Elemental composition of single-phase kidney stones. Part II

A Aleksandrova,1,2 A Tsygankova,1,2 O Lundovskaya,1 I Korolkov,1,2 E Filatov,1 E Sabantseva1 and A Gubanov1

1 Nikolaev Institute of Inorganic Chemistry SB RAS, Acad. Lavrentiev Ave. 3, Novosibirsk, 630090, Russia
2 Novosibirsk State University, Pirogov Ave. 2, Novosibirsk, 630090, Russia

E-mail: alphiya@yandex.ru

Abstract. The article is devoted to the element composition study of 5 kidney stones types. Calculi consisting of calcium oxalate, uric acid, hydroxyapatite, brushite and struvite were analysed by optical emission spectrometry with inductively coupled plasma (ICP-OES). The elements Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr and Zn were determined in each stone types. Also, correlation analysis for stones consisting of uric acid (n=100) was performed and Spearman’s rank correlation coefficient was calculated. Average and high positive correlations were found between K/Mg, Mg/Na, Mg/P, Mg/Sr, Na/P (r=0.41-0.50) and Ca/Sr (r=0.71) respectively.

1. Introduction
Kidney stones (KS) are products of pathological mineralization in the urinary system. The process of KS formation is influenced by many factors such as climate, environment, patient lifestyle and diet [1]. The chemical composition [2] of calculi depends on urinary saturation by ionic constituents of a particular type of stone. The most common KS types comprise of calcium oxalate (CaC₂O₄) and calcium phosphate, which include hydroxyapatite (Ca₁₀(OH)₂(PO₄)₆) and brushite (CaHPO₄). Struvite stones are less common and consist of magnesium ammonium phosphate (NH₄MgPO₄). There are stones consisting of uric acid, cysteine, xanthine and cholesterol besides to calculi formed by inorganic components.

The mechanisms of KS formation known do not take into account the microelements role in pathogenesis of urolithiasis. It should be noted that microelements are essential enzymes, hormones and vitamins components. Therefore, they contribute to most vital processes in the human body [3]. All blood constituents can pass when filtering through the capillaries of kidney except protein molecules due to their size. Some ions are excreted from the body with urine while glucose, amino acids, vitamins and ions Na⁺, K⁺, Cl⁻, etc. are reabsorbed back into the blood [4]. Thus, the resulting secondary urine contains some microelements possibly affecting on nucleation and crystallization processes. It is necessary to have an idea of the quantitative elemental composition of different KS types in order to identify any patterns. The inter-element correlation analysis in calcium oxalate stones [5] showed a positive average relationship between Ca/Mg, K/Na and Mg/P (correlation coefficient r=0.43-0.49).

In the present study we accumulated data for conducting similar research for uric acid stones in order to find common trends for other calculi types.

2. Design, setting, and participants
We investigated calculi of residents from all regions in Russia. At first, the X-ray analysis was performed for these calculi [6] in order to determine KS type. In this article we studied the elemental composition of single-phase KS consisted of calcium oxalate (n=100), uric acid (n=100), hydroxyapatite (n=18), brushite (n=6) and struvite (n=16). The current analysis sample size was defined by the inclusion of all patients who have calculus medical removal from the urinary system extraction. KS were collected and sent for analysis to Nikolaev Institute of Inorganic Chemistry Siberian Branch of Russian Academy of Science (NIIC SB RAS) by INVITRO-Siberia Laboratory. The samples were analyzed by ICP-OES.

3. Experimental

The matrix effects on analytes signals for each calculi type were studied in order to develop kidney stones analysis procedure. For this purpose, a spike experiment was carried out. The KS matrix in solutions varied from 10 to 1000 ppm. The samples were dissolved in high purity concentrated hydrochloric (HCl) and nitric (HNO₃) acids. The ICP-OES analysis was carried out using high-resolution spectrometer iCAP-6500 Duo (Thermo Scientific).

