Mineral identification of human kidney stones in South Brazil.

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Abstract

Kidney stones are precipitated when abnormal conditions within the urinary tract promotes local ions supersaturation, changes in the pH, and, in some cases, a differential bacterial influence. The most common minerals in kidney stones are calcium oxalates, followed by calcium phosphates, struvite, cystine and uric acid. In this study, the morphological and mineralogical characteristics of kidney stones were registered and applied to simplify their identification and facilitate the diagnosis. Furthermore, we performed isotopic analysis to verify the likelihood of external factors influencing kidney stones formation. In total, 160 samples of kidney stones from different patients above 18 years old were analyzed. We examined the morphological characterization macroscopically, based on features such as color, fabric and relative hardness. The x-ray diffraction (XRD) applied to mineral identification indicated that whewellite was present in 64% of the samples, followed by 14% uric acid stones and 10% struvite stones. The x-ray fluorescence (XRF) revealed that the majority of the kidney stones were formed by phosphates and calcium oxides, followed by magnesium, sodium and sulfur oxides. Isotopic analysis showed $\delta^{13}C$ values from − 23 to -8‰ and $\delta^{18}O$ values between − 12 and − 6‰ in different types of kidney stones. All the results have shown that it is possible to improve the discrimination of kidney stones based on some morphological features associated with chemical and isotopic composition. Furthermore, isotopic results have suggested that kidney stone formation can be associated with different diets and water intake.

Introduction

The process of kidney stone formation, called urolithiasis or nephrolithiasis, is a very common disease in the world and there is evidence of urinary stone disease since antiquity (Chandrajith et al. 2006; Moe 2006; Balaji and Mani Menon 1997). Nowadays, urinary stone disease affects 1–5% of populations in industrialized countries. In Brazil, approximately 5% of the population is affected by urolithiasis, independent of age and geographical location (Peres et al., 2003). The lifetime risk of becoming a patient is about 10–20% for men and 5–10% for women, of which approximately 50% will experience reoccurrence of the disease (Tiselius 2011; Moe 2006; Peres et al. 2003; Bellizzi et al. 1999).

Kidney stones are deposits of crystals formed inside the urinary system (Stoots et al., 2021). Even though they are organically formed, kidney stones are minerals based on a wider definition of Klein and Dutrow (2012), who stated that minerals can be formed in both ways. Calcium oxalates and phosphates represent the main constituent of kidney stones. According to Lieske et al. (1999), most kidney stones contain calcium oxalates mono-hydrated and dihydrated, or whewellite and weddellite stones, respectively. The calcium phosphates stones are the second most important group of kidney stones (Moe, 2006; Balaji and Mani Menon 1997). They occur as apatite, fluorine-apatite, hydroxyapatite, besides others rare minerals such as whitlockite (iron, magnesium, and calcium phosphate) and brushite (calcium phosphate dihydrate).

Besides oxalates and phosphates, there are other kind of minerals such as struvite stone, which is also a phosphate and contains carbonates, magnesium and ammonium. Uric acid stones normally occur in males and cystine or L-cystine is defined as an organic molecule linked by sulfur, the main compound of this mineral (Giannossi et al., 2009; Balaji and Mani Menon 1997; Krouse and Levinson 1983). Despite the great mineralogical diversity found in kidney stones, the mechanisms of formation and occurrence are still under debate. Different processes are involved in the formation or inhibition of minerals crystallization and depending on the metabolic conditions, different types of stones can precipitate (Branco et al. 2009). Geological and geochemical techniques were applied in this kidney stones research, with the purpose of characterizing and improving the identification of each mineral species precipitated inside the urinary system. The aim of this work was to examine samples of kidney stones in order to assess the mineralogical composition. We know that the mechanisms of formation of kidney stones are still under debate and few works have scanned the mineralogical composition of such minerals. Therefore, knowing the mineralogical composition could help in understanding the mechanism of lithiasis. The examination was completed by performing isotopic analysis, aiming to approach the importance of diet in mineral formation. For this purpose, we examined samples from patients from southern Brazil. In addition, the work shed light in the understanding of how the human environment is involved in the precipitation of minerals.

Mechanisms Of Stone Formation

There is no consensus regarding the formation of kidney stones, but supersaturation and crystallization are the main controls (Sivaguru et al., 2019; Branco et al. 2009; Moe 2006; Balaji and Mani Menon, 1997). Moreover, external risk factors such as diet, drinking habits, climate, concomitant diseases and medications, as well as genetic tendencies are important factors to kidney stones disease (Keshavarzi et al., 2016; Tiselius, 2011).

According to Sivaguru et al. (2019), Lieske et al. (1999), Balaji and Mani Menon (1997) and Cordua (1996), the stone formation happens with the following sequence of events: saturation of calcium and oxalate ions, supersaturation degree, nucleation, crystal growth or aggregation and crystal retention. Furthermore, in some cases multiple nucleation is considered part of stone formation (Lieske et al. 1999). In addition, sources of oxalates become dangerous when normal flora of the gastrointestinal tract is disturbed by changes in environmental conditions, and may also be affected by type of dietary intake, gender and type of gastrointestinal flora (Chandrajith et al., 2019; Sadaf et al. 2017). Khan et al. (2002) observed that altered membrane lipids promote face selective nucleation and retention of calcium oxalate crystals, an abnormal process that becomes part of the growing crystals and stones.

Temperature and pH control most of the chemical reactions inside of a human organism; however, pH is clinically more important than temperature because it directly affects the type of stone formed. The formation of the majority of kidney stones occurs under alkaline conditions, as a chemical rule, as for most elements the solubility decreases with increase of pH. In contrast, acidic conditions precipitate uric stones. An important and old hypothesis is that water hardness influences and/or inhibits kidney stones formation, mostly because of the high calcium and magnesium content that can easily precipitate in alkaline conditions (Briggs and Ficke, 1977). Some authors believe that hard-water intake inhibits or decreases the risks of calcium oxalate crystallization. According to Siener et al. (2004) magnesium and calcium bicarbonate act as inhibitors on stone formation and Irsay et al. (2014) advised patients who have nephrolithiasis to avoid reducing calcium in their diet. Instead, most authors strongly recommend cutting foods rich in oxalate such as spinach, rhubarb, black and green tea, bread, chocolate, and others. For these authors, the stone formation is related to food intake and environmental factors, not with water hardness (Bellizzi et al., 1999). On the other hand, there are surveys suggesting that hard-water intake between meals increases calcium concentration in urine and calcium oxalate supersaturation. According to the research of Mirzazadeh et al. (2012), in patients who had nephrolithiasis, drinking hard water showed a statistically significant increase in urinary calcium excretion, while patients without a history of stone precipitation did not show the same result. All studies about the influence of water hardness in stone formation concluded that magnesium in certain way inhibits the kidney stones formation mainly because magnesium is more soluble than calcium and when the amount of magnesium is low, the risk of kidney stones formation is higher (Irsay et al. 2014; Mirzazadeh et al. 2012; Siener et al. 2004; Bellizzi et al. 1999). Therefore, hardness in water can be ambiguous, presenting both negative and positive aspects, depending on its compounds. Geomicrobiology can help to better understand the precipitation and formation of kidney stones considering that mineralization can be driven by bacteria in the same way observed in the environment (Sivaguru et al., 2021; Dupraz et al., 2008). Recently, scientists combined geology, biology and medicine to map the entire process of kidney stone formation and describe the biomineralization of a kidney stone (Sivaguru et al., 2021). For example, oxalate kidney stones follow similar processes of precipitation as travertine in nature (Sivaguru et al., 2021). Biologically influenced mineralization is a passive crystallization of organic matter, whose properties just influence its growth (Dupraz et al. 2008).

In general, struvites and apatite stones are related to infectious origin, and the bacteria are responsible for calcium phosphate production. The nanobacteria have been recently associated as the nucleating agent of kidney stones based on their widespread occurrence and *in vitro* formation of stones such as apatite (Aloisi, 2008). Nanobacteria are also called “calcifying nanoparticles” (CNP) and an experiment has shown that when a sulphate-reducing bacteria is immersed in a supersaturated fluid, it produces nanometer-sized organic globules. These globules, according to Aloisi (2008), are evidence for a bacterial origin of the organic nuclei and for a passive mechanism of mineralization. In other words, there are strong evidences that the nucleus of a kidney stones is formed by a biologically-influenced mineralization.

