

# A review of urinary stone analysis techniques

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## ABSTRACT

Knowledge of the chemical composition and structure of urinary stones is of great value in the choice of treatment and prevention of recurrence. This is a brief review and a comparative study of the principles and practical application of various chemical and physical techniques used for urinary stone analysis. The different methods of classifying and grouping urinary stones by results of chemical analytic techniques are, also, compared and evaluated. In addition to reviewing various techniques used for the in-vitro analysis of removed stone samples, the newly emerging physical and radiological techniques for the in-vivo intact-stone analysis are, also, evaluated. These in-vivo techniques, particularly the rapidly advancing unenhanced spiral CT scanning, represent an important step forward towards the notion of non-destructive analysis of urinary stones while still in situ before the choice of treatment modality.

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Urolithiasis is a multi-factorial recurrent rapidly increasing problem of universal distribution that causes substantial morbidity. Therefore, it is commonly believed that biochemical studies alone are never complete but correlation of clinical and biochemical results with that of chemical composition and structure of the stones is of great value in the delineation of pathogenesis, clinical management, preventive measures and choice of treatment modality. Until the late eighties, urinary stone analysis relied more on chemical rather than physical analytic techniques. The latter were considered not optimum for the quantitative analysis<sup>1,2</sup> as it detects the constituent salts providing it is not less than 5% of the sample.<sup>1,3</sup>

**Chemical analytic techniques and classification of stones.** The chemical analytic techniques detect the individual ions.<sup>1,4</sup> In urinary stone analysis, the hospital laboratories relied only on the simple qualitative and semi-quantitative chemical analytic techniques<sup>5</sup> which only give arbitrary information on stone composition.<sup>6,7</sup> However, many investigators

appreciated the value of quantitative chemical analytic techniques and several schemes for the classification of urinary stones were suggested.<sup>5,8-13</sup> Almost all the investigators utilized the resulting percentage composition of the constituting ions in considerable calculations based on certain assumptions to arrive at estimates for compounds.<sup>2,11,14-17</sup> Hence, the comparison between these classifications is difficult as the findings of chemical analysis in each were presented in a different manner and neglecting minor components (10 or 20%) was a common practice in all of them. However, a simpler classification of urinary stones based only on the percentage composition of the constituting ions was offered.<sup>18</sup> It successfully encompassed all the stone samples subjected to wet chemical analysis and also considered all the ions present whether trace (<2%) or minimum (2-10%) amounts; hence, the various stone groups were specified with precision. In approval of this classification, the results of microanalytic technique for the determination of total carbon, hydrogen and nitrogen confirmed the previous classification

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of the stone types and categories.<sup>18</sup> In addition, the classification of 214 urinary stones using the cluster analysis technique of the results of their ex-vivo computerized tomographic (CT) scanning and chemical analysis<sup>19</sup> was generally supportive to the previous classification and to the variables used for classifying the various stone groups.

***Ionic correlations within urinary stones.*** Meanwhile, in some large lamellate urinary stones when the previous classification was applied on the chemical composition of various stone lamellae the subsequently identified groups and their lamellar arrangement pointed out to some ionic correlations which may help in predicting the stone type in recurrent stone formers.<sup>20</sup> On the other hand, in urinary stone analysis although different analytic techniques (chemical and physical) were used, they shared some similar information on the stone composition as the identification of 5 groups in the phosphate stones<sup>18,21</sup> and the presence of <2% phosphate in uric acid stones<sup>2,11,18,22-24</sup> also the presence of uric acid (<20%)<sup>11,18,23</sup> and calcium phosphate<sup>2,11,15,18,22,23,25-28</sup> in oxalate stones and 2-10% of uric acid in calcium phosphate ones,<sup>11,18</sup> furthermore, infection stones are not oxalate but phosphate or uric acid<sup>11,18,29</sup> and the latter is of very low relative frequency percentage.<sup>7,15,18,30</sup> Meanwhile the infection phosphate stones are never pure struvite<sup>18,21,29,31-33</sup> as it contains variable amounts of calcium oxalate<sup>2,18,31,32,34</sup> or more frequently calcium phosphate.<sup>2,11,15,18,21,26,27-29,32,35-37</sup>

