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CHEMICAL AND PHYSICAL IDENTIFICATION OF KIDNEY STONES: A METHODOLOGICAL INVESTIGATION USING KAMLET AND FEIGL METHODS

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ABSTRACT:
Introduction: Kidney stone formation arises from accumulating crystals and organic materials within the urinary system, constituting a prevalent global health concern. Laboratory investigations are pivotal in stone detection, aiding patient prevention and management. This study focuses on the chemical and physical identification of two kidney stones utilizing the Kamlet and Feigl methods available in the Bioclin laboratory reagent kit.
Methods: The investigation comprises two phases: physical analysis and chemical analysis. Physical analysis delineates the distinctive characteristics of each kidney stone, while chemical analysis identifies urinary crystals contributing to stone formation. The study aimed to elucidate the efficacy of these methodologies in stone identification and prevention.
Results: Both samples exhibited distinct physical properties, while chemical analysis revealed the presence of calcium oxalate crystals in both stones. Sample B contained uric acid, underscoring the method's utility in identifying stone composition and guiding preventive measures.
Conclusion: The Kamlet and Feigl methods offer a simple, quick, and safe approach to identifying kidney stone composition, facilitating tailored patient management and prevention. Incorporating these methodologies into routine laboratory diagnostics enhances patient care, mitigates disease recurrence, and improves overall quality of life.

KEYWORDS: Urinary Crystals; Identification; Physicochemical; Kidney Stones.

INTRODUCTION:

Kidney stones are made when crystals and organic matter build up in the urine tract. At least 13% of men and 7% of women will get it at some point, and up to 50% of people who get it will get it again. The most common sign is severe pain in the back, sides, belly, or when you urinate. What makes the situation worse is the constant need to urinate and the need to do it so often that blood may be in the urine. It can lead to swelling, infection, blood in the urine, and worsening kidney function. It can also move into the bladder (González-Enguita, Garcia-Giménez, Garcia-Guinea, & Correcher, 2024a, 2024b).

Kidney stones happen because of oxidative stress, inflammation, the urea cycle, the breakdown of purines, and the growth of blood vessels. The calculations show that saturation will lead to the concentration. Many things can cause kidney stones, such as age, climate, genes, job, sex, food, and metabolic diseases, that make someone more likely to get them (Balamurugan & Rathina, 2024; Menon et al., 2024).

Kidney stones forming depends on people drinking less water, which is necessary to raise the mineral levels in the urine to the point where crystals form.

Problems with biochemical processes like oxidative stress, inflammation, purine metabolism, the urea cycle, and angiogenesis can lead to kidney stones. Increasing the saturation will raise the salts in the urine, which will help the formation of rocks. Once the crystals start to stick together, a process called aggregation will happen, which is when the crystals finally join together and form large particles that make up the calculation (Mammate et al., 2024; Raj et al., 2024).

The kidneys must eliminate things that don't dissolve easily to keep water in the body. Usually, urinary stones happen when there is an imbalance between two physical qualities that are at odds with each other: the solubility and precipitation of salts. The body balances these two physical traits using normal physiological processes and some substances that stop urine from crystallizing (He, Li, Li, & Fu, 2024; Q. et al., 2024).

But some things, like what you eat, the weather, and how much you exercise, can make this function less useful. Stones form in a complicated process that includes many steps. For example, crystals form in too concentrated urine because the molecules that make up the rocks are being flushed out more quickly or there is less urine in the body. The crystals then start to stick together until a clinical stone forms (Raj et al.; Shahzadi et al., 2024).

Most of the time, calcium salts, uric acid, cysteine, and struvite make up kidney stones. Kidney stones come in four different types: struvite stones, calcium stones, cystine stones, and uric acid stones. Nearly 80% of stones are made of calcium oxalate and calcium phosphate. Uric acid (5–10%), struvite (5%), and cysteine (1%), on the other hand, are less common. Stones in a patient's urinary tract is a sign of a diseased process that causes chemical compounds to precipitate and form deposits (Kanlaya et al., 2024; Ouhammou et al., 2024).