4. Results and discussion

4.1. Matrix effects

It is known that introducing a high-salt solution into an ICP can change the discharge parameters. As a result, spectral and non-spectral interferences or effects can occur. These effects can distort the result obtained. In the present study we carried out a spike experiment for each stones type. The matrix effects of calcium oxalate (CaOx), uric acid (UA), calcium phosphate (CaP) and struvite stones (MgP) were studied. As a result, the optimal main components concentration for each KS type were selected and limits of detection (LODs) were evaluated. In the previous work we developed analysis procedure of CaOx stones. The optimal matrix concentration in solution was 100 ppm. The analysis procedure developed provides the determination of 22 analytes – Al, B, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, P, Se, Sn, Sr, Zn, Zr; the accuracy in this case does not exceed 20% [5].

UA stones have an organic matrix. That is why, the matrix effects on the most analytes determination weakly. The optimal concentration of UA was 500 ppm according to spike experiment results. In these conditions 42 analytes (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hf, Hg, In, K, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, Re, Sb, Se, Si, Sn, Sr, Ta, Te, Ti, V, W, Zn, Zr) can be reliably determined. The LODs for this calculi type varied from 0.00001 to 0.05 g/kg.

In the CaP and MgP stones the matrix base on calcium and magnesium respectively. These elements presence in significant quantities can change ICP parameters. The optimal concentration of CaP and MgP stones was 10 ppm. In these conditions 40 analytes determination (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hf, Hg, In, K, Li, Mg, Mn, Mo, Na, Nb, Ni, Pb, Rb, Re, Sb, Se, Sn, Sr, Ta, Te, Ti, V, W, Zn, Zr) is possible. The LODs in these cases varied from 0.0001 to 0.5 g/kg.

Figure 1 shows the spike experiment diagram for Cu and Zn in various matrix content. The normalized values “added” and “found” are in agreement and coincide with the confidence interval (Student's t distribution with probability P=0.95, experiments quantity n=3-5).

![Figure 1. Spike experiment results for Cu and Zn in various matrix content.](image-url)
4.2. The real samples analysis

The elemental composition study of single-phase calculi was carried out based on the developed analysis procedures for different KS types. Figure 2 shows the real sample analysis results in the form of a boxplot (software Excel 2016). The results for CaOx are highlighted in dark blue, UA – in red, hydroxyapatite CaP – in green, brushite CaP – in violet, struvite MgP – in blue. Three main quartiles were evaluated when drawing boxplot. The first quartile (Q1) is defined as the middle value between the smallest value (minimum) and the median of the data set. It is also known as the lower or 25th empirical quartile, as 25% of the data is below this line. The second quartile (Q2) is the median of a data set i.e., 50% of the data lies below this value/line/point. The third quartile (Q3) is the middle value between the median and the highest value (maximum) of the data set. It is known as the upper or 75th empirical quartile, as 75% of the data lies below this line. The Q1 (the first line), the Q2 (the second line) and the Q3 (the third line) are presented in Table 1 as concentration values for all found elements – Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr and Zn. The data is given for each type of stone under investigation. We propose to use the calculated median Q2 as an analyte average concentration in CaOx and UA stones type.

So, according to Table 1, for CaOx stones analytes Ba, Cu, Li and Mn are microelements. Their median value Q2 does not exceed 5.1·10^3 g/kg. Analytes Fe, K, Mg, Sr and Zn can be classified as semimicroelements stone. Q2 for their vary from 3.8·10^-2 to 4.5·10^1 g/kg. Q2 for analyte Na are 1.4 g/kg; and for analyte P are 3.8 g/kg.

The analytes concentration in UA is less than in the CaP and CaOx in the majority cases. UA stones contain more microelements – Ba, Cu, Fe, Mn, Sr, Zn. Their median value Q2 does not exceed 7.1·10^3 g/kg. Analytes Ca, K, Mg, Na, P can be classified as semi-microelements stone. Q2 for their vary from 1.8·10^-2 to 3.6·10^1 g/kg. The median value for most elements in UA stones is one order of magnitude lower than in CaOx stones. The analyte Li was not found in UA stones.