Sampling And Methods

Sampling

Kidney stones were obtained from patients of the Nephrolithiasis Ambulatory of the Clinical Hospital at the Federal University of Parana. A total of 160 samples were obtained from patients above 18 years old, with various lifestyles and diets. Some of them had recurrence of kidney stones. Samples were collected in the period of 2012 to 2016, and most of the patients lived in southern Brazil. All selected patients consented to the use of their kidney stones and had a medical record of a nephrolithiasis diagnosis by imaging exams. The samples were previously cleaned with alcohol after surgical procedures at the hospital and sent to Laboratory of Mineral and Rock Analysis (LAMIR) at the Geology Department of Federal University of Paraná without further cleaning.

Methods

The first step for kidney stone identification was the description of morphological characteristics and photographic imaging of samples. The aspects described were color, size, fabric, structure, porosity and relative hardness. The stone-structure analysis included the spherical and roundness, besides the general homogeneity and heterogeneity of the whole sample. The fabric definition consisted in describing the stone fragments surface, whether predominated by crystals or smooth material. Porosity was classified as null, low, medium or high according to the sample pore volume, whereas the relative hardness was determined based on Mohs Hardness Scale, using fingernail and knife scratching.

The XRD analysis was conducted to determine the mineral composition of the samples using a *PANalytica Empyrian X-celerator* diffractometer and the minerals were identified using the *X'PertHighcore* Plus software. In order to determine chemical composition (Pradhan and Ambade, 2021), semi-quantitative compositional analyses of five samples previously analyzed by XRD were also examined using x-ray fluorescence (XRF) energy-dispersive spectrometer (EDS) *AXIOS MaX DY 5297.* We interpreted the results using the *SuperQ Manager PANalytical 5.0L 5.2187.3* software. The ^{13}C and ^{18}O values of 18 samples were determined using a gas-bench coupled to a *Thermo-Scientifique Delta V Advantage Isotope Ratio MS* mass spectrometer, and the results were analyzed on *Isodat* software. We expressed the isotopic results in the conventional per mil notation with respect to PeeDee Belemnite (PDB) for carbon and standard mean ocean water (VSMOW) for oxygen.

Results

Mineralogical Classification

Firstly, all samples were classified into three groups based on the mineral percentage obtained by XRD. The results showed that almost 54% of kidney stones are monomineralic, 34% are bimineralic and 11% had three or more minerals, being classified as polymineralic. When the content of a single mineral was higher than 90%, the mineral was classified as monomineralic. In total, we identified 11 types of minerals in these kidney stones; however, among monomineralic samples, seven minerals were identified (Table 1). In bimineralic stones, the main pair was formed by whewellite and weddellite, with about 30% of occurrence. The second main pair was formed by struvite and apatite, with 20% of occurrence, and the other pairs were formed usually by whewellite or struvite associated with other minerals. The third group corresponds to polymineralic kidney stones, formed by at least three minerals. It was possible to identify ten different minerals, with whewellite and apatite prevailing, besides uric acid, whitlockite and brushite minerals.

Table 1
– Percentage of occurrence and minerals association in bimineralic kidney stones.

Minerals	**Mineralogical Group**	**Chemical Formula**	**Occurrence (%)**
Whewellite	Calcium Oxalate	$Ca(C_2O_4) \cdot (H_2O)$	64
Uric Acid	Organic Compound	$C_5H_4N_4O_3$	14
Struvite	Phosphate	$(NH_4)Mg(PO_4) \cdot 6(H_2O)$	10.5
Apatite	Phosphate	$Ca_3(PO_4)2(OH, F, Cl)$	5.8
Cystine	Organic Compound	$C_6H_{12}N_2O_4S_2$	3.5
Weddellite	Calcium Oxalate	$Ca(C_2O_4) \cdot 2(H_2O)$	1.2
Brushite	Phosphate	$Ca(HPO_4) \cdot 2H_2O$	1.2

Morphological Aspects

The mineralogical characterization of kidney stones was performed by describing aspects such as color, size, fabric, structure, porosity and hardness level, as well as “zonation”, in some samples, which contain levels, laminas or portions with distinctive aspects. The key features of each mineral group are summarized below.

Whewellite

The whewellite stones present high dissimilar features regarding color and morphology. When the samples are composed entirely of whewellite mineral, they often exhibit a dark brown to a black color on the surface, a medium to high level of roundness and sphericity and they are normally formed by an aggregate of crystals, which can be elongated or round. When other minerals are combined with whewellite, the samples show a light brown color and almost all of them have low to medium level of sphericity and roundness. In these cases, there is no prevalence with respect to fabric; in other words, some samples are formed by an aggregate of minerals whereas others have a smooth surface. Whewellite stones show low to medium porosity and relative hardness ranging from 2.5 to 3.0, furthermore, “zonation” is rarely observed (Fig. 1). Weddellite stones occur mainly mixed with whewellite, and normally exhibit a white-brownish to light brown color, with less than 1 centimeter in size and fabric characterized as an aggregate of elongated crystals (Fig. 2). Regarding to the structure, the samples are homogeneous and show low sphericity and low to medium roundness. The pore volume is high and the relative hardness is about four.

Struvite

Struvite stones often exhibit a white to white-brownish color and dimensions larger than 1 centimeter. Their surface is commonly flat, their levels of roundness and sphericity are medium and low, respectively, their porosity level is low, and the samples are heterogeneous (Fig. 3). Although a white color and a smooth surface prevails in these samples, small areas with different colors and shapes are observed and inside some stones there are concentric “zonations” emphasized mainly by the alternation of light colors. The hardness ranges about 1.5 to 2.

Apatite

Apatite stones usually occur combined with other minerals, but even when there is a dominance of apatite, morphologies are variable. The color varies from light brown to dark gray, roundness and sphericity range from medium to high and it is not possible to determine their main fabric because they exhibit flat surfaces and aggregates of crystals in the same sample (Fig. 4). The porosity varies from low to medium and concentric “zonations” are emphasized mainly by the changing of colors, which shows that apatite stones are highly heterogeneous. Their relative hardness is about 5 to 5.5.

Whitlockite

Whitlockite stones are a very rare calcium phosphate mineral, ($Ca_9(MgFe)(PO_4)_6PO_3OH$), similar to apatite stones. Some samples show a distinctive orange color, or a combination of white and orange colors; their levels of roundness and sphericity are low and medium, respectively (Fig. 5). The fabric is mainly micro-laminar with different colors and shapes, some of them with concentric “zonation” emphasized by the alternation of colors, showing, therefore, a heterogeneous structure. Their porosity level is medium and their relative hardness is about five, similar to apatite stones.

Brushite

Brushite stones display a predominantly white color on the surface occurring in aggregates or fragments of crystals with flat surfaces. Their roundness and sphericity levels are low and generally display a homogeneous matrix despite the visible concentric “zonation”. Their porosity level is high and their relative hardness is 2.5. In Fig. 6 are showed some images of brushite.

Uric Acid

Uricite or uric acid is usually light brown or slightly orange. When not fragmented, their roundness and sphericity levels are frequently high and a smooth surface predominates. Overall, the stones are homogeneous, their porosity level is low and their relative hardness is about 1 to 2 (Fig. 7).

Cystine

Cystine stones are extremely rare, with light brown color and fabric with a smooth surface or presenting a surface made of an aggregate of elongated crystals. The roundness and sphericity levels are low; usually homogeneous; with low porosity and relative hardness of about 2.

Chemical Composition

Analysis of the chemical composition of five samples examined by XRD were obtained using semi-quantitative x-ray fluorescence (XRF). Table 2 shows the main oxides (CaO, SO_3, Na_2O, P_2O_5, SiO_2, MgO, ZnO, K_2O) of some types of kidney stones and oxides prevalence.

Table 2
Chemical composition of cystine, whewellite, struvite, weddellite and apatite minerals in kidney stones obtained by XRF.