For this reason, it is essential to diagnose all stones, whether they were passed naturally or surgically removed. By accurately figuring out the structure and chemical makeup of rocks, you can determine why they formed, pick the best treatment method, and change the patient's diet to stop them from reoccurring (Mancuso et al., 2024a).

Medications may be used to ease the pain and get rid of the stone. Still, if it doesn't pass, other procedures are needed, like an endoscopic procedure or ureteroscopy, which entails putting a tube through the urethra and into the kidney, where the stones are broken up mechanically or with a laser to get rid of them. Lithotripsy is another treatment that uses shock waves to break up stones so that they are easier to pass when you urinate (Bi et al., 2024).

Based on what Gomes et al. How well kidney stones are treated rests on figuring out exactly what caused them to form in the first place. The disease can be stopped from happening again once the underlying cause of kidney stones has been identified. These can be metabolic, structural, idiopathic, or dietary. These risk factors will help with prevention and treatment (Cao et al., 2024; Robinson et al., 2024).

Because doctors don't always follow the proper steps, clinical analysis labs often don't use this method to find crystals in kidney stones. Two well-known ways in the literature can be used to determine what parts of a kidney stone are present. Kamlet and Feigl's methods use colorimetric reactions to find urinary crystals that have formed a particular stone. These reactions can show whether certain crystals like calcium oxalate, acid uric acid, phosphate, cystine, and others are present or absent. These crystals often appear in the urine and may indicate stone disease (Lapsina et al., 2024).

Identifying kidney stones has evolved into an essential part of lab investigations. These usually involve several biochemical tests used together to give clues about the type of stone and its origin. These tests are based on a method created by Kamlet and Feigl and are now available in pre-made reagent kits that can correctly and accurately figure out what kind of material kidney stones are made of (Pereira Amado et al., 2024).

A method that isn't used very often is studied in this work because it is easy to use, quick, and safe. It can help prevent problems in patients. The point of this article is to show how easy it is to figure out what causes renal lithiasis so that doctors can try to stop the disease from happening again and cut down on the number of people who have to go to the hospital by studying the physics and chemistry of these crystals in the urine (Bose et al., 2024).

MATERIALS AND METHODS:

This is a study that is qualitative and experimental and explains things. Two donated samples from two patients at a private urology clinic in Imperatriz, MA, are part of the study universe. Both samples come from kidney "stones" removed during surgery (Amado et al., 2024).

The patients were both male and female and were identified with kidney lithiasis. The stones had to be removed by surgery because they wouldn't go away on their own (through the urethra). Patients agreed to give samples by signing the informed consent document (TCLE). Since the FACIMP Ethics Board for Scientific Research approved the study, it was made public through protocol 1290830 (Pathan et al., 2025).

The Kamlet and Feigl method was used to change this study. This method tries to find out directly whether kidney stone-forming crystals are present or not. The kits with chemicals were made using the standard procedure, split into two steps: physical analysis and chemistry analysis. The kit makes it easy, quick, and safe to find Carbonate, Oxalate, Ammonium, Phosphate, Calcium, Magnesium, Urate, and Cystine (Mancuso et al., 2024b; Singh & Dash, 2024).

The two samples (A and B) were looked at separately to see how their chemistry and physical properties changed. The KIDNEY STONE Kit from Bioclin has reference number K008 and lot number 0072. It has 14 chemicals and one standard, enough for ten complete analyses. A precision analytical balance was used to weigh the numbers. Next, each sample was ground up in a porcelain bowl and put in its bowl to be used by the analysis chemist (Naranjo-Ruiz et al., 2024).

Once the stone was broken up, a small amount of each sample A and B was put into a different tube and labeled (P.A.) and (P.B.). Certain parts of the positive control sample that came with the reagent kit have been named (P.C.). Each test tube got ten drops of solution no. Five to ten drops of distilled water should be mixed and put in a bain-marie that has already been cooked to 56°C. After spinning each test tube three times for five minutes, the centrifuge was turned up to 3000 rpm for three minutes (Rath et al., 2024).

The sample was then centrifuged with the help of a pipette, and the supernatant was carefully moved into three other test tubes labeled (S.A.), (S.B.), and (S.C). We sorted the precipitate and supernatant from the samples and the control (Liu et al., 2024).