***Physical analytic techniques.*** On the contrary, for urinary stone analysis in the nineties, there was progressive increase in the use of physical techniques (infrared spectroscopy and x-ray diffraction photography) with decrease in relying on chemical methods which by now are considered to be obsolete.<sup>38,39</sup> This is expected to add more to the ease of procedures, revealing more detailed information on fine cellular structure and possibly diagnosing the stone type in-vivo especially after the recent advances in these techniques. The x-ray diffraction photography and crystallographic techniques of polarization microscopy are useful tools in the study of the crystalline structure, order of deposition of components and the nucleus.<sup>40-45</sup> However, as they need high technical skill and patience,<sup>15,21,25,26,46,47</sup> x-ray diffraction photography is only employed now, in a small number (5-9%) of specialized laboratories.<sup>39</sup> In addition, electron probe analysis and scanning electron microscopy are useful too for the study of different crystalline forms and organic matrix as well.<sup>42,48-55</sup> Accordingly, close examination of the urinary stones by scanning electron microscopy and similar high quality

sensitive analytic techniques as atomic absorption spectrophotometry,<sup>18,56,57</sup> inductivity coupled plasma atomic emission spectroscopy,<sup>18,58,59</sup> instrumental neutron activation analysis (INAA),<sup>60</sup> proton induced characteristic x-ray emission spectroscopy (PIXE)<sup>61,62</sup> and nuclear reaction analysis (NRA)<sup>62</sup> revealed several unexpected elements and trace elements mostly in the nuclear region of the stones such as Calcium, Magnesium, Sodium, Potassium, Copper, Aluminum, Zinc, Iron, Phosphorus, Silicon, Cobalt, Chlorine, Lead, Titanium, Selenium, Strontium, Boron, Tungsten, Zirconium and Uranium. However, their role in the nucleation process of stone formation is still controversial as it is supported by some investigators<sup>18,60-62</sup> and denied by others.<sup>58</sup> Meanwhile, all the previously used techniques are too cumbersome for routine purpose as they need special expertise and laborious sample preparation.<sup>7</sup> However, there is a progressive increase in the use of infrared spectroscopy technique in various biochemical laboratories<sup>38,39</sup> as it defines the stone composition with precision.<sup>18,43,63-68</sup> Meanwhile, the new technique of near-infrared reflectance spectroscopy when compared to the infrared spectroscopy is simpler and identifies, with sufficient accuracy qualitatively and semi-quantitatively, mixed urinary stones with 2 or 3 components in short time even when the stone powder is <100 micrograms.<sup>69</sup> But, the modern Fourier transform infrared spectrophotometry (FT-IR) is the most advanced technique. It allows the identification of calculi components by detailed comparison with libraries of reference spectra.<sup>70</sup> Its application pointed out to the presence of false calculi or artifacts and calculi containing drugs, metabolites or usual components around unusual nucleus. Hence, it was recommended that analysis of all calculi should be a strategy as sometimes it gives a clue to the pathogenesis. Meanwhile, for the characterization of the 2 hydrates of the calcium oxalate salt (mono- and dihydrate), thermogravimetry (TG) technique and differential thermal analysis (DTA) were considered as the best physical methods.<sup>71</sup> Hence, it was believed that TG method<sup>47,72-74</sup> may inform us about the age of the stone<sup>72,73</sup> and activity of the disease.<sup>75</sup> Furthermore, the simultaneous thermal analysis method TG-DTA would be an alternative method to the use of x-ray diffraction or Fourier transform infrared techniques for the quantitative determination of each hydrate of the calcium oxalate when present together in the presence of uric acid or magnesium ammonium phosphate.<sup>71</sup> Moreover, in urinary stone analysis testing the usefulness of dual energy radiographic densitometry and solid-state nuclear magnetic resonance spectroscopy (NMR) showed

that the former technique<sup>76</sup> is of a little value in determining the stone composition though providing information on the mineral and non-mineral phases of the calculus. However, the latter technique<sup>70,77</sup> detects and quantifies calcium oxalate, uric acid, struvite with calcium phosphate that closely resemble brushite and calcium hydroxy apatite (HA). Hence, it represents a new applicable technique.

#### ***In-vivo intact-stone analytic techniques.***

Furthermore, the exposure of the stone to a pulsed dye laser beam resulted in a fluorescent emission which appeared to be especially related to the stone composition previously determined by x-ray diffraction. Thus, it is another new technique which may have a practical in-vivo application.<sup>78</sup> Generally, in urolithiasis, the advent of the non-invasive techniques of ultrasound and CT provided an advance especially in demonstrating non-opaque urinary calculi which may be uric acid, urates, xanthine, cystine or poorly mineralized matrix and differentiating them from blood clots and tumors.<sup>79-81</sup>

In CT, it was found that in measurable non opaque calculi ( $\geq 2\text{mm}$ ) no difference in the attenuation value in vivo or in vitro and no correlation between stone size and density.<sup>82</sup> Meanwhile, sequential CT study is useful in the assessment of the position of the small calculi pre- and postoperatively especially in horseshoe or crossed fused ectopia where external and internal architectures are distorted. Also, it is useful in the dissolution therapy to determine its response<sup>83,84</sup> although a small uric acid stone below the detection threshold size (1.3 mm + 0.1) is imperceptible even with the unenhanced helical CT,<sup>85</sup> which has evolved to a well accepted alternative to intravenous urography.<sup>86</sup> In addition, CT and the more advanced unenhanced helical CT may be adjunct to the laboratory methods for the differentiation among various types of renal calculi. In vitro, by using CT, differentiation between uric acid, struvite and calcium oxalate stones with overall accuracy of 89% was possible by the mean pixel value and standard deviation of the stones pixel value.<sup>87,88</sup> However, the mean, maximum and modal pixel densities showed heterogeneity in the stone density and the uric acid stones were a distinct group. Also, the cystine stones were differentiated from struvite, calcium oxalate and brushite stones with 70% accuracy. However, calcium containing stones of various composition and struvite were not distinguished reliably.<sup>89</sup> Hence, better differentiation between various stone types was achieved on the basis of 3 parameters of CT data namely absolute CT values, dual CT values and pixel pattern. Thus, uric acid and cystine stones were significantly differentiated from the other types