Minerals	**Major Elements (%)**											
	CaO	**SO_3**	**Na_2O**	**P_2O_5**	**SiO_2**	**MgO**	**ZnO**	**K_2O**	**I**	**Cl**	**Fe_2O_3**	**Al_2O_3**
Cystine		98.9	0.6	0.4	0.1							
Whewellite	73.8	0.3	1.5	22.7	0.4	0.6	0.4					
Struvite	29.6	0.5	1.4	47.2	0.3	20.7	0.1	0.3				
Weddellite, whewellite and F-apatite	70.7	0.7	3.5	20.6	0.6	0.5	0.4		2	1		
Apatite	52.1	0.7	2.6	41.1		3.1	0.1	0.2				

The dominance of calcium and phosphorous oxides was notable in almost all samples, mainly in oxalate stones, which contain more than 70% CaO and 20% P_2O_5, the sum of which corresponds to about 90% of these samples. It was noticed that Na_2O as well as SiO_2, MgO and ZnO, were present in almost all samples, usually in small quantities. The K_2O oxide was present in struvite and apatite stones and the sample composed of weddellite, whewellite and F-apatite contained iodine and chlorine in its chemical composition in a relatively large quantity. In contrast, cystine stones were composed almost solely by sulfur, which was also present in other minerals in low quantities.

Isotopic Analysis

The isotopic analysis aimed to verify the existence of differences between isotopic results, demonstrating the possibility that diet and hydration influence the formation of kidney stones. Carbon and oxygen isotopic values could reflect the origin of water and type of food ingested by the original host. Furthermore, each step-up in the food chain is accompanied by an increase of heavy isotopes on biomass, whereas the light isotopes are catabolized (Tian et al., 2022; Tian et al., 2021; Caxito and Silva 2015). According to Caxito and Silva (2015), isotopic enrichment happens because the hydrogen and oxygen atoms from water intake are added to the atoms from food. Krouse and Levinson (1983) have analyzed $\delta^{13}C$ and $\delta^{34}S$ values in human kidney stones from North America and Mexico and they obtained different values depending on the geographical location of the patients involved and the kind of photosynthetic mechanism (C_3, C_4 and CAM) of the plants ingested by these patients. According to Krouse and Levinson (1983), the mean $\delta^{13}C$ values in oxalate stones from North America become less negative with the decreasing latitude and the same result was observed on $\delta^{13}C$ values of plants. In contrast to the results obtained from calcium oxalates (whewellite and weddellite), the uric acid stone results have shown enriched ^{13}C by up to + 7‰. Cystine stones have shown $\delta^{34}S$ values from 0 to + 18‰, whose sulfur could be derived from ingested organo-S compounds, which probably originated from sulphate in the hydrosphere at lower levels in the food chain, such as bacterial assimilation (Krouse and Levinson 1983).

In this study, eighteen samples were analyzed for carbon and oxygen stable isotopes. Table 3 shows the isotopic data and the mineralogical and chemical composition of the examined stones.

Table 3
Isotopic data of 18 kidney stones and their respective minerals and chemical formula.

Sample	Minerals (%)	$\delta^{13}C$	$\delta^{18}O$	Deviation ^{13}C	Deviation ^{18}O
556/14 G	Whewellite (92) and Weddellite (8)	-19.10	-8.29	0.03	0.03
556/14 H	Whewellite (88) and F-apatite (12)	-16.79	-9.77	0.09	0.13
556/14 K	Stru(59), F-apat(27), H-apat(15)	-15.94	-9.45	0.05	0.15
556/14 M	F-apatite (54) and Struvite (46)	-16.45	-11.13	0.08	0.52
234/15 B	Whewellite (85) and struvite (15)	-16.55	-8.06	0.05	0.05
234/15 E	Struvite (100)	-13.76	-7.74	0.01	0.06
234/15 G	Hydroxyapatite (100)	-23.53	-11.54	0.02	0.03
234/15 I	Whewellite (100)	-8.06	-12.20	0.06	0.33
234/15 K	Struvite (100)	-15.30	-6.62	0.02	0.03
234/15 L	Struvite (100)	-17.01	-6.07	0.02	0.05
234/15 M	Struvite (100)	-17.83	-9.10	0.06	0.04
234/15 O	Struvite (100)	-17.83	-7.00	0.04	0.03
234/15 U	Struvite (100)	-20.14	-9.24	0.02	0.04
556/14 J	Uric Acid (> 90)	No signal detected			
234/15 A	Cystine	No signal detected			
234/15 C	Cystine	No signal detected			
234/15 J	Uric Acid (84) and Whewellite (16)	No signal detected			
234/15 P	Uric Acid (100)	No signal detected			

Kidney stones formed by uric acid and cystine did not present any isotopic signals, despite many attempts. The other minerals analyzed presented negative values for both carbon and oxygen isotopes $\delta^{13}C$ ranging from − 24 to -8‰, while $\delta^{18}O$ values are less negative, ranging from − 12 to -6‰. The results were separated into four groups according to their mineralogical composition: whewellite, struvite, struvite and apatite, and hydroxyapatite. Results are highlighted on Fig. 9.

The R^2 value represents a trend-line indicating a general pattern of whewellite and struvite, whose values range from 0 to 1; the near-zero values are less reliable than those near to 1. The R^2 value of the whewellite group is about 0.85, and for the struvite group is about 0.24. It is notable that the latter indicates struvite stones are the most spread group and therefore, the less reliable.

In this study, the whewellite group showed a decreasing trend of whewellite composition, as shown in Fig. 10, where the minerals of this group were plotted in a single chart. The sample plotted as a red data point is formed of 100% whewellite, whereas, the green data point represents a kidney stone composed of 92% whewellite and

8% of weddellite. The yellow data point represents a stone comprised of 88% whewellite and 12% fluor-apatite and finally the blue data point represents a stone formed of 85% whewellite and 15% struvite.

According to Fig. 10, the sample that contains 100% whewellite is less negative with respect to carbon isotopes than the other three samples, whose $\delta^{13}C$ values range from about − 19 to -16‰. In contrast, in relation to oxygen isotopes, the relationship is the opposite; oxygen isotopic value of the sample with 100% whewellite is more negative than the values obtained for the other samples, whose mineralogical composition is diverse.

Discussion

After removal from patients, kidney stones were classified according to colour, external morphology, and anatomical location, most of the times without medical information of the patient. Kidney stones used in this work did not show unique and representative color. In fact, color commonly varied from light to dark brown. In addition, it was not possible to establish an average size for kidney stones because almost all samples were fractured, however, the majority of samples were less than 1 centimeter. With respect to the fabric, most of the stones were generated by an aggregate of crystals, disposed in levels or laminas that grow as semi-concentric microstructures. According to the literature, a possible mechanism of stone formation is growth in the membrane debris (Sivaguru et al., 2021; Fasano and Khan, 2001). In terms of external morphology stones are heterogeneous and without dominance regarding sphericity and roundness. The samples were classified as having low to medium porosity and their hardness varied from 1.5 to 5.5.

The wide occurrence of calcium oxalate stones and their different shapes hampers their macroscopic identification. Oxalate whewellite and weddellite stones have light to dark brown colors, usually without zonation and with a surface characterized by an aggregate of crystals, and beyond low to medium levels of roundness and sphericity. However, weddellite stones, despite rarely occurring without whewellite, have a lighter color and a higher relative hardness. According to the literature, the calcium:phosphate ratio is crucial to diagenetic transition, for example when that ratio is higher than 1.6, weddellite is more stable as monoclinic lozenge-shape crystals whewellite from tetragonal.

Apatite, struvite, brushite and whitlockite are calcium phosphates and each one exhibits different morphological characteristics. Struvite stones can be recognized by their white/light brown color, smooth surface, presence of zonation and low hardness of about 1 to 2. These features are similar to those described for brushite stones, but the latter are very rare and do not have zonation, and sometimes present aggregates of minerals on their surface. Apatite and whitlockite stones can be easily identified by their high hardness of about 5. Apatite samples are heterogeneous, although they can be distinguished from whitlockite stones because of the orange color of the latter mineral.

Uric acid and cystine stones are organic compounds with similar morphological characteristics. Both have low hardness and homogeneous structure, however, uric acid stones can be recognized by their slightly orange color and their high levels of roundness and sphericity.

The main characteristics of all kidney stones are summarized in Table 4. This table represents a general pattern of each stone, but it is important to remember that kidney stones have features related to mixing of different minerals.

Table 4. Summary of morphological characteristics of each kidney stone sample regarding its mineralogy.

