Sofia University "St. Kliment Ohridski"

Faculty of Chemistry and Pharmacy

Department of Analytical Chemistry

Laboratory of Analytical Atomic Spectrometry

ABSTRACT

of dissertation for awarding of

the degree of Doctor of Philosophy to

Valentin Georgiev Georgiev

Methods for the determination of uranium in water and food

Academic advisors:

prof. Irina Karadjova, PhD   Assoc. Prof Ivanka Dakova, Ph.D

Professional field: 4.2 Chemical Sciences (Analytical Chemistry)

Sofia, 2024

The dissertation contains 117 pages, illustrated is with 18 figures,28tables. The bibliographic reference covers 188 literature sources.

The PhD thesis was discussed, accepted and directed for defense in front of a scientific jury by the Council of the Analytical Chemistry Department at the Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski", held on 25.01.2024

The public defense of the PhD Thesis will take place on ……………………….. from ………… h in the Meeting Hall of the Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", Sofia, 1, J. Boucher" Blvd.

The materials are available in the office of the Faculty of Chemistry and Pharmacy at Sofia Unuversity "St. Kliment Ohridski", 1, J. BoucherBlvd., Office 107, and on the websiteof FCP.

List of abbreviations used

ACN – acetonitrile

AIBN – azobisisobutyronitrile

EDTA – ethylenediaminetetraacetic acid

ICP-MS – Inductively coupled plasma mass spectrometry

ICP-OES – inductively coupled plasma optical emission spectroscopy

IIP – Ion-imprinted polymer

IPDM – intraparticle diffusion model

LOD – limit of detection

LOQ – limit of quantification

MAA – Methacrylic acid

PAR – 4-(2-Pyridylazo)resorcinol

PFO – pseudo-first-order model

PSO – pseudo-second-order model

SEM – scanning electron microscopy

SPE – Solid-phase extraction

TMPTMA – Trimethylolpropane trimethacrylate

Contents

I. Introduction

Uranium is the 49th most abundant element in the earth's crust with a similar abundance to tin and arsenic. It is relatively abundant in seawater: ranking 26th, similar to that of arsenic and vanadium. Uranium is lithophile, reflecting its tendency to remain close to the earth's surface and be strongly bound to oxygen. It occurs naturally in soils, minerals, rocks and water, and as a result of anthropogenic contamination it can be detected in phosphate fertilizers, mine waste, fly ash from power plants and as a result of military use. Uranium is a radiotoxic and hemotoxic element that exists in the environment in the form of three radioactive isotopes: ^{238}U (99.27%), ^{235}U (0.72%) and ^{234}U (0.0057%). In natural undisturbed uranium, the radioactivity contributions of ^{238}U, ^{235}U, and ^{234}U are 47.3%, 2.2%, and 50.5%, respectively.

Uranium occurs in oxidation states III, IV, V and VI, but the dominant species in the nature are IV and VI. Uranium is one of the main contributors to the earth's natural radioactivity. Human exposure to environmental uranium is considered a radiological health risk, although there are few epidemiological studies that have been able to demonstrate the resulting harm, even in an occupational context [1]. Drinking water is estimated to provide about 70% of the amount of U in the standard human diet [2]. Health effects of chemical exposure include nephritis (kidney disease) and changes in bone structure [3-8]. Ingested uranium is considered less toxic than inhaled uranium, partly due to the relatively low gastrointestinal absorption of uranium compounds (about 1%) [9]. Animal and human studies have shown that the bioaccumulation of uranium and its toxicity depend on the chemical species. In general, uranium bound in calcium-carbonate complexes has lower toxicity. Epidemiological studies and toxicity tests show a significant difference in the concentration levels of uranium causing harmful effects. As a result, the accepted permissible concentrations of uranium in environmental samples, food and beverages vary in a very wide range from 2 µg/L for Japan to 1700 µg/L for Russia.

The most significant research to limit human exposure to uranium is related to established permissible concentrations for uranium content in drinking water. The latest results of the studies of the World Health Organization (WHO) show that the chemical toxicity of uranium is a significant potential risk for human health. The World Health Organization (WHO) has established a Tolerable Daily Intake for uranium of 0.6 µg/kg body weight per day. This value is based on the lowest observed adverse effect level for

nephrotoxicity (degenerative lesions in the proximal convoluted tubules of the kidney). Accumulated data from epidemiological studies in human populations and further statistical processing, as well as data on average intake of drinking water, lead to the conclusion for a permissible value for the content of uranium in drinking water of 30 μg/L (fourth edition, WHO, 2017). The same value is suggested by the EU Directive for Uranium in Water Intended for Human Consumption as well as the US-EPA Maximum Contaminant Level (MCL) for Uranium in Drinking Water. The standard for U in drinking water in Canada is 20 μg/L; in Germany this value is 10 μg/L [10].

Uranium concentrations in surface waters vary widely depending on local geology and land use: from <0.002 to 21.4 μg/L for Europe. Isolated high concentrations are observed in areas with known uranium mineralization, for example the Essonne valley - France; Southern Poland, Southwestern Spain. Groundwater, as a rule, contains significantly higher amounts of U than surface water. Concentrations vary considerably and are often above the permissible limits for drinking water. Uranium has not been assessed as a priority pollutant for the aquatic environment (Water Framework Directive, 2000), but it is accepted as a specific pollutant for most member countries. Accepted environmental quality standards range from 0.15 μg/L to 24 μg/L depending on the methodology used and laboratory ecotoxicity data. For Bulgaria, the accepted value is 5 μg/L (Regulation H-4).

The significant spread of this toxic element in the environment is due to the development of nuclear energy and the mining industry for the extraction of uranium. Uranium extraction technology in 50% of uranium mines is based on in situ extraction with sulfuric or hydrochloric acid, which leads to a significant spread of soluble uranium species in groundwater. The uranium mines closure without their proper remediation leads to further spread of uranium in surface waters.

The strict quality control of both the environment and the products intended for human consumption requires reliable analytical methods for uranium determination. The developed analytical procedures should meet a number of requirements: to be fast and to allow determination in a wide concentration range, to ensure accurate results and to be applicable in routine analytical practice.

The analytical methods used for uranium determination can be divided in two large groups:

- ✓ direct methods for uranium determination using an analytical method with high sensitivity

✓ methods based on preliminary separation and enrichment of uranium with subsequent instrumental measurement. These methods allow to achieve the lower limits of determination necessary for the assessment of background uranium contents in the environment. In addition, these methods allow the determination of the chemical species of uranium in the sample using suitable sorbents with high selectivity.

The current PhD thesis is focused on the investigation of the possibilities for uranium determination by:

- Direct measurement of uranium concentration by ICP-MS, which includes the study of matrix interferences observed in the presence of macrocomponents in natural waters, as well as the possibility for their corrections and the ability to obtain accurate results.

- Determination of uranium after enrichment by solid phase extraction and instrumental measurement. A new sorbent: an ion imprinted polymer has been synthesized and characterized for the selective concentration of uranium. Its application will allow the determination of uranium at low concentration levels by applying cheaper and more accessible instrumental methods than ICP-MS.

II. Aims and Tasks

The aim of the thesis is to propose and optimize suitable methods for the routine determination of uranium in a wide concentration range, which are characterized by low determination limits, corresponding to the nationally or internationally accepted permissible uranium contents, as well as corresponding with possibilities to determine background uranium concentrations. The methods should allow rapid analysis of a significant number of samples for the purposes of drinking water monitoring, monitoring of food, beverage and environmental samples.

The most suitable method for drinking water and environmental samples monitoring is the direct quadrupole ICP-MS - it provides the required low limits of determination, it has a long linear part of the calibration curve, it is characterized by high speed of analysis, and possibility for automated sample feeding. The method is relatively expensive and implies a high cost of analysis. In order to ensure determination of background concentrations of uranium and to establish compliance with the surface water quality standard (according to

Regulation H-4, 2012, Directive 2009/90/EU), the method should be characterized with a determination limit in the range 0.05 - 1.6 µg /L or it is necessary to be applied a preliminary procedure for uranium enrichment. In the last case, it is possible to be used a less expensive spectral method such as ICP-OES.

Bearing in mind these aspects, as well as the fact that the obtained results and the developed analytical procedures will be applied in the practice of the laboratories controlling uranium content, the following tasks of the presented thesis have been formulated:

1. **Application of ICP-MS for the determination of uranium in drinking, bottled, groundwater and surface water intended for human consumption.**
   The following tasks should be completed to reach this goal:

- Investigation of matrix interferences in the determination of uranium in highly mineralized waters by ICP-MS.

- Correction of estimated interferences by applying internal standard calibration method, including selection of appropriate internal standard.

- Application of the proposed procedure for the determination of uranium in different types of water on the territory of Bulgaria.