It should be noted that data volume for hydroxyapatite, brushite and struvite is insufficient (n<20). Therefore, we can use calculated Q2 for these as an analyte average concentration. The hydroxyapatite composition has a higher elements content. Only Li and Mn can be classified as microelements. Their median value Q2 does not exceed 2.4·10^3 g/kg. The analytes Ba, Fe and Sr can be classified as semi-microelements stone. Q2 for their vary from 1.2·10^-2 to 3.0·10^1 g/kg. The Q2 for K, Mg, Na and Zn in this type of stone vary from 1.2 to 8.5 g/kg. For brushite analytes Li and Mn are microelements. Their median value Q2 vary from 1.2 to 6.4·10^3 g/kg. The analytes Ba, Fe, K, Mg, Na, Sr, Zn can be classified as semi-microelements stone. Q2 for their vary from 1.0·10^-2 to 8.2·10^1 g/kg. Struvite stones contain Ba and Li at the level of 4.7·10^-3 g/kg. The Q2 for semi-microelements Fe, Sr and Zn vary from 1.2·10^-2 to 6.0·10^-2 g/kg. The Q2 for Ca, K and Na vary from 1.4 to 10.7 g/kg.

**Table 1.** The quartiles values as microelement concentrations, g/kg.

| Element | CaOx | UA | Hydroxyapatite | Brushite | Struvite |
|---------|------|----|----------------|----------|----------|
| Ba      | 7.0·10^-4 | 1.0·10^-4 | 9.4·10^-3 | 8.0·10^-4 | 4.0·10^-3 |
|         | 1.4·10^-3 | 3.0·10^-4 | 1.2·10^-2 | 1.0·10^-2 | 7.0·10^-3 |
|         | 5.5·10^-3 | 6.8·10^-4 | 2.2·10^-2 | 2.5·10^-2 | 1.2·10^-2 |
| Ca      | matrix | 3.6·10^-1 | matrix | matrix | 10.7 |
|         | 7.5·10^-1 |      |          |         | 23.2 |
| Cu      | 4.0·10^-3 | 1.4·10^-3 |        |        |       |
|         | 5.1·10^-3 | 2.4·10^-3 | not found | not found | not found |
|         | 5.7·10^-3 | 3.3·10^-3 |          |        |       |
| Fe      | 2.6·10^-2 | 4.5·10^-3 | 5.2·10^-2 | 1.3·10^-2 | 1.0·10^-2 |
|         | 3.8·10^-2 | 7.1·10^-3 | 1.2·10^-1 | 4.4·10^-2 | 1.2·10^-2 |
|         | 7.2·10^-2 | 1.4·10^-2 | 1.6·10^-1 | 2.4·10^-2 | 2.0·10^-2 |
| K       | 2.7·10^-1 | 2.0·10^-1 | 1.1      | 3.0·10^-1 | 2.8 |
|         | 3.5·10^-1 | 2.7·10^-4 | 1.9      | 4.0·10^-4 | 5.0 |
Spearman coefficient of strontium with precipitates of calcium carbonate and phosphate [8]. These factors structure. It should be noted that the CO$_2$ in the +2 oxidation state. The ionic radius of Sr$^{2+}$ is 1.12 Å, which is very close to the radius of Ca$^{2+}$ – 0.99 Å. Strontium has similar chemical behavior to calcium [7] when it’s entering in the many minerals structure. It should be noted that the CO$_3^{2-}$ and PO$_4^{3-}$ ions present in blood promotes the coprecipitation of strontium with precipitates of calcium carbonate and phosphate [8]. These factors may result in the Spearman coefficient obtained value. Another interesting fact that should be noted is an average positive correlation for Ca/Sr in detail. It is known that strontium occurs only in UA stones. The Spearman’s rank correlation coefficient was calculated for establishing inter-element dependencies in UA stones. The values are given in Table 2. A weak positive correlation (are highlighted in violet) was found between elements Ca/Mg, Ca/P, Cu/K, Cu/Mg, Cu/P, Fe/Mg, K/Na, K/P, K/Sr, Na/Sr, P/Sr and P/Zn (r = 0.21–0.41). An average positive correlation (in green) was estimated for analyte pairs K/Mg, Mg/Na, Mg/P, Mg/Sr, Na/P (r = 0.41–0.50). Finally, a high positive correlation (in blue) was established between Ca and Sr (r = 0.71). The dependence for other elements pairs is not statistically significant.