Minerals	**Features**						
	Color	**Fabric (Surface)**	**Structure**		**Porosity Level**	**Relative Hardness**	**Zonation**
			Roundness and Sphericity Level	**Homogeneous or heterogeneous**			
Whewellite	Light to dark brown	Aggregate of crystals	Low to medium for both	Heterogeneous	Low - Medium	2.5 - 3.0	Rare
Weddellite	White-brownish to Light Brown	Aggregate of elongated crystals	Sphericity - low Roundness - low to medium	Homogeneous	High	4.0	Not observed
Struvite	White to White-brownish	Smooth	Sphericity - low Roundness - medium	Heterogeneous	Low	1.5 - 2.0	Observed
Apatite	Light brown to Dark gray	Smooth - Aggregate of crystals	Medium to high for both	Heterogeneous	Low - Medium	5.0 - 5.5	Observed
Whitlockite	Orange to Light brown	Mostly smooth	Sphericity - medium Roundness - low	Heterogeneous	Medium	5.0	Observed
Brushite	White	Smooth - Aggregate of crystals	Low for both	Homogeneous	High	2.5	Not observed
Uric Acid	Light brown to Slightly orange	Smooth	High for both	Homogeneous	Low	1.0 - 2.0	Not observed
Cystine	Light brown	Smooth - Aggregate of crystals	Low for both	Homogeneous	Low	2.0	Not observed

The principal macro-characteristic to differentiate minerals in kidney stones is the relative hardness. Using the hardness, stones can be subdivided into three groups: low (< 2), medium (between 2 and 5) and high (> 5) relative hardness. After, according to the color, it is possible to recognize and differentiate each mineral, as shown in Fig. 11.

The minerals can be separated by using the relative hardness and information about the main color. The first step separates the kidney stones with the low relative hardness, which can be struvite, uric acid and cystine according to Table 3. The cystine stone does not appear in Fig. 4 because it is rare; however, it can be distinguished by its light brown color and its low roundness and low sphericity.

The second step to identify the kidney stone minerals permits distinguishing high and medium relative hardness using at knife to scratch the stone. If possible to scratch, the sample is composed probably of whewellite or weddellite. On the other hand, if the stone has a high relative hardness and the knife cannot scratch it, the sample is composed of apatite, fluorine-apatite, hydroxyapatite or whitlockite.

From FRX results, it is noted that Na_2O as well as SiO_2, MgO and ZnO are present in almost all samples usually in small quantities. However, struvite stones contain more than 20% of MgO, and this could be related to its origin, since struvite stones are often formed by urinary infection, which is associated with presence of bacteria.

In contrast, cystine stones are composed almost solely of sulfur, which is also present in other minerals in low quantities. Cystine stones are made of hard organic compounds generated by cystinuria disease (Balaji e Mani Menon, 1997). The results obtained by isotopic analyses have demonstrated that the kidney stones formed by uric acid and cystine do not show any isotopic signals. Both are organic compounds and their chemical bonding could be responsible for the unsuccessful reaction between them and the phosphoric acid (H_3PO_4), which does not allow the CO_2 release. On the other hand, Krouse and Levinson (1983) have found isotopic carbon signs in uric acid stones, whose $\delta^{13}C$ values are up to + 7‰.

The pair formed by struvite and apatite have similar isotopic values, with just a little difference between $\delta^{18}O$ values. The hydroxyapatite minerals have shown the most negative values for both isotopes, with $\delta^{13}C$ and $\delta^{18}O$ values equal to -23.53 and − 11.54‰, respectively. Whewellite and struvite groups are the most representative, with four and six samples, respectively. Whewellite stones have shown $\delta^{13}C$ values similar to those described by Krouse and Levinson (1983), whose values range from − 24 to -10‰.

The struvite group composed of 100% struvite mineral samples exhibited different isotopic results, with a low R^2 value, as shown in Fig. 2. Nevertheless, despite the low reliable trend-line, this group has the lowest $\delta^{18}O$ values and the data points are plotted close to each other. The fact that struvite group displays relevant differences in the isotopic values suggests an external influence during generation as different geochemical composition of the water and other liquids or a distinct liquid absorption by the cellular membranes.

Conclusion

Despite the substantial incidence of nephrolithiasis across the world, there is not a common understanding about the interactions and mechanisms acting in the generation of kidney stones. The application of a geological and chemical assessment was effective producing a morphological and mineralogical characterization of the kidney stones, as well as an attempt to verify an external environmental interference producing variations in isotopic values. The results of mineralogical composition have shown similar minerals to those mentioned in literature. Samples were divided into three groups, with respect to the mineral prevalence: monomineralic, bimineralic and polymineralic stones. Whewellite represents more than 60% of monomineralic stones, followed by 14% uric acid, 10.5% struvite, 5.8% apatite, besides cystine, weddellite and brushite. Among bimineralic stones, the pair formed by whewellite and weddellite predominates, representing more than 30% of them, followed by the struvite and apatite pair, with 20% of occurrence. In polymineralic stones, whewellite is also present in each sample, showing the great prevalence of this mineral in kidney stones of south Brazil.

Chemical composition analyses have exhibited the prevalence of CaO, SO_3, Na_2O, P_2O_5, SiO_2, MgO, ZnO, K_2O oxides. Most of the samples are formed by calcium oxalate and phosphate, which supports the chemical results obtained, whose main elements were calcium and phosphorus. Cystine stones are characterized as a relatively soft organic product composed predominantly by SO_3 (99–100%). Struvite stone is the second most important mineral occurrence in quantity after the calcium oxalate stones. Struvite is the only stone that contains MgO in the composition. The observation of the different morphological aspects of kidney stones permitted the establishment of some patterns for each mineral, mainly based on relative hardness, color and fabric. Struvite and uric acid stones have low hardness and they can be recognized by the color, since uric acid stones tend to be orange-brownish and the struvite stones are white. Apatite stones have high relative hardness, while whewellite stones have medium relative hardness. A mineral micro-laminar zonation is more representative of apatite than in whewellite stones.

Isotopic analysis revealed that cystine and uric acid stones did not show any isotopic signs, which could be related to their chemical compositions, which do not easily produce CO_2 during heating experiments due to their chemical formulas, since these stones are complex organic compounds. The other kidney stones have shown a wide range of oxygen and carbon values. However, despite that broad distribution and the low number of samples, the four groups previously defined by their mineralogical composition have shown slightly different results, which could be considered as patterns in this work. Samples with 100% whewellite revealed less negative values with respect to carbon isotopes, while struvite showed the less negative values for oxygen. This study demonstrated the significant use of mineralogical and chemical composition associated with external aspects of kidney stones to assist in the identification of stones and streamline medical treatment. It is recommended, in order to completely understand kidney stone formation, associate with diet and age, considering that diet is one of the main factors of lithiasis.

Declarations

Conflict of interest-We declare that there is no conflict of interest in this manuscript.

Funding information-Funding in this research is not applicable.

Data availability

The authors commit to provide all data when be requested.

Author contribution

Isis Armosntrong-responsible for data collecting

Mauricio de Carvalho-interpretation of data and discussion

Anelize Manuela Bahniuk Rumbelsperger-interpretation of data and discussion

Sandro Froehner-discussion

Jose Manoel dos Reis Neto-encouraged the project.

Animal research-No animal was used to produce data in this research.

Consent to participate and publish-The authors consent in publish the data and their participation in the manuscript.