2. **Development of an analytical procedure for the selective enrichment of uranium with subsequent instrumental determination by ICP-MS, ICP-OES.**
   The following tasks should be completed to reach this goal:

- Synthesis and characterization of a new U(VI) ion-imprinted polymer providing high selectivity of uranium sorption.

- Evaluation of its extraction efficiency and selectivity.

- Optimization of conditions for quantitative sorption/desorption of U(VI) during solid-phase extraction with U(VI) ion-imprinted polymer.

- Elucidation of the sorption mechanism of U(VI) ions on U(VI) ion-imprinted polymer.

- Development of an analytical procedure for solid phase extraction of U(VI).

- Application of the procedure for the determination of uranium in waters, wines and honey. Validation of the developed procedures.

III. Materials and methods

1. Synthesis of U(VI) ion-imprinted polymer

The scheme for the synthesis of the imprinted and non-imprinted polymer gels is presented in Figure 1.
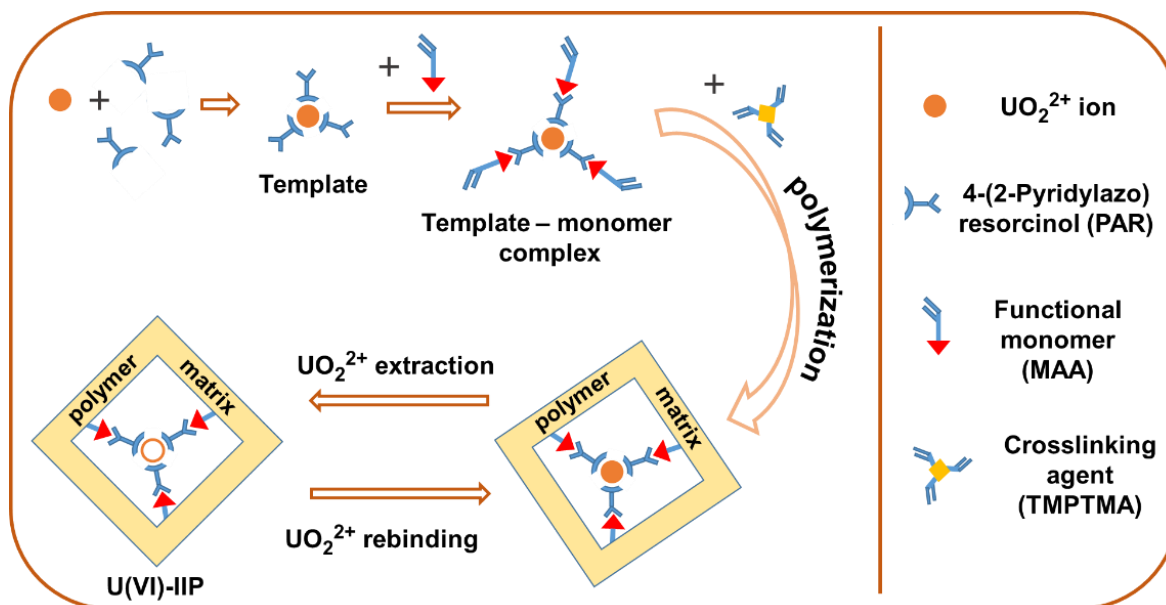

Figure 1. Schematic diagram of U(VI) ion-imprinted polymer gel synthesis

The U(VI) ion-imprinted polymer was synthesized by the dispersion copolymerization method using 25 mL ACN as porogenic solvent, 70 mg AIBN as initiator, 2.16 mmol MAA as functional monomer, and 0.96 mmol TMPTMA as crosslinker in the presence of U(VI) complex) with PAR (U(VI)-PAR, 0.12 mmol) as template. The resulting solution was saturated with dry nitrogen for 15 minutes. The copolymerization process was carried out at 60 ºC for 24 hours. As a next step, the polymer particles were separated by centrifugation of the resulting suspension and washed with ACN to remove unreacted monomer molecules. The uranyl ion was removed from the polymer matrix by multiple sequential elution with 3 mol/L HCl. Elution was repeated until the concentration of uranium in the eluate reached values lower than the limit of quantification (LOQ) when measured by ICP-OES. The control non-imprinted polymer (NIIP) was synthesized in an identical manner but in the absence of the complex of U(VI) with PAR. Finally, the imprinted and non-imprinted polymers were dried at 60 ºC in a vacuum dryer.

2. Analytical procedures

2.1 Analytical procedure for the direct determination of uranium by ICP-MS

The daily optimization of the instrumental parameters was carried out according to the manufacturer's recommendations in order to achieve the requirements and the maximum signal-to-noise ratio. The following isotopes were selected for the experiments: [139]La, [185]Re, [205]Tl and [238]U. All water samples were filtered and acidified with conc. HNO3 prior to analysis. The internal standards used were added so that in the final solution their concentration was 10 µg/L. The spectrophotometric measurement of the uranium content was carried out according to an established procedure [12] after concentration with the proposed new sorbent.

2.2 Assessment of the extraction efficiency of the sorbent

The extraction characteristics of the sorbents were studied during solid-phase extraction in static mode. 200 µL of a working solution containing U(VI) with a concentration of 100 mg/L was added to 10 mL of the test solution so that the U(VI) content of the final solution was 20 µg. The desired pH value of the solution (in the range 3-9) is achieved by adding solutions of 1 mol/L HNO3 or 1 mol/L NH4OH. 100 mg of polymer particles from the synthesized sorbents were added to the obtained solution, after which the solid phase extraction was carried out. The samples were mixed continuously using a shaker for 30 minutes. Subsequently, the suspension was centrifuged at 5000 rpm. in 20 minutes. The solution obtained after sorption (effluent) was decanted and separated for determination of U content by ICP-OES. To evaluate the sorption stage, the degree of sorption was calculated according to the formula:

$$D_S = \frac{A_i - A_{eff}}{A_i} \times 100 \ ,$$

where Ai is the mass of U(VI) ions in the starting solution, µg;

A_{eff} is the mass of U(VI) ions in the effluent, µg.

The U(VI)-loaded polymer particles were then washed twice with double-distilled water and an eluent was added to them. This is followed by another stirring for 30 minutes and centrifugation. The eluate was decanted and its uranium content was measured by ICP-OES. To evaluate the degree of desorption, the degree of elution was calculated according to the following formula:

$$D_E = \frac{A_{el}}{A_i - A_{eff}} \times 100 \, ,$$

where A_{el} e is the mass of U(VI) ions in the eluate, µg.

2.3 Investigations on the mechanism of U(VI) sorption onto U(VI)-IIP

The adsorption capacities of the synthesized U(VI)-IIP and NIIP were determined using the following procedure: 100 mg of sorbent was added to 10 mL of a solution containing U(VI) of increasing concentration (from 2 to 30 mg/L) under optimal experimental conditions and a temperature of 25 ºC. The equilibrium concentration of U(VI) after the adsorption process was measured by ICP-OES. The maximum adsorption capacity of U(VI)-IIP and NIIP ($Q_{max,exp}$), defined as the amount of uranyl ions adsorbed per gram of sorbent, was calculated by the following equation:

$$Q_{max,exp} = \frac{(C_0 - C_e) \cdot V}{m} \, ,$$

where $Q_{max,exp}$ is the mass of sorbed U(VI) per gram of sorbent, mg/g;

V is the volume of the solution, L;

m is the mass of the sorbent, g;

C_0 and C_e are the initial and equilibrium concentrations of U(VI) in the solution, mg/L.

The kinetics of the sorption/desorption processes was studied according to the following procedure: 100 mg of sorbent was added to 10 mL of a pH 7 solution containing 20 µg of U(VI) ions. A solid-phase extraction was carried out, as the sorption time, resp. desorption, was varied in the time interval 5 - 45 minutes. The temperature during the experiments was kept constant: 25 ºC. U content in effluents and eluates after sorption and elution was measured by ICP-OES.

2.4 **Assessment of matrix interferences**

The potential matrix interferences on the determination of uranium were studied by mixing 10 mL of a solution containing 20 µg U(VI) with a solution containing macro components typically found in surface and groundwater (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, SO_4^{2-}, a mixture of humic acids) at different concentration levels. Solid-phase extraction was carried out according to procedure 2.2. under the established optimal parameters. The concentration of U in the eluate and effluate was measured by ICP-OES.

2.5 **Analytical procedure for the determination of uranium in waters after enrichment on U(VI)-IIP**

The water sample of 30 mL was quantitatively transferred to a centrifuge tube and the pH of the solution was adjusted to 7 by adding 1 mol/L HNO3 or 1 mol/L NH₄OH. Sorbent 100 mg was added to the solution and mixture stirred for 30 minutes. The resulting suspension was centrifuged, the polymer particles were separated and washed twice with double-distilled water. The sorbent particles were eluted with 2 mL of 2 mol/L HCl. The U content in the eluate was measured by ICP-OES or ICP-MS depending on the expected concentration.