( $p < 0.001$ ). The struvite stones with low or moderate calcium phosphate were identified correctly with 80% accuracy. However, the following stones calcium oxalate, calcium phosphate and struvite with high calcium phosphate could not be differentiated from each other.<sup>90</sup> Meanwhile in vivo, using the advanced unenhanced helical CT showed significant difference ( $p = 0.0367$ ) between the Hounsfield unit (HU) of the calculi predominantly composed of uric acid and that of the calculi predominantly composed of calcium oxalate.<sup>91</sup> Furthermore, no significant difference was noted between the Hounsfield unit values of calcium oxalate and calcium phosphate; hence, they were considered collectively as calcium stones.<sup>92</sup> However, significant difference ( $p = 0.006$ ) was found between the mean HU density of calcium stones and uric acid stones. Also, the difference between the mean HU density of calcium stones and struvite stones was significant ( $p = 0.073$ ). Otherwise, no significant differences were found among other stone types.<sup>92</sup> Accordingly, as it was not possible to identify the various urinary stone types by either the mean HU values or HU density, a new technique was offered using more than one parameter of CT data namely absolute HU values, HU density, dual CT values<sup>93</sup> using a small collimation size.<sup>93,94</sup> By the absolute HU values and HU density it was possible to differentiate with statistical significance between each of the following pure stone types uric acid (UA), calcium oxalate monohydrate (COM) and struvite in addition to their differentiation from most of the following mixed stones; COM 60-90% + hydroxyapatite (HA), COM 40-90% + UA, UA + COM <40% and struvite + COM + HA. Thus, differentiation with statistical significance was possible between pure UA and all mixed stone types except UA + COM <40% also between pure COM and mixed UA + COM <40%; and also pure struvite from all mixed stone types except mixed struvite stones. On the other hand, the dual CT values were not as good as the absolute HU values and HU density in the determination of stone composition on the basis of single CT evaluation. Furthermore, during spiral CT examination the recent advances in hardware and software technology enabled 3 dimensional figures of urinary stones. Hence, subsequent determination of linear dimensions along 3 coordinate axes and volumetric analysis of the calculi with high degree of accuracy becomes possible.<sup>95</sup> Accordingly, in vitro using microcomputed tomography (microCT),<sup>96</sup> an excellent information of the surface and internal structural deposition pattern was available from high resolution 3 dimensional images of intact urinary stones. Furthermore, the following 6 minerals UA, COM, COD, cystine, struvite and HA, which may be present in pure or heterogenous urinary stones were

identified and they had non-overlapping ranges of micro attenuation values. Therefore, this technique represents an important step forward towards the notion of non-destructive stone analysis in patients by the helical CT before the choice of treatment modality. In addition, another novel technique for intact stone analysis is the x-ray coherent scatter analysis using monoenergetic x-ray from the standard diagnostic x-ray equipment. As the coherent scatter properties depend on the molecular structure of the scattering media each of the stone components; COM, cystine, Magnesium ammonium phosphate and calcium phosphate demonstrated a distinct coherent scatter pattern which matches that of a pure chemical sample.<sup>68</sup> However, instead of monoenergetic x-ray, when using polyenergetic x-ray from the diagnostic x-ray equipment,<sup>97</sup> the coherent scatter analysis becomes as sensitive to stone component structure as the monoenergetic x-ray diffraction analysis. Therefore, it can be used in classifying urinary calculi by composition as it can detect the multiple stone components and show its structural arrangement.<sup>97</sup> Hence, with the use of appropriate collimation size, coherent scatter analysis may represent another technique for intact stone analysis in patients.

On the other hand, the relatively new technique of fiberoptic infrared spectroscopy<sup>98</sup> represents a new trend in urolithiasis. It is an alternative method to stone analysis especially after the use of Extra Corporeal Shock Wave Lithotripsy (ESWL), the best treatment choice, as the stone is fragmented into small particles. Therefore, the fiberoptic infrared spectroscopy technique is designed to detect qualitatively and quantitatively the various urinary salts present in fresh urine sample (without processing) with an average error of 20%.<sup>98</sup> However, further clinical research on this technique is essential to evaluate its potential.

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