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Figures

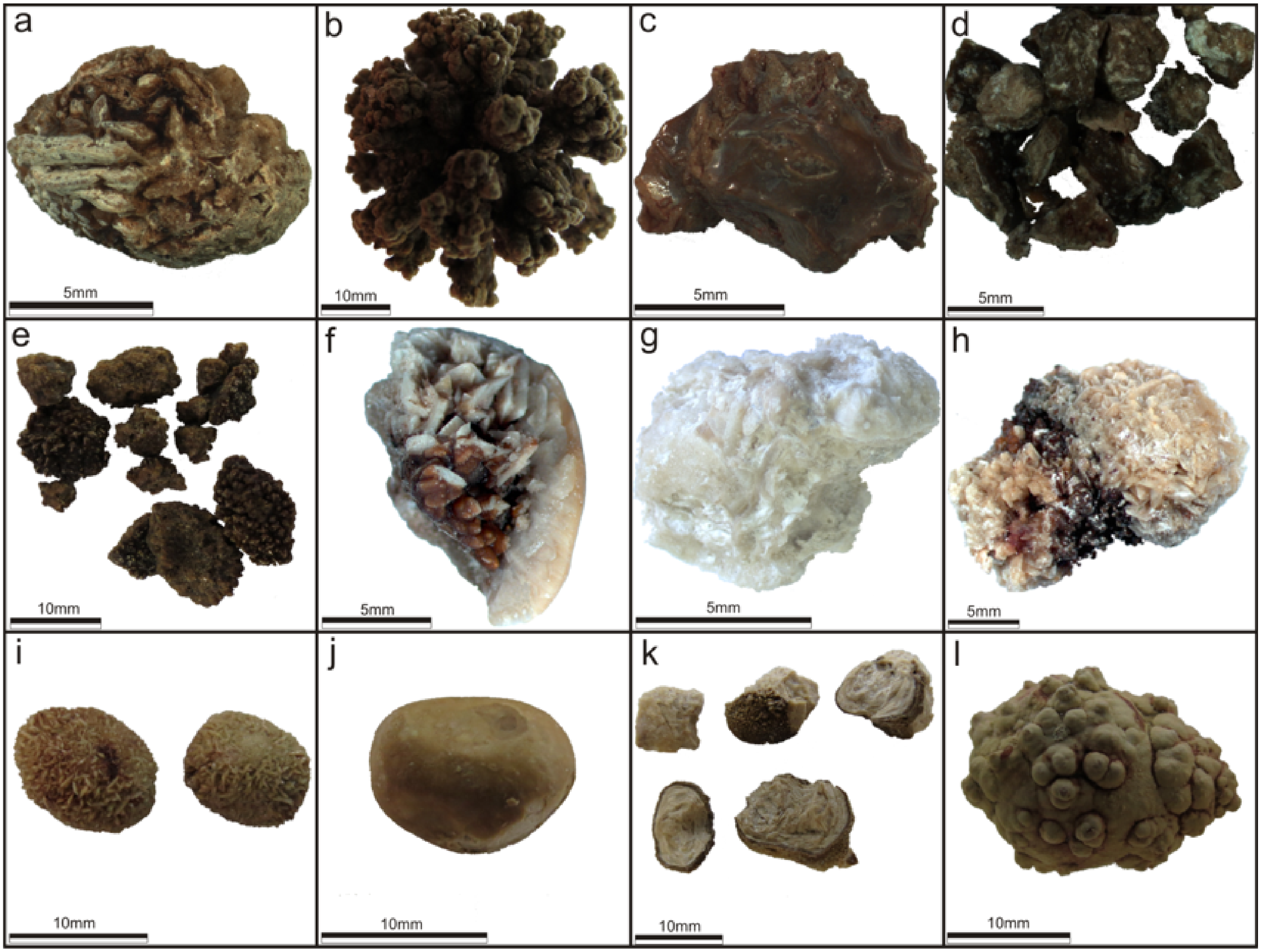

Figure 1

Images of whewellite minerals demonstrating the morphological difference. The kidney stones of the images from (A) to (E) are comprised by 100% of whewellite mineral, whereas the other stones have different values of whewellite.

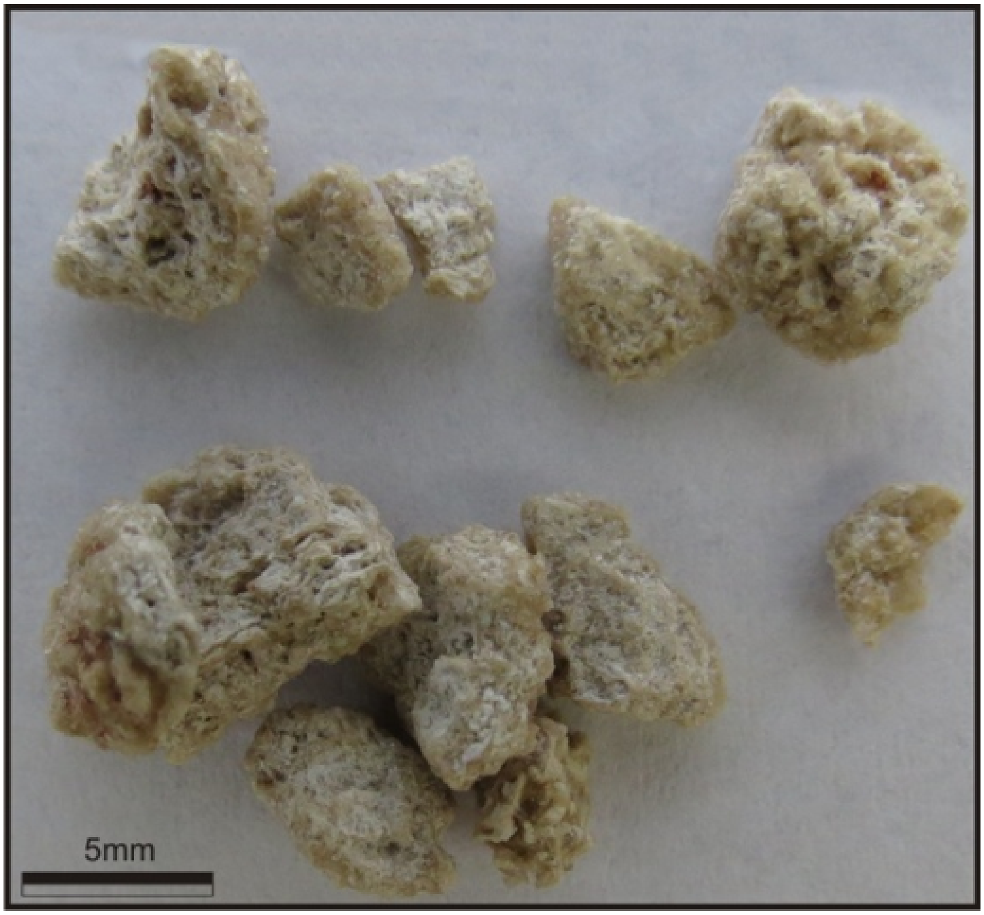

Figure 2

Weddellite minerals from a bimineralic sample, composed by 70% of weddellite and 30% of apatite.