2.6 **Analytical procedure for the determination of uranium in wine after concentration on U(VI)-IIP without prior decomposition of the wine sample**

The wine sample (white, red, rosé) of 20 mL was transferred into a centrifuge tube, and the pH of the solution was adjusted to 7. The sorbent amount of 100 mg was added to the wine sample and the mixture was placed on a shaker mixer for 30 minutes. After centrifugation the sorbent particles were washed twice with doubly distilled water, 2 mL of 2 mol/L HCl were added and mixture stirred for 30 minutes. After centrifugation the eluted U was measured by ICP-OES or ICP-MS.

2.7 **Analytical procedure for the determination of uranium in honey after U enrichment on U(VI)-IIP, without prior decomposition of the honey sample**

The sample of 5 g of bee honey was dissolved under low heating in 100 mL of distilled water (the possible inhomogeneity of the sample is taken into account). An aliquot sample of 20 mL was taken from the prepared solution for uranium determination. The sample was transferred to a centrifuge tube and the pH of the medium was adjusted to 7. The procedure already described was then carried out.

IV. Results and Discussion

1. Direct determination of uranium by ICP-MS

1.1. Optimization of ICP-MS instrumental parameters for determination of uranium content in environmental samples

Several parameters, such as the dwell time, the number of readings per replicate, the number of replicates and the sample flow rate were optimized using solution of uranium with concentration 10 µg/L. Results obtained showed that the optimal parameters are 50 ms dwell time, 10 scans per replicate, 10 replicates per one sample and a sample flow of 1.1 mL/min.

The attenuation system is calibrated with two solutions containing both elements of interest (analyte and internal standards being tested). These two solutions should differ in their concentration at least 50 times, the analyte concentration has to be 50 times more diluted than the concentration of internal standard. Also, the analyte concentration must fall within the range of the detector without attenuation. Due to these considerations, concentrations of 1 and 50 µg/L were chosen for both solutions.

1.2. Development of a procedure for direct ICP-MS determination of uranium content in drinking, surface and groundwater

First calibration of uranium is performed with calibration standards of: 0, 10, 25 and 50 µg/L and the calibration line obtained is $Ir = (4269 \pm 325) + 181400 * conc$, where concentration is expressed in µg/L, regression coefficient achieved is 0.99997. Calibration curve constructed for lower concentration range e.g. 0.05–10 µg/L showed almost identical calibration equation.

Based on the resulting calibration curve, a limit of quantification (as a sum of the blank value and 10 times its standard deviation value) of 0.05 µg/L was calculated.

The effect of macro elements Na, K, Ca and Mg as nitrates on the uranium measurement was studied at concentration levels of 50 and 100 mg/L of Na, K, Ca and Mg. Results obtained are depicted in Fig. 1 for Ca and Mg as examples. The slope of the calibration curves decreases significantly in the presence of 50 mg/L and even more significantly in the presence of 100 mg/L of the studied matrix elements. Most likely, the high mineralization leads to a decrease in the ionization efficiency of the argon plasma. Also, the physical effects, such as the partial blocking of the openings of the "sampler" and "skimmer" cones, as well as the presence of the so-called "space charge" effect (due to electrostatic repulsion between cations present in the interface region) contribute most to this phenomenon.

The most noticeable decrease in the slope is observed for the salts $Ca(NO_3)_2$ and $Mg(NO_3)_2$, which is most likely due to the significantly higher boiling point of their oxides, compared to the boiling point of Na_2O and K_2O. These temperatures are respectively for MgO: 3600 K, for CaO: 2850 K, for Na_2O: 1950 K and for K_2O: 1327 K. Therefore, upon interaction of the respective element with oxygen and the formation of oxide in the colder regions of the argon plasma, these oxides probably physically accumulate on the two cones in the interface region (sampler and skimmer), leading to their partial blocking and suppression of the uranium signal, as in the case, the more resistant oxides MgO and CaO would be more difficult to remove, resulting in a significantly stronger signal intensity suppression effect.

The effect of matrix interferences presented as the ratio of the slope of the calibration curve in the presence of matrix (b_m) and for aqueous standard solutions (b_0) are shown in Table 1.

Table 1. Ratio of the slope of the curves with the studied matrix element in the corresponding concentration to the slope of the curve obtained with pure 2 % HNO_3

Matrix	Slope ratio (b_m/b_0) in the presence of the corresponding matrix
50 mg/L $NaNO_3$	0.95 ± 0.2
100 mg/L $NaNO_3$	0.91 ± 0.3
50 mg/L KNO_3	0.94 ± 0.2
100 mg/L KNO_3	0.92 ± 0.3
50 mg/L $Ca(NO_3)_2$	0.91 ± 0.3
100 mg/L $Ca(NO_3)_2$	0.82 ± 0.4
50 mg/L $Mg(NO_3)_2$	0.83 ± 0.5
100 mg/L $Mg(NO_3)_2$	0.73 ± 0.2

The observed matrix effects, called for the calibration method using an internal standard. Experiments were carried out with the model solutions to determine the most suitable internal standard element and its optimal concentration. The internal standards investigated in the present work are La, Re and Tl. They were prepared individually at concentrations of 10 mg/L from Merck's Titrisol. Initially, a considerable number of different water samples were analyzed to prove the absence of La, Re and Tl in them. Different

concentrations (10-30 µg/L) of the elements selected as internal standards were added to the model solutions containing the interfering elements $Ca(NO_3)_2$ and $Mg(NO_3)_2$ at a concentration of 50 mg/L and 100 mg/L. The experiments performed showed that the optimal concentration for the internal standard is 10 µg/L.

The results obtained for the slope ratios in the presence of the internal standards are shown in Table 2.

In addition, model solutions containing 50 µg/L uranium and 100 mg/L $Ca(NO_3)_2$ and $Mg(NO_3)_2$ in the presence of 10 µg/L of the internal standard was measured against a calibration curve constructed with water standards in 2% HNO_3. The results for the measured uranium concentration in the two solutions without internal standard as well as with internal standard correction are shown in Table 3.

Table 2. Ratio of the slope of the curves with the investigated matrix element in the corresponding concentration to the slope of the curve obtained with pure 2% HNO_3 for each of the investigated standards

Matrix	Slope ratio (b_m/b_0) in the presence of the corresponding matrix		
	10 µg/L ^{139}La	10 µg/L ^{185}Re	10 µg/L ^{205}Tl
50 mg/L $Ca(NO_3)_2$	0.95 ± 0.07	0.99 ± 0.05	1.02 ± 0.07
100 mg/L $Ca(NO_3)_2$	0.82 ± 0.07	0.98 ± 0.05	1.07 ± 0.08
50 mg/L $Mg(NO_3)_2$	0.95 ± 0.08	0.99 ± 0.04	0.99 ± 0.07
100 mg/L $Mg(NO_3)_2$	0.81 ± 0.02	0.98 ± 0.03	1.12 ± 0.08

Table 3. Comparison of results for analyzed model solutions of uranium 50 µg/L and $Ca(NO_3)_2$ and $Mg(NO_3)_2$ matrices with the investigated internal standards

Model solution	Recovery for ^{238}U without correction	Recovery with ^{139}La	Recovery with ^{185}Re	Recovery with ^{205}Tl
50 µg/L U + 100 mg/L $Ca(NO_3)_2$	96 ± 7	89 ± 5	101 ± 8	107 ± 9
50 µg/L U + 100 mg/L $Mg(NO_3)_2$	89 ± 5	93 ± 4	100 ± 7	109 ± 9

As seen in Table 3, the matrix interferences from $Ca(NO_3)_2$ and $Mg(NO_3)_2$ on the results for ^{238}U are confirmed, again being more significant for $Mg(NO_3)_2$. As would be expected, La is not a suitable internal standard for the determination of U by ICP-MS in water samples. Despite their relatively close ionization potentials, the mass of La is significantly lower than that of uranium, which means that it is even more affected by the matrix interferences (the effect on lighter elements is more pronounced in ICP-MS) and moreover, it is detected far earlier than ^{238}U by the electron multiplier due to the fact that it passes through the quadrupole significantly earlier. In theory, ^{205}Tl should be the best suited for the intended purpose. As can be seen, however, the data showed that the ^{205}Tl corrected results for uranium are higher both in the solution containing $Ca(NO_3)_2$ and in the solution containing $Mg(NO_3)_2$. The suggested reasons for these results might be: different chemical behavior of the elements in the system, such as the presence of a "memory effect", the formation of oxides at a different extent for both elements, affecting the accuracy of the result. According to the obtained experimental results, ^{185}Re most precisely corrects the matrix influence of $Ca(NO_3)_2$ and $Mg(NO_3)_2$ on the determination of uranium with the mass spectrometer used in our case. The mass of ^{185}Re closer to the mass of ^{238}U than La, which turns out to be a more significant feature than the first ionization potential for reliable interference correction.

