Use of Infrared Spectroscopy for the Identification of Urinary Tract Calculi

FATEH UDDIN KHAND, MUHAMMAD YAR KHUHAWAR, SAIFULLAH MEMON AND AHMED FAROOQ ANSARI*

*Institute of Chemistry, *Department of Pharmacy, University of Sindh, Jamshoro, Pakistan.*

(Received 9th May, 1990, revised 11th October, 1990)

Summary: The potentials of infrared spectroscopy (IR) for the qualitative identification of calcium oxalate (mono- and di-hydrate), calcium hydrogen phosphate, magnesium ammonium phosphate, hydroxyapatite, carbonateapatite, calcium carbonate, ammonium acid urate, sodium acid urate and uric acid present in total 188 urinary tract calculi of patients of Hyderabad (Sindh), have been investigated. Specific bands within 4000-250 cm^{-1}, have been applied for the identification of each of the components present individually or in two to four component mixtures in the calculi. The results obtained were compared with that of wet chemical method, and a good correlation of both the methods has been noted. However, the convenience of complete and accurate analysis at mg level of the sample is the major advantage of IR technique.

Introduction

The analysis of renal calculi by infrared spectroscopy (IR) was briefly reported by Beischer [1] using nujol mulling technique as back as 1955. Weissman *et al* [2], Klein *et al* [3], Tsay [4], Takasaki [5], and Modlin [6] have performed analysis of renal stones by IR with paste and KBr tablet method. Bellanto *et al* [7], and Bollain *et al* [8], have identified with IR the different types of oxalates, phosphates and urates in urinary calculi. Oliver and Sweet [9], proposed a systematic scheme for the qualitative identification and interpretation of the IR spectra of calculi which was applied by Gault *et al* [10], and compared with wet chemical analysis. Later, Corns [11] compared the results of IR studies to that of X-ray diffraction and wet chemical analysis and suggested that combination of methods may be adapted for complete analysis. Recently, Berthelot *et al* [12], have reported computer aided analysis of urinary calculi.

In the present study IR has been utilized for the qualitative analysis of urinary tract calculi obtained from the patients of Hyderabad (Sindh) and adjoining areas and the results are compared with that of wet chemical analysis, in terms of reliability and the ease of analysis for identification of single- and mixed - component urinary calculi.

Experimental

A total of 188 calculi, from upper urinary tract (124) and lower urinary tract (64) were either obtained from the patients admitted in the Surgical Units of Liaquat Medical College Hospital, Jamshoro, or supplied by the patients themselves. The calculi received were washed with doubly distilled deionized water and dried in air. Samples weighing less than 500 mg were ground to a fine powder in an agate mortar, while the larger were cut into two equal halves with a fine Jeweller's saw to select representative material for IR studies. The outer layer was obtained by scraping the surface with sharp knife and the material from inner and central layers was taken with sharp preparation needles.

The IR of all the samples were recorded on Hitachi 250-60 IR spectrophotometer in the range 4000-250 cm^{-1} using well known KBr disc technique [7]; samples (1-2 mg) mixed with dried KBr (200-300 mg) were homogenized in agate pestle and mortar, placed in the evacuated die, and pressed at 10 t/cm^2 for two minutes for disc preparation.

The standard IR spectra of uric acid, hydroxyapatite, calcium carbonate, L-aspartic acid, L-glutamic acid (Merck), calcium oxalate monohydrate, L-cystine, albumin, magnesium ammonium phosphate hexahydrate (B.D.H.), calcium hydrogen phosphate (Hopkin and Williams) and xanthine (Sigma) were also recorded accordingly.

Wet chemical analysis for calcium, magnesium, ammonium, phosphate, oxalate, carbonate, uric acid/urate and cystine were carried out as reported earlier [13].

*To whom all correspondance should be addressed.

