

## ANALYSIS OF URINARY CALCULI IN MAURITIUS

*by*

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### ABSTRACT

Urinary calculi are often small and can be composed of many different constituents. The highly variable composition has led to the development of different chemical and physical methods for calculi analysis. In Mauritius, urinary calculi are routinely analysed for cations and anions using qualitative wet chemical spot tests as outlined in standard textbooks of clinical chemistry. However these procedures have been found not to be very accurate and reliable. The present study reports a comparative study of analysis of urinary calculi obtained after surgery from patients in Mauritius using wet chemical tests, spectrophotoscopic and analytical methods. Our results show that the spectrophotoscopic and analytical techniques are more sensitive and reliable for renal stone composition. Based on these findings we propose that in addition to routine chemical tests, analysts should also consider using spectrophotoscopic and analytical techniques for further characterisation of the different constituents of renal stones.

**Keywords :** Stone analysis, urinary calculi, wet chemical tests, spectrophotometry, calcium, oxalate, phosphate.

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## INTRODUCTION

Urinary stones may be regarded as an example of biomineralization that involves the formation of inorganic minerals by living organisms (Lieske *et al.* 1995). However, kidney stone is a pathological manifestation of the phenomenon, exhibiting features typical of uncontrolled biomineralization (Karlsen *et al.* 1995). Characteristically, stones are composed of mineral crystals aggregated into random clumps of varying sizes, that are formed within the kidney in a relatively open environment by processes not orchestrated by specialised cellular or macromolecular machinery (Phulwinder & Ryall, 1994).

The high and rising incidence of upper renal stones in developing countries has been extensively reported (Michaels *et al.* 1994). Current evidence suggests that between 5-10% of the population in the Western world are at risks of forming a kidney stone at some time during their life (Hofbauer *et al.* 1994). Accurate stone analysis is therefore essential for the investigation and management of the stone-forming patient (Vergauwe *et al.* 1994).

A wide spectrum of techniques ranging from the routine wet chemical 'spot tests' to highly sophisticated spectrophotoscopic techniques are now available to the chemical analyst for stone analysis. An external quality assessment scheme has shown that less than 50% of returns from departments involved with the use of 'spot tests' approach are totally correct (Samuell & Kasidas, 1995). According to the authors many factors may have contributed to the problem including inherent sensitivity, non-specificity of the reactions used inadequate quality control and infrequent performance. Furthermore these methods are time consuming and wasteful of precious stone material.

Sophisticated techniques such as thermogravimetric analysis and Fourier Transform infrared spectroscopy are also available for renal stone analysis. These methods have the abilities to generate accurate quantitative analyses (Samuell & Kasidas, 1995). However the commonest approach in general laboratories has been to use simple inexpensive wet chemistry tests.

In Mauritius general laboratories routinely perform stone analysis by 'spot tests'. However, clinicians have complained that these procedures were unsatisfactory and stone analysis reports that described anions without cations or *vice versa*, or combinations of components. This has resulted in circumstances whereby clinicians have failed to insist on stone analysis or largely ignoring the results when produced. In part this may be due to the poor quality of data generated.

The present work was undertaken as a comparative study between routine wet chemical 'spot tests' and a spectrophotoscopic technique for renal stone analysis in Mauritian patients.

## METHODOLOGY

We obtained 12 kidney stones from patients aged between 23-53yr after open surgery during the period 1995 -1998. Stone analysis was done at the University of Mauritius laboratories using wet spot chemical tests, spectrophotoscopic and analytical techniques.

Infrared spectra of all urinary calculi were recorded as KBr pellets using a MATTSON 1000 series Single Beam FT/IR spectrophotometer. Metal ions were detected by atomic absorption using a UNICAM 929 model. Presence of phosphate was determined using a UV-Visible spectrophotometer (PHILIPS PU 8710 UV/VISIBLE data station).

Metal ions and phosphate in the renal stones were studied after a standard digestion process using concentrated nitric acid at 250°C for at least 30min. However, prior to determining presence of phosphate, digested solutions were first neutralised using M sodium hydroxide. 1.5ml of ascorbic acid solution (7% w/v) followed by 1.5ml of mixed reagent (45ml of molybdate dissolved in 200ml of concentrated sulphuric acid followed by 5ml of tartrate solution) was added for colour development. Absorbance of the resulting coloured solution was then read at a wavelength of 882nm.

Qualitative wet chemical analysis for cations and anions in the urinary calculi was carried out as per the routine technique commonly used in Biochemistry laboratories in Mauritius.

## RESULTS

All data are presented descriptively. No attempt has been made to perform any statistical analysis. Infra red spectrophotometry was used to determine the presence of uric acid and oxalate in the renal stones. Upon analysis of infrared spectra only one of the samples showed an intense peak in the region of 1350cm<sup>-1</sup> indicating presence of uric acid. Furthermore, seven out of the twelve renal stones showed a strong peak in the region of 1310-1315cm<sup>-1</sup> indicating the presence of oxalate. These data were concordant with wet chemical analysis (Table 1).