Despite the reliable correction of matrix interferences, the application of an appropriate internal standard is also a useful method for the minimization of the drift of the signal over time that occurs due to changing instrument characteristics. To evaluate this capability, Re and Tl were added to a solution with a uranium concentration of 10 µg/L, and this solution was measured for 20, 40 and 60 minutes after the initial calibration, in a number of real water samples. The obtained results are shown in Table 4.

Table 4. Results obtained for a standard solution of 10 µg/L within 60 minutes after calibration and drift correction with Re and Tl

	Elapsed time since initial calibration [min]		
	20	**40**	**60**
Without correction	9.2 ± 0.2 µg/L	8.9 ± 0.2 µg/L	8.4 ± 0.2 µg/L
Correction with Re	10.2 ± 0.2 µg/L	10.2 ± 0.2 µg/L	10.0 ± 0.2 µg/L
Correction with Tl	10.5 ± 0.2 µg/L	10.7 ± 0.2 µg/L	11.1 ± 0.2 µg/L

The results show that within a 60-minute determination of uranium in natural water samples, system drift leads the result for a 10 µg/L standard to drop to 8.4 ± 0.1 µg/L. In the presence of Tl, the results for uranium increased within the investigated period to 11.1 ± 0.1 µg/L. In this case, Re was again proved to be the most effective as an internal standard. Precise results were achieved within 60 minutes with variation within ± 0.22 µg/L, with the final result obtained at the end of the period being 9.95 µg/L.

1.3. Validation of the method for direct analysis of uranium by ICP-MS and its application to real samples

Uncertainty budget of the method for direct determination of U was calculated at three points of the calibration interval. The selected concentrations were 2, 20 and 40 µg/L.

The obtained values for percentage expanded uncertainty in the three selected points are respectively: for 2 µg/L – 9.65 %; for 20 µg/L – 8.59%; for 40 µg/L – 8.64 %. At all three selected points, the contributions of the different components on the method uncertainty have a similar behavior and a similar relative contribution. For all three points, the largest contribution is from intra-laboratory reproducibility – about 3.5%. Next in importance is the intra-laboratory bias of the method (Bias). Its contribution varies between 1.5 and 2.5 %.

The accuracy of the proposed analytical method has been proven by several different approaches. Parallel analysis of several real water samples for the uranium content with a developed method for direct ICP-MS determination of U and method based on enrichment of U using the synthesized U(VI)-IIP and subsequent determination by UV-VIS spectrophotometry using the reagent arsenazo III, which forms a violet complex with U (IV). A comparison between the results obtained after ICP-MS and UV-VIS measurements is shown in Table 5.

Table 5. Results (mean±sd) for uranium concentration in natural waters measured by ICP-MS and UV-VIS (three parallel measurements)

Sample code	U [µg/L] ICP-MS	U [µg/L] UV-VIS
B 1	18 ± 1	16 ± 3
B 2	14 ± 1	13 ± 1
B 3	14 ± 1	14 ± 2
B 4	26 ± 2	30 ± 3
B 5	69 ± 5	77 ± 8
B 6	24 ± 2	26 ± 3

As can be seen from the results in Table 5, the uranium content measured by ICP-MS using the internal standard Re match those obtained by the standard procedure based on UV-VIS spectrophotometry (Student's t-test). For this comparison, waters with a high uranium content were used, since the UV-VIS method is characterized by a significantly higher detection limit.

The advantages of the proposed analytical procedure over the standard spectrophotometric method are the speed of the measurement, the lower uncertainty, the labor-intensiveness associated with the preparations in the UV-VIS-method and significantly lower limit of quantification achieved.

Participation in an interlaboratory comparison for the determination of natural uranium content in water, showed very good agreement between the obtained and certified values as shown in Table 6. The calculated value for the z-score is 0.35.

Table 6. Results for uranium concentration in PT AQ629

AQ629	U [µg/L]
Measured value	34.7 ± 2.7
Certified value	35.6 ± 1.8

The accuracy of the method was also proven by the analysis of a certified reference material SLRS-6 (river water), and the results obtained are shown in Table 7.

Table 7. Results for uranium concentration in certified reference material SLRS-6

SLRS-6	U [µg/L]
Measured value	0.0712 ± 0.0042
Certified value	0.0698 ± 0.0034

The proposed analytical procedure was applied to measure the content of natural uranium in mineral, drinking and groundwater from different regions in Bulgaria.

2. Application of solid-phase extraction for uranium determination with U(VI) ion-imprinted polymer

2.1 Synthesis of U(VI) ion-imprinted polymer

In the present thesis, 4-(2-pyridylazo)resorcinol (PAR) was selected as a new ligand for the synthesis of IIP for U. The polymer synthesis was carried out using the so-called "trapping" technique. The first step consists in the formation of a complex between the target ion (UO_2^{2+}, hereafter referred to as U(VI)) and the specific chelating agent – PAR in ACN solution. The second step is the formation of a prepolymerization complex, between the functional monomer (MAA) and the template molecule (the U(VI)-PAR complex). During the next step, dispersion copolymerization takes place between the formed prepolymerization complex and the cross-linking agent (TMPTMA) and the three-dimensional polymer network is created. Finally, the U(VI) ions are eluted from the resulting polymer gel, leaving in its cavities with a size corresponding to that of the template ion and containing specific binding centers with functional groups whose spatial orientation corresponds to that of the U(VI)-PAR complex. The control non-imprinted polymer was prepared using the same procedure, however without uranium (NIIP).

In order to evaluate the efficiency of the U(VI)-IIP synthesis procedure, as well as the properties of the resulting polymer gels, several polymers were synthesized by varying the amount of the functional monomer. The amount of other reagents was constant: 70 mg AIBN; 25 mL ACN; 0.96 mmol TMPTMA; 0.12 mmol U(VI)-PAR. The conditions for the preparation of the polymer gels, their nitrogen content, the specific surface area (S_{BET}), the total pore volume (V_{total}), the average pore diameter ($D_{average}$) and the adsorption capacity of U(VI)-IIPs are presented.

The degree of incorporation of the template molecule into the polymer network was assessed by elemental analysis. The obtained nitrogen content values (between 2.25 and 3.10 wt.%) indicate that the PAR ligand is successfully "trapped" in the imprinted polymer. The results showed that as the amount of functional monomer (MAA) increases, both the nitrogen content (and therefore the content of the U(VI)-PAR complex in the polymer network) and the sorption capacity of U(VI)-IIP increased. This is probably due to the formation of larger amounts of the prepolymerization complex between MAA and U(VI)-PAR. At the same time, the change in the mole ratios between U(VI)-PAR:MAA:TMPTMA did not significantly affect the values of S_{BET}, V_{total} and $D_{average}$. These results give reason to choose the molar ratio 0.12:2.16:0.96 (U(VI)-PAR:MAA:TMPTMA) as optimal for carrying out the synthesis of U(VI)-IIP.

2.2 Characterization of the composition and structure of the sorbents

Scanning electron microscopy (SEM) was used to examine the shape and morphology of the ion-imprinted and control polymer particles. Figure 2 shows electron micrographs of U(VI)-IIP (A) and NIIP (B).
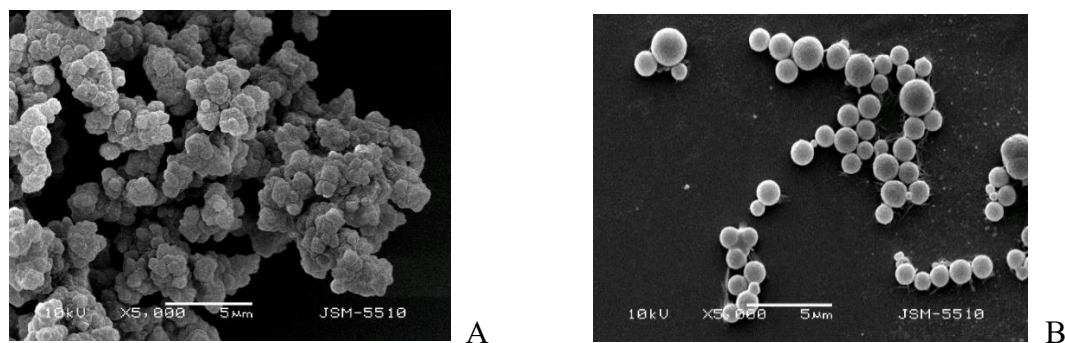
 A

 B

Figure 2 shows electron micrographs of U(VI)-IIP (A) and NIIP (B) when zoomed in x5000.