The first thing we did was look for carbonate, oxalate, calcium, and magnesium in the residue, as we will explain below:

Carbonate: Ten drops of reagent no. 1 were put into the three test tubes labeled as precipitates (P.A.), (P.B.), and (P.C.). Then, the bottom of each tube was watched to see if gas was leaving, which would mean the carbonate was positive. Finally, ten drops of water that had been distilled were added, mixed well, and put under the alcohol lamp's light with the help of a Bunsen flame. The tube was removed and set away to cool as soon as it started to boil. Three tubes were used for the whole process. This helped break up the sample stuck at the bottom of the testing tube, which could then be used as a sample for more studies (Karantas et al., 2024).

Oxalate: 0.1 ml of the hot solution above plus three drops of reagent no. 2. The creation of intense turbidity or white precipitate shows the presence of oxalate.

Calcium: 5 drops of reagent no. 1 mixed with 0.1 ml of the hot sample. 6. The fact that the white powder forms shows that calcium is present.

Magnesium: 0.02 ml of the heated sample was put into an Erlenmeyer flask, and 20 ml of distilled water that had been weighed into a beaker was added. One drop of reagent number was added after it was mixed well. 5 to water down the sample. A test tube got seven drops of solution no. Seven drops of reagent no. 8 were mixed in, and 0.05 ml of the diluted sample was added to the Erlenmeyer jar while stirred. When the color purple shows up, it means that magnesium is present (Ida & Yamane, 2024).

The last step was to look at the residue and see if Urates, Cystine, Ammonium, and Phosphate were present or not, as explained below:

Urates: After spinning in a tube, 0.1 ml of the supernatant was moved to be separated and set aside. Five drops of reagent number 10 and five of reagent number 11 were added. Urates are present because of their solid blue color (Punzi et al., 2024).

Cystine: Reagent number 12 and a drop of 13 were added to the tube along with 0.1 ml of the supernatant. After five minutes, add two drops of reagent number 14. Cystine is present as soon as the red solid color shows up. "The whole procedure had to be done under a fume hood because reagent no. 13 contained NaCN, which is against the law according to RDC 306/2004 ANVISA;" (Matejić et al., 2024).

Ammonium: A test tube was filled with 0.1 ml of residue, ten drops of homogenized distilled water, and five drops of reagent no. 9. The presence of a yellow residue shows that ammonium is present.

Phosphates: To make phosphonates, 0.1 ml of supernatant, 1 ml of pure water, and one drop of reagent N° 1 were mixed. Then, two drops of reagent N° 3 were added, and the mixture was mixed to add two drops of reagent N° 4. After two minutes of rest, add two drops of reagent no. 5. The fact that the blue color shows up means phosphate is present (Raghav et al., 2024).

Excavations	Reagents	Reaction
1st	One drop of NaCO3 solution and two drops of uric acid reagent.	Immediate, intense blue staining indicates uric acid and urates.
2nd	Three drops of HCl, let it cool, and add one pinch of MnO2	Tiny bubbles released from the bottom reveal oxalates
3rd	Five drops of molybdate solution and heating over a flame	Clear yellow precipitate means phosphates
4th	One drop of ammonia + 1 drop of NaCN, wait 5 min, and add three drops of Na nitroprusside.	The beetroot-red color indicates cystine.
5th	Add ten drops of HCl Divide this acid extract into two parts, distribute them into two excavations (A and B) and add:	Frothy fizz indicates carbonates
a.	Three drops of NaOH solution and three drops of Nessler's reagent	Yellow or orange precipitate indicates ammonia. Light, white precipitate means calcium oxalate.

b.	Three drops of NaOH solution	Thick precipitate, calcium phosphate

Chart 1: *Simplified identification of kidney stones*

RESULTS AND DISCUSSIONS:

The steps suggested by the methodology were used to report the findings, broken down into chemistry and physics for each sample. The stone that was physically analyzed (sample A) has an uneven shape, and the surface looks like it has holes in it. The colors on the surface vary from dark yellow to light brown. The second stone that was physically analyzed (sample B) differs greatly from the first. It has a smooth, oval shape and only one color that changes shades of yellow. It is also the biggest of the two samples, which means it is the biggest in how GOOD is (Medjoubi et al., 2024).