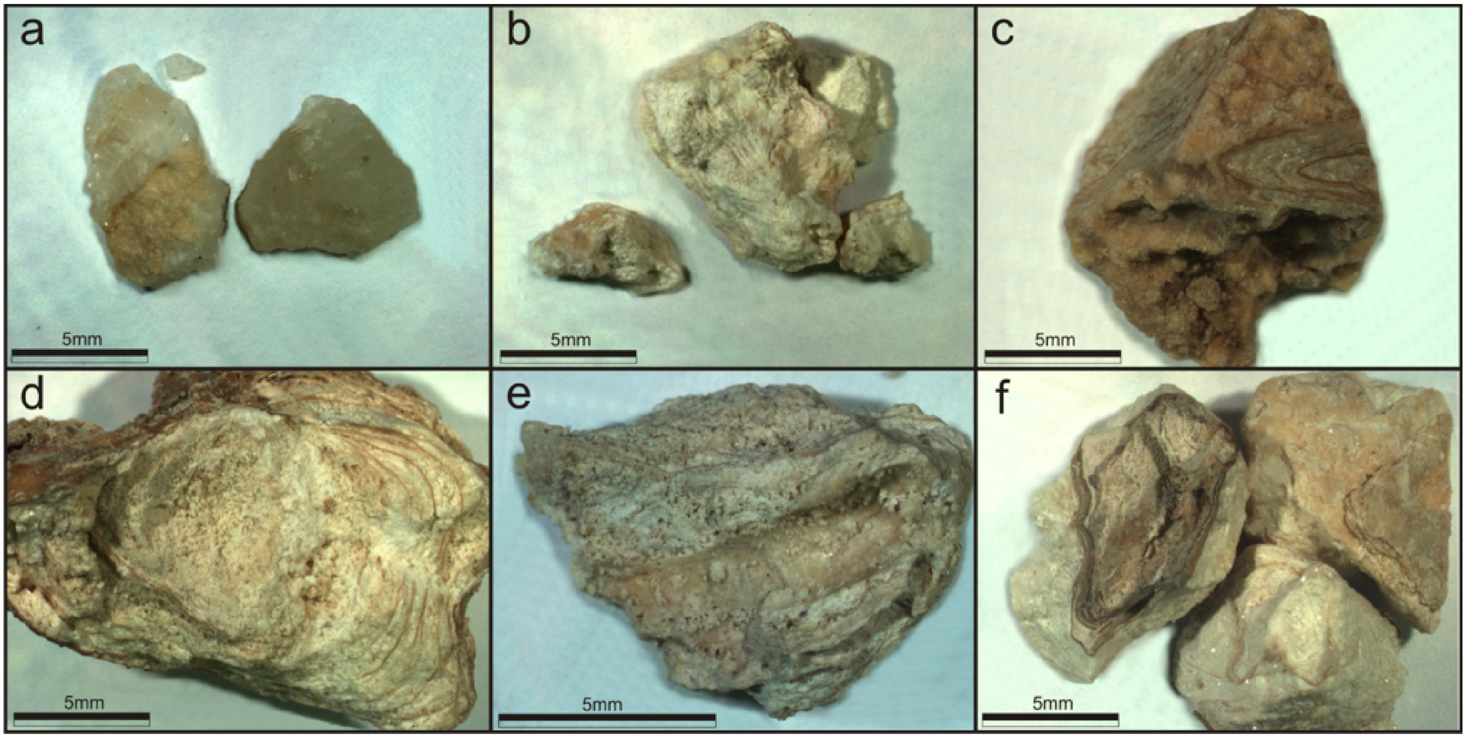

Figure 3

Kidney stones composed by 100% of struvite mineral with strong morphological differences between them and predominant light white to cream colors.

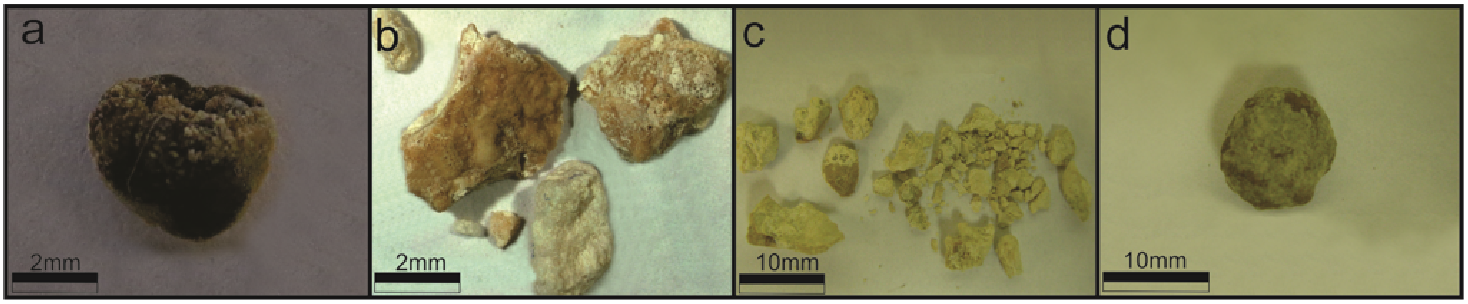

Figure 4

Template showing images of apatite minerals. The kidney stones are composed by 100% of apatite mineral and even though having the same composition, the samples display substantial morphological differences between them.

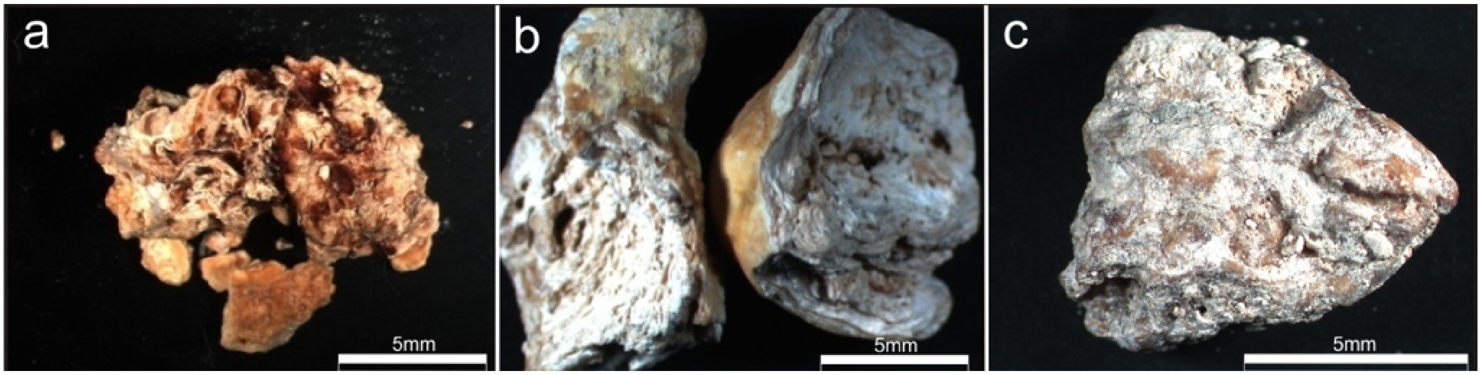

Figure 5

Template showing images of whitlockite minerals. Kidney stone (A) is more orange with about 80% of whitlockite mineral, while the images (B) and (C) have less than 40% of whitlockite mineral.

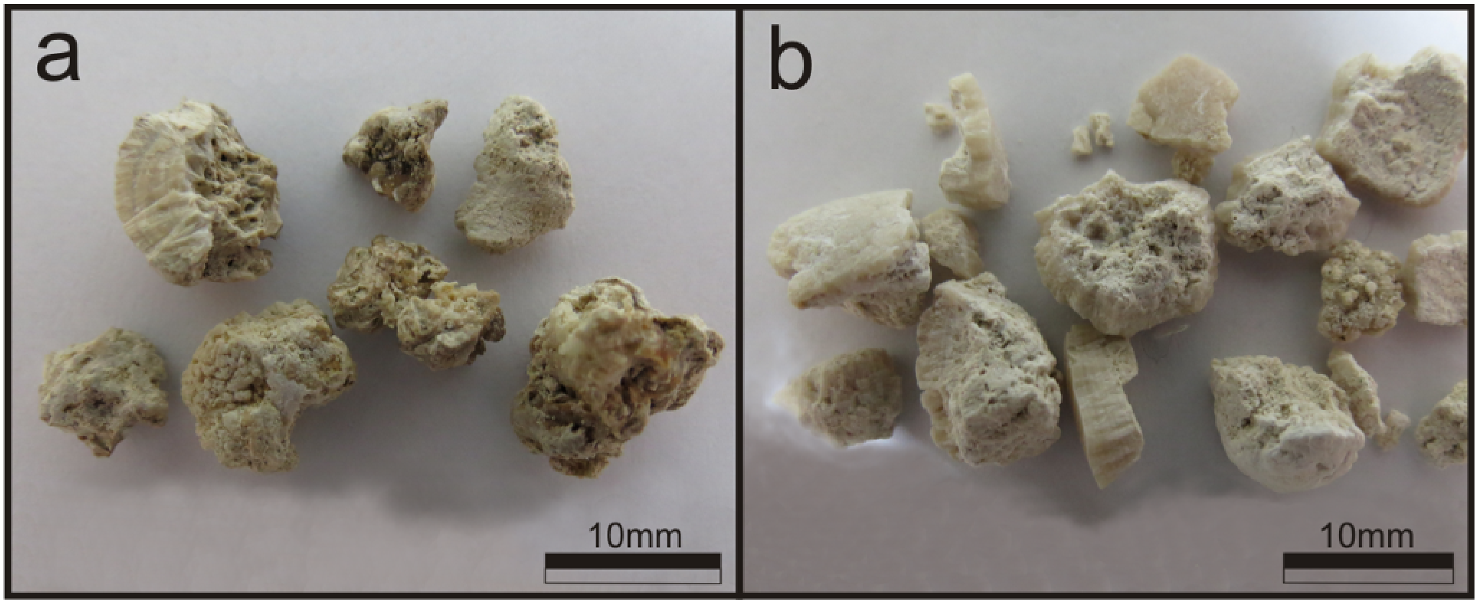

Figure 6

Template showing images of brushite minerals. The stone of the image (A) is composed by 78% of brushite, besides apatite and whewellite. The stone of the image (B) is composed by 97% of brushite and 3% of apatite.