As can be seen from Figure 2, the particles of the non-imprinted polymer gel have a shape close to spherical. Their average diameter determined by micrographic analysis is about 1.2 µm. At the same time, the surface structure and morphology of U(VI)-IIP is different. As can be seen, U(VI)-IIP is in the form of larger aggregates made up of particles with a close to spherical shape that are stucked together.

The results of the BET analysis show that the values of the specific particle surface area ($S_{BET} = 6.5$ m^2/g) and the total pore volume ($V_{total} = 0.05$ cm^3/g) of U(VI)-IIP are smaller than those of the non-imprinted polymer ($S_{BET} = 27$ m^2/g and $V_{total} = 0.10$ cm^3/g), which is characteristic of the imprinted copolymer gels and can be explained by the incorporation of the U(VI)-PAR complex into the copolymer network. This leads to a partial filling of the pores and, accordingly, to a lower adsorption of N$_2$. The average pore diameter ($D_{average}$) has values of 22 nm and 15 nm for U(VI)-IIP and NIIP, respectively, confirming their mesoporous structure.

2.3 Extraction efficiency and optimization of solid phase extraction

2.3.1 Effect of pH on SPE

The effect of the sample pH on U(VI) adsorption onto the prepared U(VI)-IIP and NIIP was examined in the pH range 3.0–9.0 and the results are displayed on Figure 3.
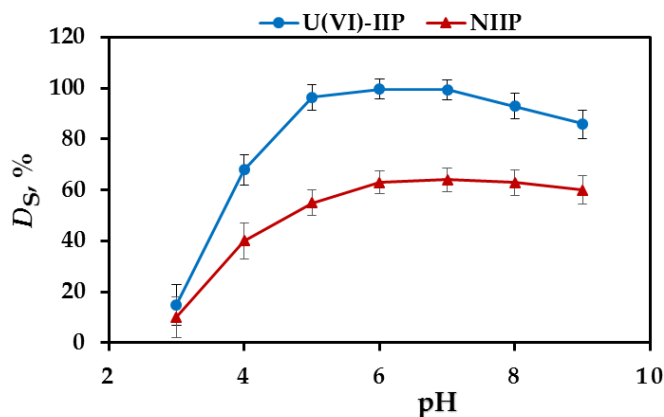
Figure 3. Effect of pH on the degree of sorption (D_S, %) of U(VI) ions on U(VI)-IIP and NIIP (three parallel experiments)

It is seen that the degree of sorption increased with pH increase, reaching maximum at pH 5–7 and thereafter decreased. These results can be explained by the combined effect of the chemistry of uranyl ions in aqueous solutions and their interactions with both the "trapped" chelating agent and the functional groups present in the polymer network. The changes in adsorption might be related to the pH dependent protonation of chelating ligands and functional monomers in the sorbents. At low pH, the active binding sites in the chelating agents PAR are protonated and positively charged (pK_{a1}(PAR) = 3.53 and the adsorption of U(VI) on the sorbent particles is prevented due to the electrostatic repulsion between them. When pH increases, the sorbent surface becomes less positively charged, due to partial deprotonation increasing the ability of the chelating ligands to form complexes with the U(VI) ions. The effect of pH on the degree of protonation of the carboxylic groups in the functional monomer MAA is similar. At pH > 5, the active binding sites in U(VI)-IIP are deprotonated, which ensures the interaction with uranyl ions existing as positively charged species (UO_2^{2+}, $[UO_2(OH)]^+$, $[(UO_2)_2(OH)_2]^{2+}$, $[(UO_2)_3(OH)_5]^+$ and $[(UO_2)_4(OH)_7]^+$. These cationic species retained on the U(VI)-IIP particles by complex formation with PAR molecules and by the electrostatic attraction with deprotonated carboxylic group in MAA. At any pH, the sorption affinity of the U(VI)-IIP towards U(VI) ion was higher than that of the NIIP. Quantitative U(VI) sorption (>95%) was achieved in the pH range of 5–7 with U(VI)-IIP, while the extraction efficiency of the NIIP was around 63% for NIIP (Figure 3). The degree of sorption for both U(VI)-IIP and NIIP decreased at pH > 7, most probably related to the partial hydrolysis of uranyl ions forming $UO_2(OH)_2$ and the presence of negatively charged species

22

such as $[UO_2(OH)_3]^-$, $[(UO_2)_2(OH)_4]^{2-}$ and $[(UO_2)_3(OH)_7]^-$. Finally, the pH of 7 was selected as optimal for the SPE of U(VI) by U(VI)-IIP and NIIP in the further investigations.

From the literature review for U(VI)-imprinted polymers, it was found that the most commonly used reagents for elution of the template ion from the sorbents were solutions of HCl, HClO$_4$ and HNO$_3$ with different concentrations. In this thesis, solutions of hydrochloric acid, sodium and ammonium salts of EDTA (EDTA-Na$^+$ and EDTA-NH$_4$$^+$) were tested as elution agents according to the described procedure 2.3. The results of the conducted experiments are shown in Table 8.

Table 8. Elution rate of U(VI) from U(VI)-IIP with different elution agents.

eluent	concentration, mol/L	D_E, %
HCl	0.5	48±4
	1.0	83±3
	2.0	96±2
	3.0	99±2
EDTA-Na$^+$ (pH 5)	0.1	42±5
	0.2	56±4
EDTA-NH$_4$$^+$ (pH 8)	0.1	64±4
	0.2	82±3

As can be seen from the results, when using EDTA-Na$^+$ or EDTA-NH$_4$$^+$ as an eluent, the highest degree of elution was 82%. This means that no quantitative elution of U(VI) from U(VI)-IIP takes place with EDTA. This result can be explained by the fact that part of the formed specific cavities are located in the bulk of the polymer network and are practically inaccessible to the relatively large EDTA molecule. Quantitative desorption ($D_E > 95\%$) of U(VI) from U(VI)-IIP was only achieved by using HCl with a concentration above 2 mol/L. This is also the chosen eluent in the present study.

Quantitative elution with 5 mL of 2 mol/HCl was achieved in 30 min.

2.4 Study of the mechanism of sorption

2.4.1 Modeling of U(VI) ion sorption kinetics

The kinetics experiments were carried out for U(VI)-IIP and NIIP according to the procedure 2.2. The samples were stirred vigorously for time intervals 5, 10, 15, 20, 25, 30, 35, 40 and 45 min to determine the effect of the contact time on the sorbent binding capacity. The obtained results are shown in Figure 4.
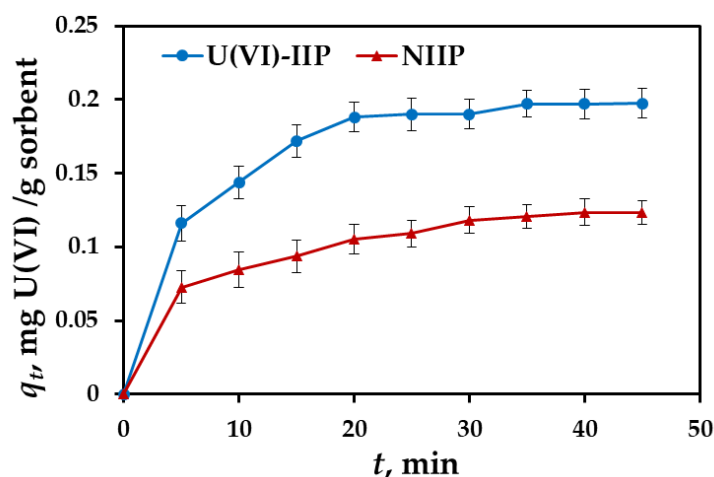
Figure 4. Kinetics of the adsorption of uranyl cations on U(VI)-IIP and NIIP (pH 7; 100 mg sorbent; 10 mL sample; C0 = 2 mg U(VI)/L; temperature 25 °C; three parallel experiments).

It can be seen that the adsorption capacity of U(VI)-IIP towards U(VI) increased rapidly in the first 20 min, then increased at a slower pace and remained unchanged after 30 min. The initial fast adsorption is due to the presence of larger number and more easily accessible specific binding sites on the surface of polymer particles. The results presented in Figure 4 also showed that the adsorption rate of NIIP is slower than that of U(VI)-IIP and its adsorption capacity remains unchanged after 40 min. This can be explained by the lack of imprinted binding sites on the surface of NIIP.

In order to determine the controlling mechanism of the adsorption process such as mass transfer and chemical reaction, pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models were applied to fit the data obtained from adsorption kinetic experiments. PFO model postulated that the rate of occupation of the adsorption sites is proportional to the number of unoccupied sites, while PSO is based on the assumption that the adsorption rate is controlled by the chemical adsorption mechanism. The linear form of equations for these models can be represented as:

PFO-model:

$$\ln(q_e - q_t) = \ln q_e + k_1 \times t$$

PSO-model:

$$\frac{t}{q_t} = \frac{1}{k_2 \times q_e^2} + \frac{t}{q_e}$$

where:

q_e is the amount of U(VI) ions sorbed on a given amount of sorbent at equilibrium, mg/g;

q_t is the amount of U(VI) ions sorbed on a given amount of sorbent at time t, mg/g;

k_1 is the rate constant of the pseudo-first-order kinetic model, min^{-1};

k_2 is the rate constant of the pseudo-second-order kinetic model, g/mg·min.