Table 2. Spearman correlation coefficient for UA stones.

| Element | Ba | Ca | Cu | Fe | K | Mg | Na | P | Sr | Legend |
|---------|----|----|----|----|---|----|----|---|----|--------|
| Ca      | -0.001 | - | - | - | - | - | - | - | - | Weak   |
| Cu      | 0.21 | -0.02 | - | - | - | - | - | - | - | positive |
| Fe      | 0.19 | -0.06 | -0.02 | - | - | - | - | - | - |     |
| K       | 0.10 | 0.19 | 0.23 | 0.09 | - | - | - | - | - | Average |
| Mg      | 0.13 | 0.34 | 0.28 | 0.22 | 0.46 | - | - | - | - |     |
| Na      | -0.04 | 0.17 | 0.09 | 0.20 | 0.38 | 0.41 | - | - | - | positive |
| P       | 0.08 | 0.31 | 0.29 | -0.01 | 0.38 | 0.50 | 0.45 | - | - |     |
| Sr      | 0.04 | 0.71 | 0.02 | -0.06 | 0.26 | 0.44 | 0.21 | 0.34 | - | High   |
| Zn      | 0.08 | 0.12 | 0.12 | 0.16 | 0.20 | 0.19 | 0.15 | 0.27 | 0.13 | positive |

4.3. Correlation analysis

We consider the high positive correlation for Ca/Sr in detail. It is known that strontium occurs only in the +2 oxidation state. The ionic radius of Sr$^{2+}$ is 1.12 Å, which is very close to the radius of Ca$^{2+}$ – 0.99 Å. Strontium has similar chemical behavior to calcium [7] when it’s entering in the many minerals structure. It should be noted that the CO$_3^{2-}$ and PO$_4^{3-}$ ions present in blood promotes the coprecipitation of strontium with precipitates of calcium carbonate and phosphate [8]. These factors may result in the Spearman coefficient obtained value. Another interesting fact that should be noted is an average positive correlation for Ca/Sr in detail. It is known that strontium occurs only in UA stones.
correlation between Mg and P. The values of Spearman’s rank correlation coefficient in both CaOx and UA stones are quite close. In the first case \( r=0.49 \) while in the second one \( r=0.50 \).

**Figure 2.** Boxplot for the elements Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr, Zn.

5. Conclusion

It should be noted that the ICP-OES method is highly informative. It enables to quantify analytes in a wide range of concentrations in various objects including kidney stones. In the present study we established the optimal concentration of uric acid (500 ppm), calcium phosphate (10 ppm) and struvite stones (10 ppm) in the solution for ICP-OES quantification of a wide analytes list. The ICP OES procedure developed allows to determine 42 analytes in UA and 40 – in CaP and MgP stones. The analytes reliable determination was confirmed by spike experiment and precision did not exceed 20%.

Using ICP-OES procedure we determined the elemental composition of 100 UA stones; 18 hydroxyapatite and 6 brushite stones; 16 struvite stones. The boxplot based on real sample analysis results was plotted. It was defined that the analytes Ba, Ca, Fe, K, Mg, Na, P, Sr, and Zn occur in all
calculi type, meanwhile Cu and Mn were found only in CaOx and UA stones. The analytes concentration in UA is less than in the CaP and CaOx in the majority cases.

The correlation analysis (Spearman rank correlation) showed a weak dependence between elements Ca/Mg, Ca/P, Cu/K, Cu/Mg, Cu/P, Fe/Mg, K/Na, K/P, K/Sr, Na/Sr, P/Sr, P/Zn (r = 0.21÷0.41); an average correlation for pairs K/Mg, Mg/Na, Mg/P, Mg/Sr, Na/P (r = 0.41÷0.50); high correlation between Ca and Sr (r = 0.71). All correlations are positive.

References
[1] Moe O W 2006 The lancet 367 (9507) 333
[2] Korago A A 1992 Introduction to biomineralogy (St. Petersburg: Nedra) p 280
[3] Skalny A V 2004 Chemical elements in human physiology and ecology (Moscow: Oniks) p 216
[4] Rouiller C 1969 General anatomy and histology of the kidney (Elsevier: Academic Press) pp 61-156
[5] Tsygankova A, Lundovskaya O, Aleksandrova A et al 2020 Journal of Physics: Conference Series 1611(1) 012055
[6] Tsygankova A R, Korolkov I V, Gubanov A I et al 2019 JPSR 11 1721
[7] Nielsen, S P 2004 Bone 35(3) 583
[8] Zachara J M, Cowan C E, Resch, C T 1991 Geoichimica et cosmochimica acta 55(6) 1549