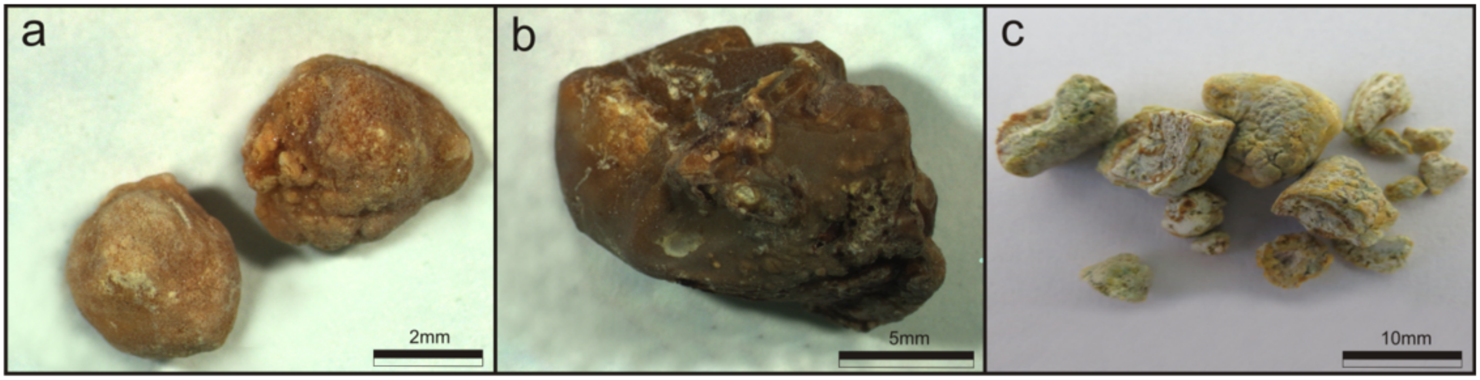

Figure 7

Images of uric acid or uricite minerals. Kidney stones of the images (A) are composed of 100% uricite, while images (B) and (C) are composed of 70% to 90% uricite.

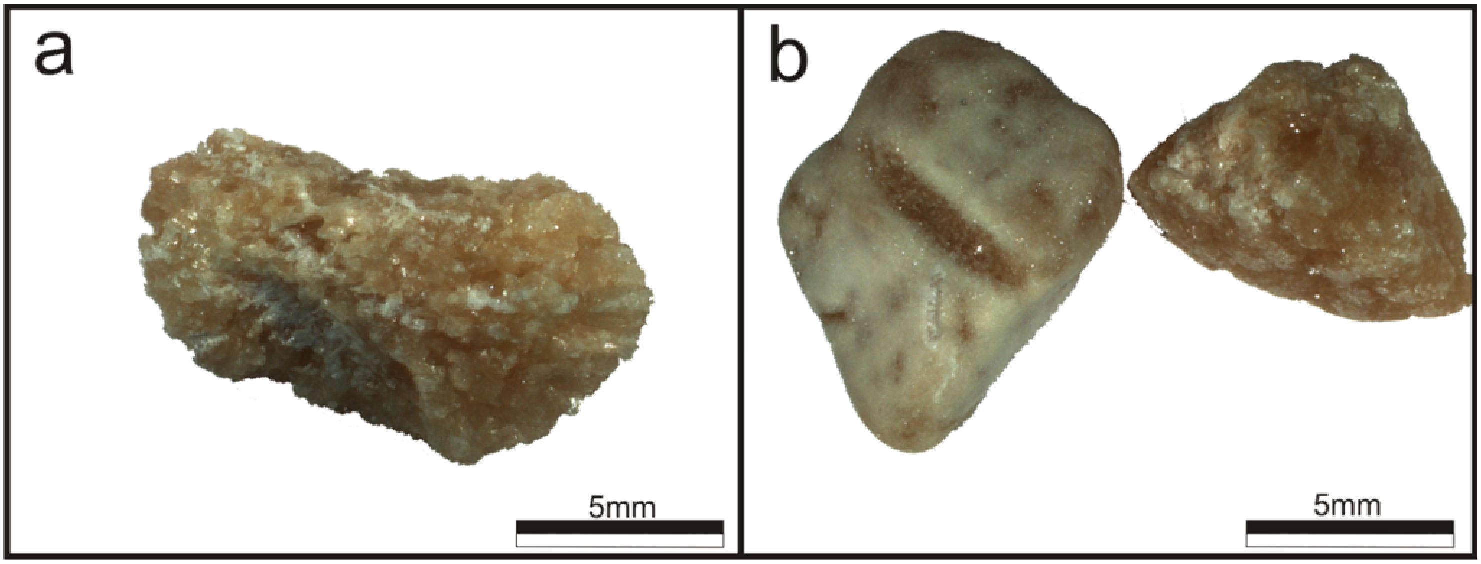

Figure 8

Images of kidney stones composed by 100% of cystine mineral, showing similar morphological features.

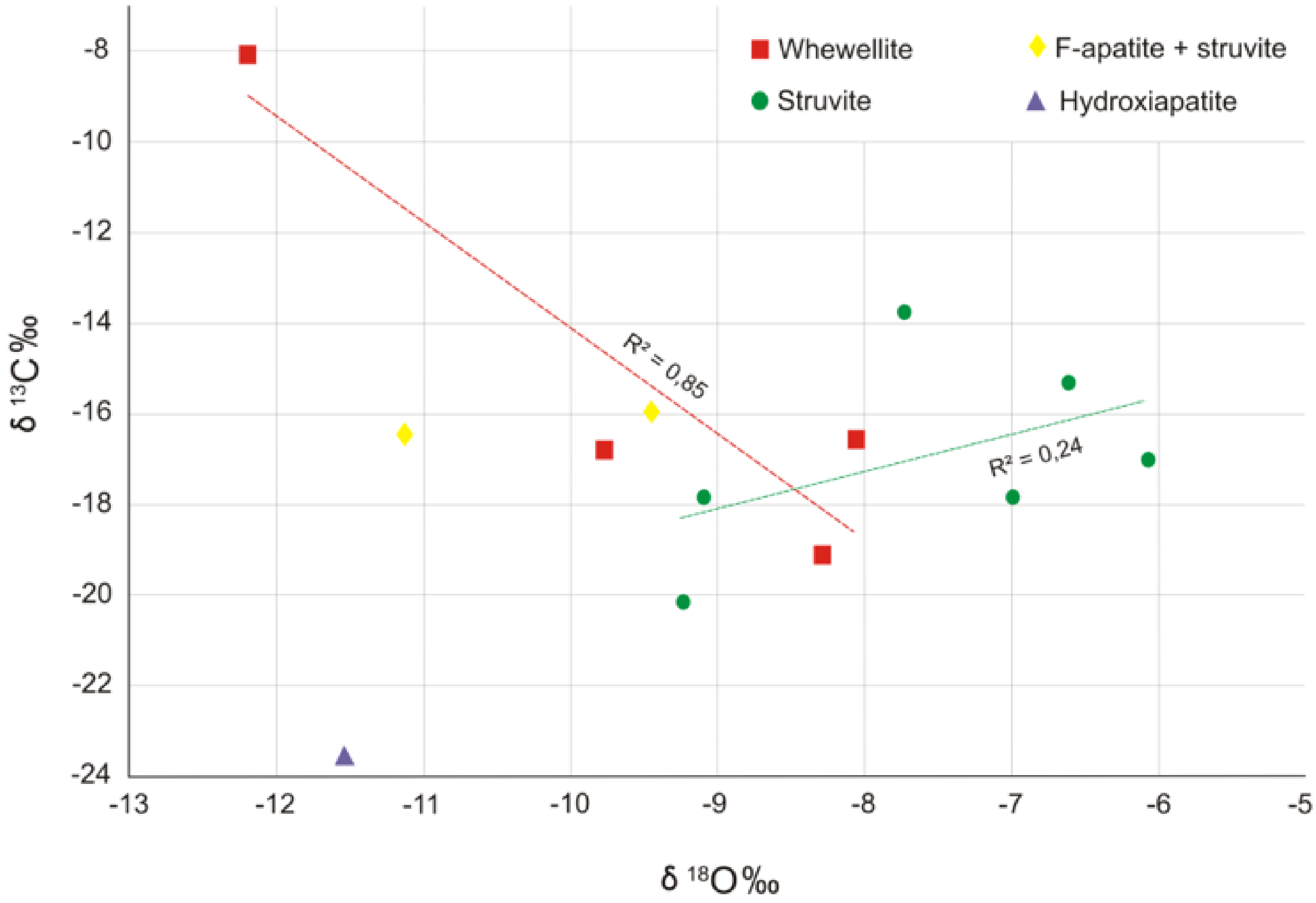

Figure 9

$\delta^{13}C$ versus $\delta^{18}O$ isotopic values of 13 kidney stones separated into four groups: Hydroxyapatite, F-apatite + Struvite, Struvite and Whewellite.