The values of q_e and k_1 are determined from the intercept and slope of the graph in coordinates $\ln(q_e - q_t)$ versus t when applying the PFO model. The values of q_e and k_2 are determined from the slope and intercept of the plot in t/q_t versus t when applying the PSO model.

In order to select the most suitable model describing the adsorption process, both the values of the coefficient of correlation (R^2) and the equilibrium adsorption capacity predicted by the respective model are taken into account ($q_{e,calc}$). Results obtained presented in Table 9

Table 9. Comparison of kinetic parameters of pseudo-first-order, pseudo-second-order, and intraparticle diffusion models and experimental and calculated values of qe. Conditions of the experiments: pH=7, amount of sorbent – 100 mg, 10 mL sample, $C_0 = 2$ mg U(VI)/L, temperature – 25 °C

model	parameters	U(VI)-IIP	NIIP
PFO-model	$q_{e,exp}$ (mg/g)	0.19	0.13
	$q_{e,calc}$ (mg/g)	4.20	11.00
	k_1 (min^{-1})	0.151	0.072
	R^2	0.945	0.974
PSO-model	$q_{e,exp}$ (mg/g)	0.19	0.13
	$q_{e,calc}$ (mg/g)	0.21	0.14
	k_2 (g/mg·min)	1.078	1.108
	R^2	0.998	0.996
IPDM region 1	k_{diff} (mg/g·min$^{1/2}$)	0.033	0.014
	C (mg/g)	0.042	0.040
	R^2	0.996	0.990

IPDM region 2	k_{diff} (mg/g·min$^{1/2}$)	0.005	0.008
	C (mg/g)	0.164	0.072
	R^2	0.790	0.844

Comparison of the results obtained shows that the pseudo-second-order equation appears to be the better-fitting model considering the higher values of the correlation coefficients R^2 and the calculated value of $q_{e,calc}$, which is closer to the experimental result ($q_{e,exp}$). These results prove that the rate limiting step is strong interactions between incorporated in polymer matrix chelating ligand PAR and U(VI) ions.

To evaluate the role of the diffusion process on the adsorption of U(VI) on U(VI)-IIP and NIIP, the intra particle diffusion model (IPDM) was also used. The linear form of the equation describing this model is:

$$q_t = k_{diff} \times t^{1/2} + C$$

where: k_{diff} is the intra-particle diffusion rate constant (mg/g·min1/2) and intercept C, obtained by extrapolation of the linear portion of the plot of q^t versus $t^{1/2}$, is an indicator to express the boundary layer thickness. The plot q_t versus $t^{1/2}$ shows that there are two distinct linear parts in the graph with different slopes, which convincingly proves the involvement of more than one step in the adsorption process. The first region could be related to the external mass transfer of the analyte (from bulk solution to the adsorption surface), while the second region could be explained by the internal diffusion of the analyte into the cavities of the polymer gel.

The results presented in Table 9 showed that for both sorbent materials the calculated k_{diff} is higher for the first adsorption step than for the second step. This proves that the first step occurs at a higher adsorption rate. The boundary layer thickness values (C) are different from zero, indicating that the adsorption of U(VI) ions on the polymer gels is achieved by surface adsorption, which is controlled by the mass transfer resistance in the external liquid film and by pore diffusion.

2.4.2. Capacity and adsorption isotherms

In order to evaluate the effect of initial U(VI) concentration on the adsorption capacity of U(VI)-IIP and NIIP, batch experiments were conducted according to the procedure described in Section 2.3. Adsorption isotherms constructed with the experimental data showed that the amount of adsorbed U(VI) per unit mass of the sorbent increased with the initial concentration of U(VI), and reached plateau values, determining the maximal experimental

adsorption capacity, $Q_{max,exp}$ (Figure 5). The value of $Q_{max,exp}$ of U(VI)-IIP is higher than that of NIIP—1.89 mg/g vs. 1.35 mg/g. These results confirm that the cavities created after removal of the template U(VI) ions from the polymer network ensures higher affinity of U(VI) ions to the imprinted than to the non-imprinted polymer gels.
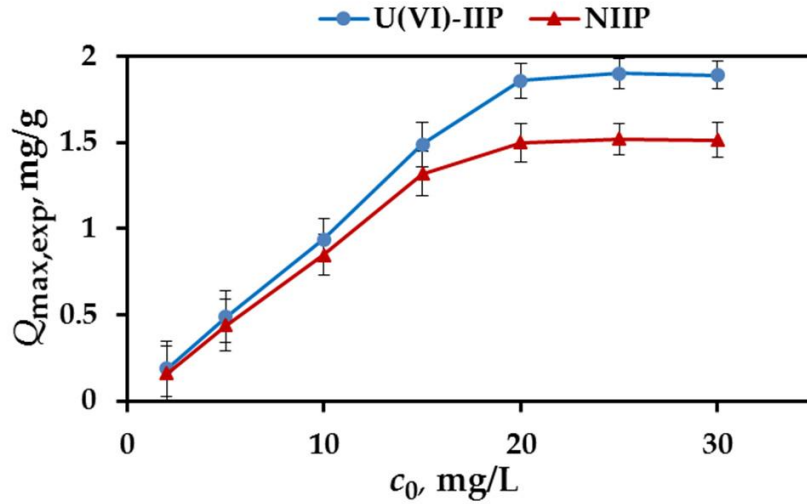

Figure 5. Effect of the initial concentration of U(VI) on the adsorption capacities of U(VI)-IIP and NIIP (pH=7, sorption time – 30 minutes, temperature – 25 °C, three parallel experiments) Freundlich and Langmuir adsorption isotherm models were used to describe the relationship between equilibrium concentration and adsorption capacity during the adsorption process. The applicability of the isotherm models was studied by judging the correlation coefficients, R^2 values.

The Langmuir isotherm theory assumes that the sorption process occurs in a surface monolayer of homogenous sites which number is fixed. It can be expressed in linear form as Equation (8):

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_{max}} + \frac{1}{b \times Q_{max}}$$

where: C_e is the equilibrium concentration of U(VI) in the solution, mg/L;

Q_e is the adsorption capacity of U(VI)-ions sorbed on the polymer at equilibrium, mg/L;

Q_{max} is the theoretical maximum adsorption capacity, mg/g;

b is the Langmuir constant, L/mg.

The Freundlich isotherm model can be applied to multilayer adsorption, with non-uniform distribution of adsorption heat and affinities over the heterogeneous surface. The linearized

Freundlich equation is expressed by Equation (7) as follows: ln Qe = ln kF + n −1 · lnCe (7), kF and n are Freundlich constants incorporating all factors that affect the adsorption process such as capacity and intensity.

The linearized Freundlich equation is expressed as follows:

$$\ln Q_e = \ln k_F + n^{-1} \times \ln C_e$$

where: C_e (mg/L) is the equilibrium concentration of U(VI) in the solution,

Q_e (mg/g) is the adsorption capacity of the adsorbed U(VI) ions onto the sorbents at equilibrium

k_F and n are Freundlich constants, incorporating all factors that affect the adsorption process such as capacity and intensity..

The calculated parameters for both models are shown in Table 10. Analyzing the data presented, the correlation coefficients obtained for Langmuir isotherm (0.9986) have higher values compared with the values obtained when experimental data are modeled using Freundlich isotherm (0.9128). This might be accepted as a proof that sorption process occurs as a surface monolayer on a homogeneous site. The theoretical adsorption capacities $Q_{max,teor}$ agreed very well with experimentally obtained values, thus confirming the validity of assumptions for adsorption in monomolecular layer.

Table 10. Values of the experimental adsorption capacities and the parameters of the Langmuir and Freundlich isotherm models for U(VI)-IIP and NIIP at a temperature of 25 °C

Polymer	$Q_{max,exp}$ mg/g	Langmuir adsorbtion model				Freundlich adsorbtion model		
		$Q_{max,teor}$ mg/g	b L/mg	R^2	R_L	k_F	n	R^2
U(VI)-IIP	1.89	1.91	1.80	0.9986	0.02-0.22	20.25	2.35	0.9128
NIIP	1.35	1.37	4.85	0.9997	0.01-0.10	1.83	3.42	0.8507

To predict the favorability of an adsorption system, the essential characteristics of the Langmuir equation can be expressed in term of a dimensionless factor, R_L, which was defined as:

$$R_L = \frac{1}{1 + b \times C_0}$$

According to the literature, the isotherm is irreversible, favorable, linear or unfavorable if R_L = 0, 0 < R_L < 1, R_L = 1 or R_L greater than 1, respectively. As seen in Table 10, the R_L values are in the range of 0 < R_L < 1 indicating that the adsorption of U(VI) ions on U(VI)-IIP and NIIP is favorable.

