doses, in which postradiation changes (formation of sclerotic sections) could take place. Owing to their biological peculiarities, the incorporation of the increased quantities of a number of trace elements occurs in these sclerotic sections. Some of them can be toxic and in turn promote diseases.

It was found in this study that tissues with a higher concentration of Ca are characterized in general by

higher contents of Sr, Zn, As, Cr, Ni, Cd, Pb, U. In Fig. 2-4 the dependence of Pb, As and U concentration on Ca concentration are presented. Of course, the concentrations of trace elements strongly depend on the environmental conditions and people's diets. However, the correlation between calcium and elements presented here is clearly seen. The established changes of chemical composition could promote pathology development due to the mutagenic and cancerogenic properties of some toxic elements (As, Cr, Ni, Pb, U).

CONCLUSION

ICP-QMS was applied for the routine determination of minor and trace elements in 90 thyroid samples. The high sensitivities achieved at small solution uptake rates allowed ultra-small quantities of biological tissue down to 1 mg to be analysed. Measurement accuracy was verified by measurements of NIST standard Reference Materials.

In comparison to other analytical methods, e.g. neutron activation analysis, which requires several sample irradiations to cover such a wide range of elements, ICP-MS is faster and cheaper (no irradiation facility such as a nuclear reactor is required), and is well suited for routine multielement measurements of many small samples. Moreover ICP-MS permits a number of important trace elements to be determined, among them Ni, Cu, Sn, Sr, Cd, Hg, Pb, in addition to INAA.

ICP-QMS allows the survey analysis of the chemical composition of tissue sections with different morphological structure and the study of the connection between tissue structure and chemical composition. The results obtained will be used as initial material for further specific studies of the role of particular elements in thyroid pathology development.

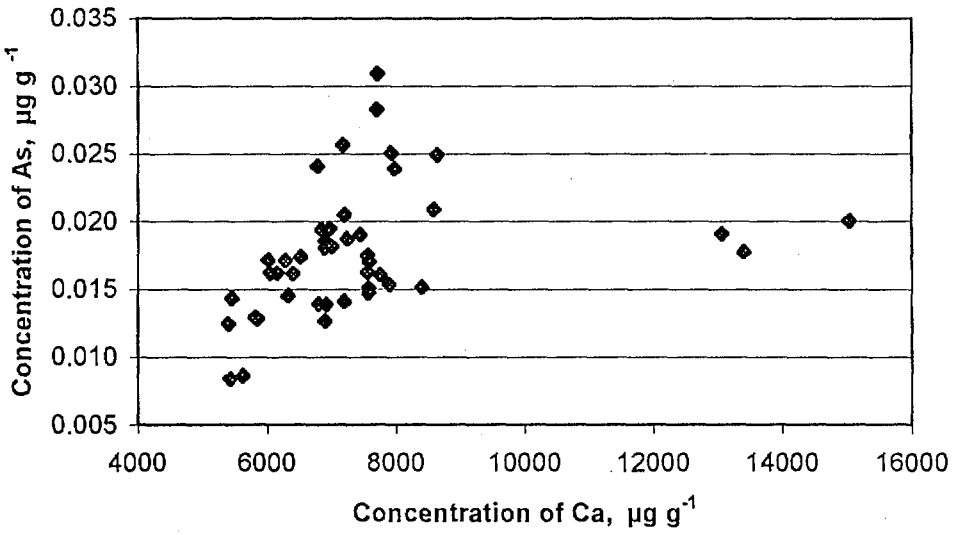

Fig.2. Dependence of As concentration on Ca concentration in analysed human thyroid tissues.

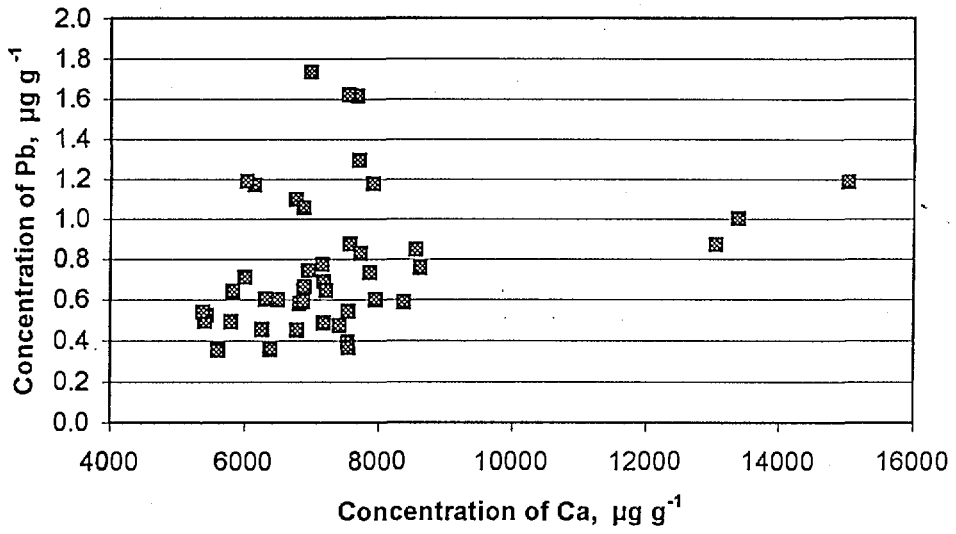

Fig.3. Dependence of Pb concentration on Ca concentration in analysed human thyroid tissues.

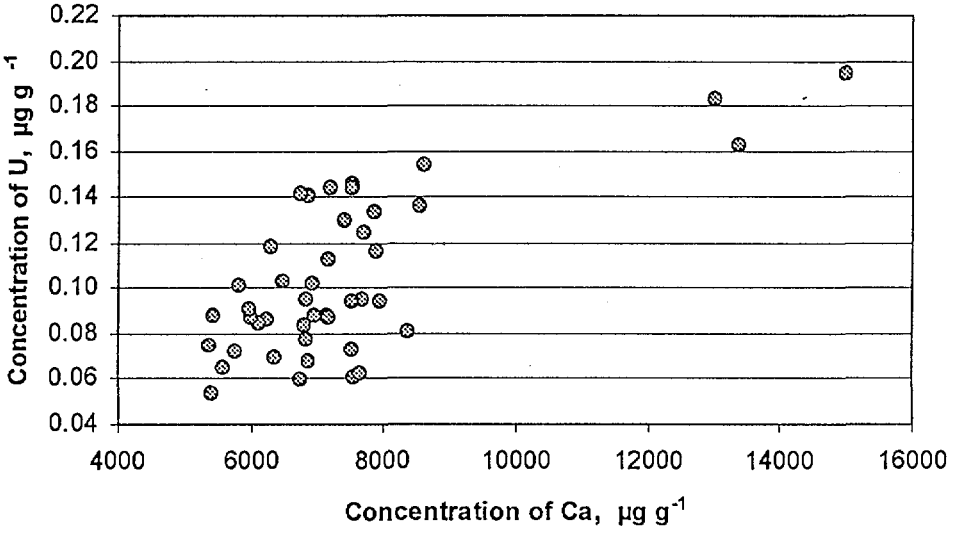

Fig.4. Dependence of U concentration on Ca concentration in analysed human thyroid tissues.

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