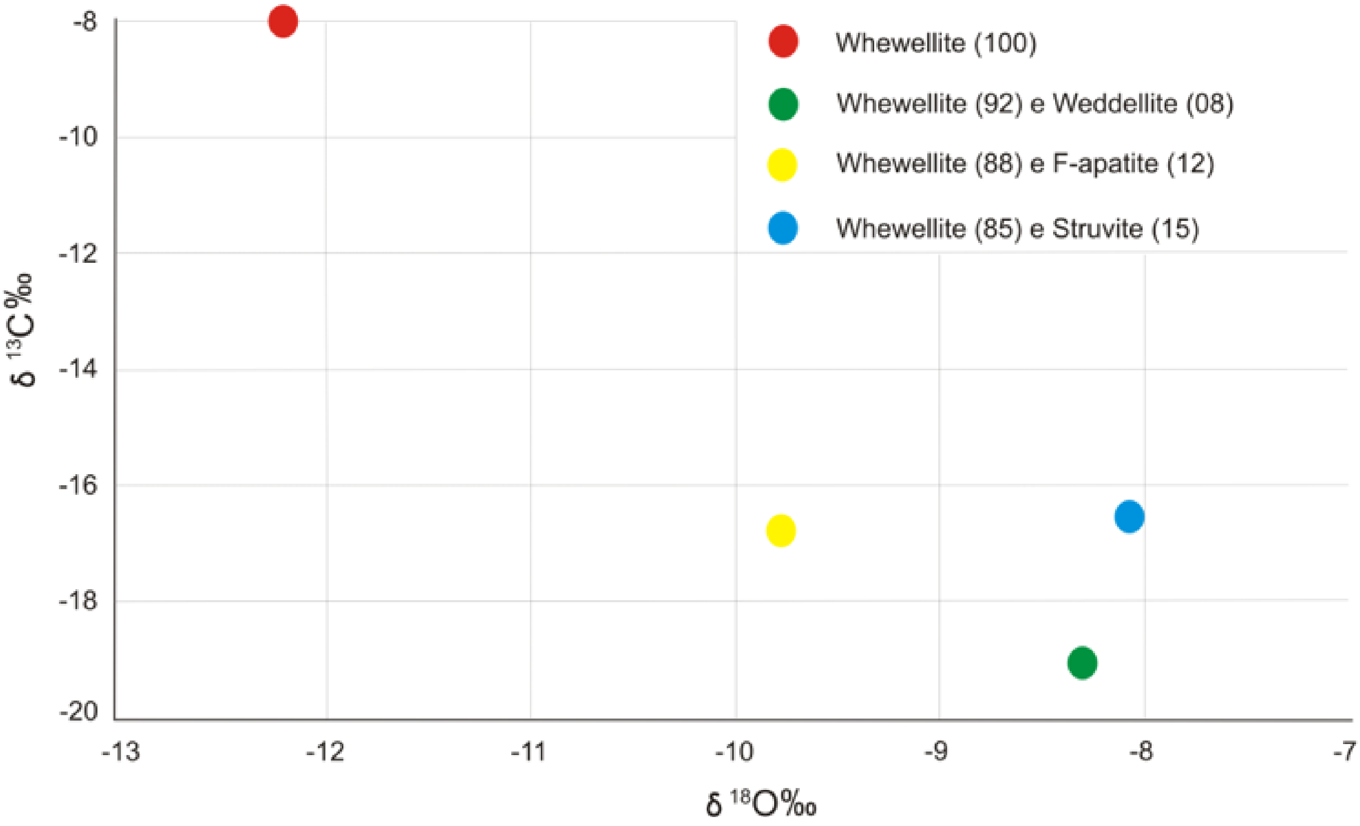

Figure 10

$\delta^{13}C$ versus $\delta^{18}O$ values of whewellite group stones, with decrease whewellite percentage from left to right. The numbers inside the brackets represent the percentage of each mineral.

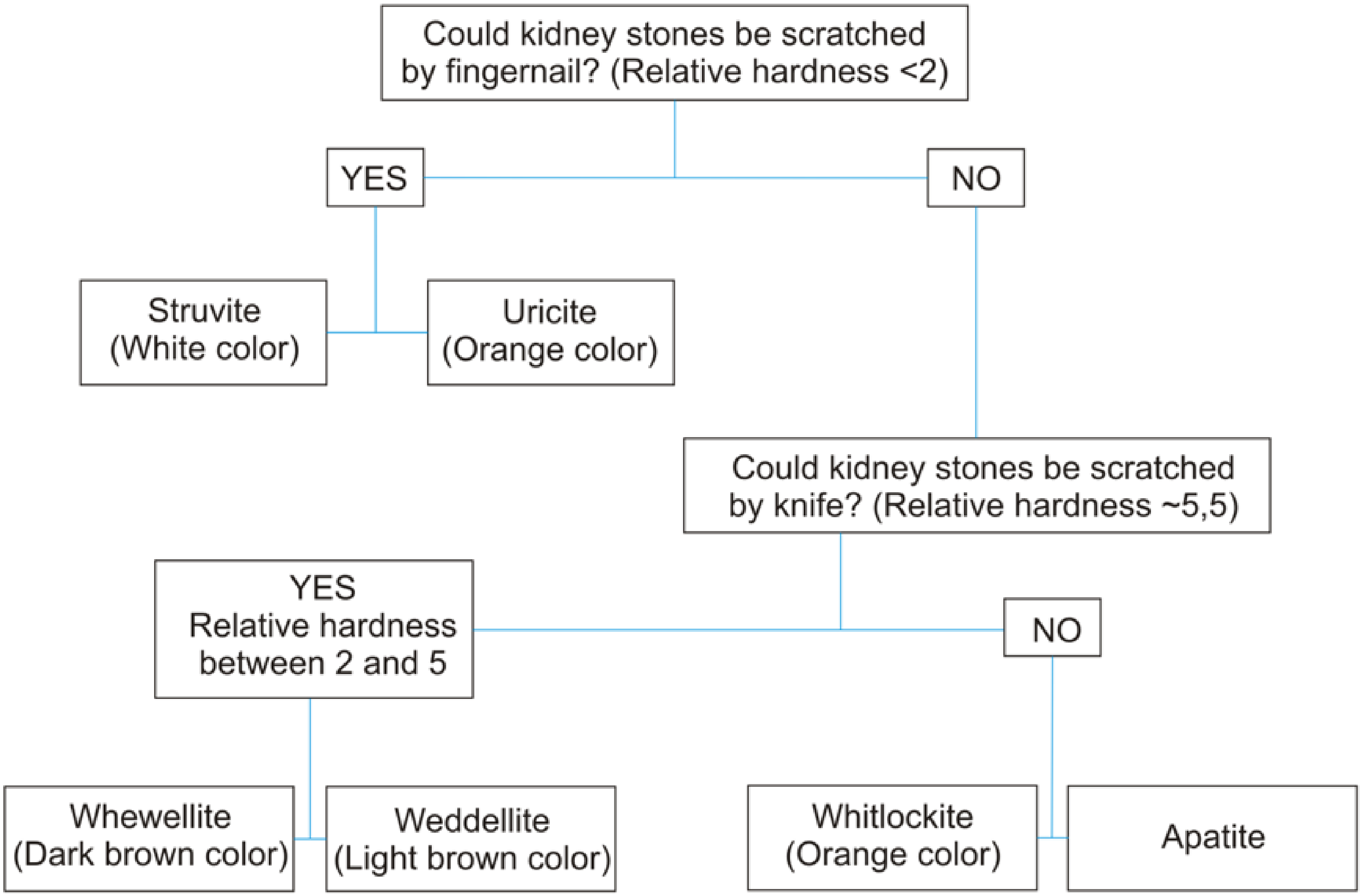

Figure 11

Morphological classification of kidney stones using relative hardness and color aspects.