2.4.3 Influence of matrix components on the sorption process of U(VI)

Potential interferences from major cations and anions in waters/wine/honey (known to form complexes with U(VI)) were studied at different relevant concentration levels. The experimental procedure follows the steps described in paragraph 2.2. The experimental data obtained are summarized in Table 11.

Table 11. Analytical yields of U(VI) in the presence of various macro components and organic substances found in natural waters

interferant	Analytical yields of U(VI), %			
	10 mg/L	50 mg/L	100 mg/L	200 mg/L
HCO_3^-	98 ± 2	95 ± 3	90 ± 3	85 ± 4
SO_4^{2-}	> 99	> 99	91 ± 3	93 ± 3
Cl^-	> 99	> 99	> 99	98 ± 2
Na^+	> 99	> 99	98 ± 2	96 ± 3
K^+	> 99	> 99	97 ± 2	97 ± 2
Ca^{2+}	> 99	> 99	97 ± 3	95 ± 3
Mg^{2+}	> 99	> 99	96 ± 3	95 ± 2
Tartarate	> 99	> 99	93 ± 3	92 ± 4
Humic acids, 2 mg/L	98 ± 2			

The degree of interference depends on the type of interfering component and its concentration. The degree of sorption of U(VI) remains high (in the range of 85 – 99%) for most of the investigated matrices, which is a proof of the selectivity of the synthesized sorbent. According to the obtained data, CO_3^{2-} and HCO_3^- lead to the most serious interference and to reduced recovery. This is probably due to the competition between the chelating agent PAR, incorporated into the polymeric network of U(VI)-IIP, and CO_3^{2-} and HCO_3^- anions, which also form complexes with the U(VI) ions. For comparison, the stability

constants of the carbonate complexes of uranium ($\beta(UO_2(CO_3)_3{}^{4-}) = 10^{21,60}$, $\beta(UO_2(CO_3)_2{}^{2-}) = 10^{16,94}$ [15] are higher than the value of the stability constant of U(VI) with PAR (β(U(VI)-PAR) = $10^{12,50}$ [16]. At higher concentrations of sulfate ions (100 mg/L and 200 mg/L) lower recovery values were also found (91 ± 3 and 93 ± 3, respectively), which can be explained by competitive complexation between U(VI) and $SO_4{}^{2-}$ ($\beta = 10^{3,0}$ [17].

Finally, in order to show the combined action of the potential interfering substances, experiments were carried out with mineral waters with known additions of U(VI). As can be seen from the results in Table 12, standard addition calibration method should be used for waters with high mineralization.

Table 12. Analytical application of the proposed IIP for the determination of uranium in various mineral waters

Mineral water	$HCO_3{}^-$, mg/L	$CO_3{}^{2-}$, mg/L	$SO_4{}^{2-}$, mg/L	Cl^-, mg/L	Recovery, %
„Gorna Bania"	17	22	22	9	95 ± 2
„Bankya"	62	12	51	10	94 ± 2
„Devin"	89	21	28	11	91 ± 3
„Bachkovo"	92	18	31	7	90 ± 2
„Hisar"	120	15	21	9	88 ± 2

2.4.4 Analytical procedure for the determination of U(VI) in waters

In order to investigate the applicability of the newly synthesized U(VI) imprinted polymer for the determination of uranium content in water samples optimization studies were performed. The experiments performed showed that the maximum sample volume that could be used was 50 mL. At a larger volume, the degree of sorption is below 95%. It was found that the optimal volume of the elution reagent 2 mol L^{-1} HCl is 2 mL, ensuring both quantitative desorption and the possibility to measure uranium by ICP-OES or ICP-MS. The developed procedure was applied for the determination of U in river/lake/sea water samples. The collected samples were filtered through a 0.22 µm pore size membrane filter (Millipore, Burlington, MA, USA) and spiked with U(VI) at different concentration levels. Each experiment was performed in triplicate, and a new sorbent was used for each individual sample (Table 13).

Table 13. Analytical recoveries of U(VI) in water samples

Water source	Spike (μg/L)	Recovery, %	RSD, %
Iskar river near the village of Rebarkovo	1.0	95	4
Maritsa river - Raduil village	0.5	92	5
Dam Ogosta	0.2	91	5
Black Sea (Gulf of Burgas)	2.0	93	2
Tap water - Sofia	0.2	94	3

The results in Table 13 showed that the recoveries for all investigated water types varied between 91 and 95% and depends on the degree of mineralization. For water samples with high HCO_3^- where the reoveries are below 90 % standard addition calibration method should be used. The developed analytical procedure is characterized with a good reproducibility (RSD) in the range between 2 and 5 %. The high recoveries achieved for the different water samples indicated that the synthesized polymer gel can be used in monitoring programs.

2.4.5 Analytical procedure for the determination of U(VI) in red, white and rosé wine

The experiments were carried for potential application of IIP for U determination in wine without preliminary digestion. Wine samples (red, rose and white) were spiked with known amount of U and pass through the proposed analytical procedure (see Section 2.6). The results obtained are presented in Table 14 and demonstrate that maximal sample volume is 20 mL with recoveries achieved above 92%. The lower sample volume is explained with organic components of wine which might be sorbed on the surface of the sorbent and blocked partially its active centers.

Table 14. Analytical yield of uranium in various wines

type of wine	Recovery, % [mean + sd]		
	10 mL sample volume	20 mL sample volume	30 mL sample volume
Red (Merlot)	97 ± 3	92 ± 3	80 ± 5
Rosé	98 ± 2	94 ± 3	82 ± 5
White (Muscat)	98 ± 2	93 ± 3	81 ± 5

2.4.6 Analytical procedure for the determination of U(VI) in honey samples

The potential application of IIP for U determination in honey without preliminary digestion is tested for 5% aqueous solutions of honey obtained after simple dissolution of 5 g honey in 100 mL distilled water (see paragraph 2.7). The results achieved, presented in Table 18 showed that 5% aqueous solution of honey ensures a quantitative recovery if 20 mL sample is used. Probably in this case as well, as with the wine samples, some of the organic compounds in the honey are sorbed on the surface of the sorbent and partially blocking the active centers.

Table 15. Analytical recovery of uranium in different honeys samples

Honey (5 % water solution)	Recovery, % [mean + sd]		
	10 mL sample volume	20 mL sample volume	30 mL sample volume
Linden honey	> 99	96 ± 2	84 ± 4
Rapeseed honey	> 99	97 ± 2	86 ± 4
Sunflower honey	> 99	96 ± 2	85 ± 5

It should be emphasized that the proposed procedures for the determination of uranium are applied without prior decomposition of the sample, might be carried out in one reaction vessel, which minimizes the possibilities for analyte loss or possible external contamination. The high analytical recoveries achieved for wine and honey samples confirmed the applicability of the procedure for quality control of these products for U content - element hazardous to human health with a relatively uncontrolled distribution in the environment.

2.5 Analytical figures of merit

An analytical procedure was developed for U determination in surface/ground waters, wine and honey based on sorption on U(VI)-IIP, see Section 2.7. Limit of detection/quantification (LOD/LOQ), for U, defined as three/ten times the standard deviation of the blank signal (optimal sorbent amount 100 mg, eluted with 2 mL 2 mol/L HCl) using ICP-OES as instrumental method are: 0.05/0.15 µg/L for surface/ground waters, 0.07/0.2 µg/L for wines and 1.0/3.0 µg/kg for honey. As can be seen even by using less expensive method such as ICP-OES almost background values for U might be determined in waters and really low levels of U content in wine and honey might be reached. The calibration graphs were linear from the LOQ to 30 µg/L (maximum concentration assayed) for waters and wine

and from LOQ to 50 μg/kg for honey. The relative standard deviations varied in the range between 5 to 9% for waters, 5–11% for wines and 6–11% for honey. The validity of results for U content in waters obtained by the proposed analytical method was checked by parallel analysis using Alpha spectrometry. In order to compare results achieved by spectrometry they were recalculated in Bq/L. Very good agreement achieved between parallel results as presented in Table 16 confirmed the versality and applicability of analytical method for U determination based on newly synthesized U(VI)- IIP.