**Table 1.** Constituents of urinary calculi based on qualitative wet chemical analysis

<b>Sample No</b>	<b>Carbonate</b>	<b>Calcium oxalate</b>	<b>Non-Oxalate calcium</b>	<b>Magnesium</b>	<b>Magnesium phosphate</b>	<b>Phosphate</b>	<b>Uric acid</b>
01	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	POS.
02	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.
03	POS.	POS.	POS.	NEG.	NEG.	NEG.	NEG.
04	NEG.	POS.	POS.	NEG.	NEG.	NEG.	POS.
05	NEG.	POS.	NEG.	NEG.	NEG.	NEG.	NEG.
06	NEG.	NEG.	NEG.	POS.	NEG.	NEG.	NEG.
07	NEG.	NEG.	NEG.	POS.	NEG.	NEG.	NEG.
08	NEG.	NEG.	POS.	NEG.	NEG.	NEG.	NEG.
09*							
10*							
11	NEG.	POS.	POS.	NEG.	NEG.	NEG.	NEG.
12	POS.	POS.	POS.	NEG.	NEG.	NEG.	NEG.

\* *Insufficient material for analysis*

**Table 2.** Percentage of metal ions and phosphate in urinary calculi

Samples	Group II metal ions		Group I metal ions		Transition metal ions					Heavy metal ions	
	<i>Ca</i>	<i>Mg</i>	<i>Na</i>	<i>K</i>	<i>Cr</i>	<i>Mn</i>	<i>Fe</i>	<i>Cu</i>	<i>Zn</i>	<i>Pb</i>	$PO_4^{3-}$
1	0.16	-	0.061	-	$1.9 \times 10^{-3}$	-	$3.0 \times 10^{-3}$	$3.6 \times 10^{-4}$	-	-	1.0
2	0.29	0.017	0.615	0.03	-	-	0.01	$2.7 \times 10^{-3}$	-	-	1.7
3	20.56	-	-	-	0.017	-	0.0174	-	-	-	11.4
4	32.18	0.112	0.258	-	-	-	-	-	0.028	-	28.2
5	14.05	$3.7 \times 10^{-4}$	0.236	-	-	-	-	$4 \times 10^{-4}$	0.002	-	7.1
6	12.95	0.179	0.662	-	$2.8 \times 10^{-3}$	-	0.016	-	0.799	-	67.6
7	-	2.588	0.628	-	0.01	0.07	5.23	$5.9 \times 10^{-3}$	0.007	-	16.0
8	4.15	4.591	0.715	0.56	-	-	-	$4.5 \times 10^{-5}$	0.034	-	61.5
9	15.21	-	-	-	-	-	$7.7 \times 10^{-3}$	$4.8 \times 10^{-4}$	-	-	18.5
10	19.13	$3.3 \times 10^{-3}$	0.121	-	$9.6 \times 10^{-3}$	-	0.04	-	0.023	-	5.6
11	25.93	0.041	0.172	-	$2.7 \times 10^{-3}$	-	$9.5 \times 10^{-4}$	$2.5 \times 10^{-4}$	-	-	24.4
12	32.81	0.026	0.201	0.06	$1.5 \times 10^{-3}$	-	-	$2.6 \times 10^{-4}$	0.029	-	26.9

UV spectrophotometry proved to be a highly sensitive method for the detection of phosphate. Concentration of phosphate in the urinary calculi was in the range of 10-700  $\mu\text{g/g}$ . However wet chemical analysis could not reveal presence of phosphate in the renal stones.

Presence of different metal ions in the stones was also studied (Table 2). It was found that these urinary calculi contain mostly calcium as cation. Percentage by mass of magnesium ion in the stones was found to be less than 4% (Table 2).

Group I metals namely sodium and potassium if present were found in low concentrations. Concentration of transition metal ions such as iron, manganese, copper and zinc were almost negligible. From a combination of spectrophotoscopic and analytical study it was found that six of the urinary calculi were a calcium oxalate stone and the composition of one of the stone was calcium phosphate.

## DISCUSSION

Urinary calculi are often small in size and can be composed of a number of different constituents (Gault *et al.* 1995). The highly variable composition has led to the use of different methods for analysis of urinary calculi. In this study we have tried to evaluate the reliability of the chemical wet chemistry tests routinely employed in Mauritius by biochemistry laboratories for analysis of renal stones.

A high percentage of the urinary calculi investigated in this study were found to consist mainly of either a calcium oxalate or a calcium phosphate salt. These findings are consistent with urinary stone compositions reported in the literature (Samuell & Kasidas, 1995).

Our results also show that the chemical methods employed in the present work are destructive and need several mg of sample. The methods also presented with the disadvantage of not being suitable for analysis of very tiny stones. Furthermore our results tend to confirm that the qualitative chemical analysis methods used are not accurate and can lead to clinical significant errors. The chemical analysis methods did not make it possible to distinguish mineral constituents with similar type of composition; for example the calcium phosphates, from each other. However spectrophotoscopic and analytical methods used in this study had the advantage of requiring the use of smaller amount of sample and enabled characterisation and identification of different constituents of the renal stones.

Since knowledge of stone composition is a key factor in directing a logical pattern of further investigation and treatment, we recommend that in addition to the routine

wet chemistry analysis, spectrophotoscopic techniques may also be used. This will undoubtedly generate more sensitive and reliable results, which will enable clinicians to focus the mind on the metabolic abnormalities that may be responsible for development of urinary calculi.

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