Variables	**Sample A**	**Sample B**
Weight	0.184g	0.223g
Form	Irregular	Oval
Color	Dark brown	faded yellow
Surface	Porous	Lisa
Consistency	Petrea	Petrea
Dimensions	0.8cm x 0.7cm	0.9cm x 0.7cm
Appearance after sectioned	Presence of grey powder with irregular colouring	Uniform colouring

Table 1 *– Physical analysis - Sample A and B*

More significant than a microscope, the two samples are physically different. The stones' weight and shape are two essential features that make them unique. These features vary from individual to individual and are directly linked to the metabolic condition of the person where the stone is found. You can get kidney stones because of how much water you drink and other essential factors. It's important to note that the same patient can form distinct physical rocks at different times. This doesn't mean they can't include parts of various kinds of stones (Kumari et al., 2024).

Figure 1 *– Samples A and B, respectively*

The chemical analysis constitutes the second and most crucial phase of the methodology, where it will be possible to qualitatively diagnose the presence or absence of some urinary crystals in samples A and B (Budiman et al., 2024).

VARIABLES	**SAMPLE A**	**SAMPLE B**

CARBONATE	-	-
OXALATE	+	+
PHOSPHATE	-	-
CALCIUM	+	+
MAGNESIUM	-	+
AMMONIUM	-	-
URATES	-	+
CYSTINE	-	-

Table 2- *Chemical analysis for the search for crystals in sample A*

The OXALATE test showed that sample A was positive, with a white residue and cloudiness. The CALCIUM test also showed that sample A was positive, with a white deposit on the bottom of the tube being tested, which was demonstrated by the positive outcome of the method (Jing & Xiaoke). This proves that the thing that was looked at does contain calcium oxalate.

Different kinds of urine crystals were found in Sample B. The first crystal found was OXALATE, which had a lot of cloudiness in part being tested and a light white residue that confirmed its presence with the beneficial control of the method (Duque-Sanchez et al., 2024).

It was also proven that the sample had CALCIUM by finding a white residue that looked like the positive control. Additionally, a purple color was seen when it tested positive for MAGNESIUM, and a strong blue color was seen when it tested positive for URATES. As expected, calcium oxide, magnesium, and urates (uric acid) were found in all of the tested sample B material (Kalashgrani et al., 2024).

Nath et al. did a physicochemical study of kidney stone fragments and got similar results. The samples showed that they contained calcium carbonate, calcium, and magnesium. Most kidney stones comprise calcium salts, uric acid, and cystine. Magnesium and phosphates are next on the list. A study by Ansari et al. looked at 1050 kidney stones from people in North Delhi. They found that 93.04% of the stones were calcium oxalate, 0.95% were uric acid, and 2.76% had a mixed pattern (Ayyamperumal et al., 2024).

Similarly, Rao et al. looked at 51 stones and found that 96% were calcium oxalate. Nevertheless, urate, carbonate, phosphate, and ammonium could not be seen in samples A and B. Only magnesium and urate were present in sample B. This is because the sample size was not as large as those of Nath et al., where all compounds were found. However, all compounds were found because they were present in some samples but not others (M. et al., Du, Chen, & Zhang, 2024).

Kidney stones come in four different types: struvite stones, calcium stones, cystine stones, and uric acid stones. That made of calcium is the most common. That made of cystine shows up in people with cystinuria. That made of struvite grows the fastest and can block parts of the urine system. And that made of uric acid is more common in men. This study looks at two types of stones: sample A is made of calcium oxalate, and sample B is made of calcium oxalate and uric acid (Marchetti et al., 2024).

CONCLUSIONS:

Based on the findings, it is essential to facilitate the diagnosis of kidney stones based on the physico-chemical analysis of the crystals. Through the methodological application used, the doctor will be able to carry out the diagnosis more simply and assertively, also providing for preventing future kidney stones.

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