Table 16. Comparison between the proposed procedure and a standard α-spectrometric method

Uranium, Bq/L	Sample 1		Sample 2	
	U(VI) –IIP method	α-spectrometry	U(VI) –IIP method	α-spectrometry
^{234}U	0.29 ± 0.02	0.27 ± 0.03	0.48 ± 0.03	0.50 ± 0.04
^{238}U	0.28 ± 0.03	0.27 ± 0.02	0.47 ± 0.04	0.46 ± 0.03

The results obtained indicated that IIP for U might be used for at least 20 adsorption/desorption cycles without significant (less than 10%) change of adsorption capacity and extraction efficiency. The repeatability of the synthesis procedure was also checked by using IIPs obtained from different batches for parallel determination of U in water, wine and honey samples. Statistically unsignificant differences were found between analytical results obtained for U content. Most probably optimal reagents content as well as simplicity of synthesis procedure ensures this high repeatably.

To prove the accuracy of the proposed procedure, a comparative analysis of parallel samples of two surface waters was conducted. A standardized procedure based on alpha spectrometric measurement was used as a comparative method. For convenience, the results obtained from the ICP-MS measurements after performing the solid-phase extraction with U(VI)-IIP have been recalculated in Bq/L. The results of the parallel analysis using the two methods (Table 16) show good agreement, which confirms the accuracy and applicability of the analytical method for the determination of U using the newly synthesized U(VI)-IIP.

Determination of uranium in wine

As far as data for valence state of U in wine and honey is not known the reliability of data for U content might be confirmed only after complete sample digestion and measurement

by more sensitive instrumental method. That is why the validity of results for wine and honey were confirmed by parallel analysis using sample digestion and ICP-MS. Very good agreement between results achieved (see Table 17 and Table 18) verified the applicability of proposed method for U determination in wine and honey without preliminary sample digestion and by using cheaper measurement method as ICP-OES.

Table 17. Comparison between the proposed analytical procedure for wine and a method based on acid digestion and ICP-MS analysis

Sample	Uranium concentration	
	Proposed analytical method, µg/L	ICP-MS with acid digestion, µg/L
Red wine (Cabernet)	0.74 ± 0.04	0.77 ± 0.02
White wine (Muscat)	0.43 ± 0.03	0.41 ± 0.02

Table 18. Comparison between the proposed analytical procedure for honey and a method based on acid digestion and ICP-MS analysis

Sample	Uranium concentration	
	Proposed analytical method, µg/L	ICP-MS with acid digestion, µg/L
Sunflower honey	1.43 ± 0.12	1.51 ± 0.08
Linden honey	3.52 ± 0.21	3.58 ± 0.09

Analytical applicability of the sorbent

To test the reusability of the U(VI)-IIP, the sorbent was applied for several adsorption/desorption cycles using 2 mol/L HCl for elution. The results of these tests show that the printed polymer can be used for at least 20 sorption/elution cycles, within these cycles the adsorption capacity is maintained up to 90% of its initial value.

The repeatability of the synthesis procedure was also checked by using IIPs obtained from different batches for parallel determination of U in water, wine and honey samples. Statistically unsignificant differences were found between analytical results obtained for U content. Most probably optimal reagents content as well as simplicity of synthesis procedure ensures this high repeatably.

Finally, the developed analytical procedures were applied for U determination in water, wines and honey samples. Results showed that U content in waters from uncontaminated regions vary between 0.01 and 5 µg/L, and in polluted ones - between 20 and 400 µg/L. Uranium concentrations in analyzed wines from Bulgarian regions are also low – up to 3.5 µg/L, with the average value being 0.224 µg/L. The U content in different monofloral honeys (lime, rapeseed and sunflower) from Bulgaria vary between 1.23 and 12.32 µg/kg.

V. Conclusions

Results obtained from the research studies might be concluded as follow:

1. An analytical method for the direct determination of uranium in natural waters by ICP-MS was developed. Internal standard calibration method is proposed to overcome the matrix interreferences observe. Rhenium is proposed as most effective internal standard compared to La and Tl.

2. The method is validated by comparative analysis using an alternative method based on the spectrophotometric determination of uranium, participation in an interlaboratory comparison and analysis of a certified reference material (river water). The extended uncertainty was evaluated at three concentration levels and the method was applied to determine the uranium content in mineral, drinking and groundwater from different regions in Bulgaria.

3. A novel U(VI) ion-imprinted polymer was synthesized by dispersion radical copolymerization of methacrylic acid (functional monomer) and trimethylolpropane trimethacrylate (crosslinking agent). A complex of U(VI) with 4-(2-pyridylazo)resorcinol was used for the first time as a template species. The synthesis procedure is relatively easy to perform, and the sorbent is characterized by high stability, low cost and ability to be reused.

4. The newly synthesized sorbent has a high extraction efficiency for the target ion U(VI) under the following optimal experimental conditions: 100 mg sorbent, sorption at pH 7 and elution with 2 mol/L HCl. Experiments with model solutions

prove the quantitative sorption of U(VI) on polymer particles in the presence of various macro components, such as SO_4^{2-}, HCO_3^-, Ca^{2+}, Mg^{2+}.

5. Studies performed showed that the sorption process proceeds as a surface monolayer on homogeneous binding sites, and the rate-limiting step is the formation of complexes between U(VI) ions and the chelating ligand PAR incorporated into the polymer matrix.

6. An analytical procedure was developed for the determination of uranium content in different types of natural waters, white, red and rosé wine, as well as in different monofloral honeys without preliminary digestion, based on solid-phase extraction with the newly synthesized sorbent and subsequent ICP-OES measurement. The proposed analytical procedures are characterized with low limits of determination (LOQ) for uranium: 0.15 µg/L for groundwater and surface water, 0.2 µg/L for wines and 3.0 µg/kg for honeys. The accuracy for water samples was confirmed by comparative analysis of parallel sample with a method based on alpha spectrometry, and for wine and honey samples with ICP-MS measurements of preliminary mineralized samples and ICP-MS measurement.

VI.   Publications

1.   Georgiev V., Dakova I., Karadjova I. Uranium Determination in Waters, Wine and Honey by Solid Phase Extraction with New Ion Imprinted Polymer (2022) *Molecules,* 27 (17), art. no. 5516

2.   Georgiev V., Dakova I., Karadjova I. ON ICP-MS DETERMINATION OF URANIUM IN DRINKING, GROUND AND MINERAL WATERS (2023) *Comptes Rendus de L'Academie Bulgare des Sciences*, 76 (4), pp. 525 - 531

VII.   Participation in conferences and scientific forums

1.   V. Georgiev, I. Dakova, I. Karadjova. Ion imprinted polymers for uranium determination by ICP-MS. Chemistry World Conference (September 06-08, 2021) - Online Event.

2.   Valentin Georgiev, Irina Karadvova Poster Matrix interferences on the ICP-MS measurement of uranium in various matrices INFRAMAT-2021 08/09-10/09 2021, Pravets, Bulgaria

3.   V. Georgiev, I. Dakova, I. Karadjova, M. Karadjov. Uranium content in traditional Bulgarian food and beverages determined by ICP-OES - matrix interferences, calibration procedure, concentration levels. Ninth International Conference on Environmental Management, Engineering, Planning and Economics (CEMEPE 2022) and SECOTOX Conference в (03.06.2022 – 09.06.2022), Mykonos, Greece.

4.   I. Karadjova, V. Georgiev, I. Dakova. U determination in wine and honey from Bulgaria. "Chemistry World Conference" (Chemistry 2022) (13-14 June, 2022) Rome, Italy. (oral poster presentation)

5.   V. Georgiev, I. Dakova, I. Karadjova, M. Karadjov. Organic wine – microelements content dependence on the bentonite used. 8th EuChemS Chemistry Congress (ECC8) (28 August - 1 September, 2022), Lisbon, Portugal.

6.   V. Georgiev, I. Dakova, I. Karadjova. Ion imprinted polymers for uranium determination by solid phase extraction. Eleventh National Conference on Chemistry (11NCC) (23–24 June 2022), Sofia, Bulgaria.

7.   V. Georgiev, I. Dakova, I. Karadjova, V. Dakov. Selective solid phase extraction of U(VI) ions based on new ion-imprinted polymer and its application for determination of uranium in waters, wine and honey. Euroanalysis XXI (26–31 August 2023), Geneva, Switzerland.

VIII. Participation in projects

1. Scientific Research Fund - Ministry of Education and Science, Contract No. 80-10-149/23.05.2022 - "New intelligent materials for speciation analysis of antimony and tin"

Leader: Tanya Vasileva Yordanova, assistant professor, PhD

2. INFRAMAT - modern research infrastructure in support of science, culture and technological development, part of the National Roadmap for Scientific Infrastructure - Ministry of Education and Science (PMS No. 354 of 29.06.2017)

Coordinator: Prof. DSci Vesela Tsakova

3. Sofia University "St. Kliment Ohridski" RESEARCH PROJECT IN SUPPORT OF DOCTORAL STUDENTS Contract No. 80-10-101/13.05.2022 - "Ion imprinted polymers for the determination of Uranium by solid phase extraction"

Leader: Assoc. Prof. Dr. Ivanka Grigorova Dakova