Table-1: IR spectral bands of the compounds identified in urinary stones

Compound	Main spectral bands	Diagnostic bands
Calcium oxalate monohydrate (COM)	3490(w),3435(w),3340(w), 3260(w), 3060(w), 1640-20(bs), 1318(ss),950(w), 885(w),780(ss), 660 (bm), 600-595(bw), 520(ss).	3490(w), 3435(w), 3340(w),3260(w), 3060(w),1318(ss), 780(ss), 520 (ss).
Calcium oxalate dihydrate (COD)	3450(bw), 1640-20(bs), 1320(ss),780(w), 625-620 (bw), 520(w).	3450(bw), 1320(ss), 780(w),520 (w).
Calcium hydrogen phosphate (CP)	3510(vw), 3000(w), 2840 (bw), 1635(bw), 1400(bw), 1350 (bw), 1130(sm), 1065 (sm), 995 (w), 890(sm), 580 (m), 560 (m), 520 (w), 400 (sm).	1130(sm), 1065 (sm), 995(w), 890 (sm).
Magnesium ammonium phosphate hexahydrate (MAP)	3420(m), 3220(vw), 2900(vw), 2770(vw), 1650(m), 1470(m), 1430(w), 1105(w), 1055(m), 980(w), 950 (w), 765(m), 630(m), 570(m), 340(m).	3420(m), 1470(m), 1430(w),1055(m), 765(m).
Hydroxyapatite (HA)	3570 (sw), 3450-3400(w),1630(w), 1450(w), 1410(w),1095(sw), 1033-1025(bs), 960(sw), 870(sw), 600(sm), 565(sm), 465 (bw).	3570(sw),1095(sw), 1033-1025(bs), 960(sw), 870(sw).
Carbonateapatite(CA)	3450(bw),2520(w),1455(w), 1420(sw), 1090(sw), 1040(ss), 960(sw),875(sw) 710(sw),605(sm), 568(sm), 480(w)	1455(w),1420(sw),1040(ss).
Calcium carbonate (CC)	2520(vw), 1420(m), 850 (m), 710 (m), 300 (vw).	1420(m), 850(m), 710 (m).
Uric acid (UA)	3015(bm), 2825 (w), 1670(bs), 1590(sm), 1482(w), 1435(w),1400(w),1350(sm), 1305(sm), 1220(w), 1120(ss), 1025(sm), 995(ss), 878(sm), 780(ss), 740 (ss), 705(sm), 618 (sm), 575 (sm), 525 (sm), 475 (ss), 395 (w), 350 (w).	3015(bm), 1305(sm), 1220(w), 1120(ss), 1025(sm), 705(sm), 575(sm), 475 (ss).
Ammonium acid urate (AU)	3050(m), 2900 (w), 2800 (w), 1650-1640(s), 1595 (w), 1535(w), 1345(sw), 1270 (w), 1135(sm), 1005 (sm), 880 (sm), 635 (w), 598 (sm), 490 (sm), 400 (w), 350 (w), three bifids at 1430, 1390, 790, 765 and 755, 725.	1535(w), 1270 (w), 635 (w).
Sodium acid urate (SU),	3600(sw), 3050(bw), 1732 (sw), 1655 (m), 1605(vw), 1520(sw), 1425(w),1380(m), 1345(w), 1252 (sw), 1195 (vw), 1130 (vw), 1000 (sm), 880 (sw), 825 (vw), 795 (w), 765 (w), 740 (sm), 718 (sm), 655 (vw), 620 (vw), 595 (sm), 525 (sm), 490 (m), 360 (vw), 330 (vw), 305 (vw).	3600(sw), 1732(sw), 1520 (sw), 1252(sw), 1195(vw), 655 (vw).

Results and Discussion

The main and diagnostic spectral bands of the compounds identified in urinary tract calculi are given in Table 1. The compounds, calcium oxalate (dihydrate), carbonateapatite, ammonium acid urate and sodium acid urate (Table 1) were not available with us. The urinary stone spectra of each of these compounds has been identified by comparing with standard spectra reported earlier [7,8,14].

Single component calculi were easily identified by matching IR spectra of these with that of the standards (for instance see figs. 1 to 3), and/or comparing the main spectral bands (Table 1). However, only 16.5% of calculi were seen to be composed of a single crystalline component and the rest were mixtures of 2-4 components (Table 2) with COM + HA,COM + AU, UA, + AU, HA + COM + AU and COM + UA + AU as the most frequent combinations.

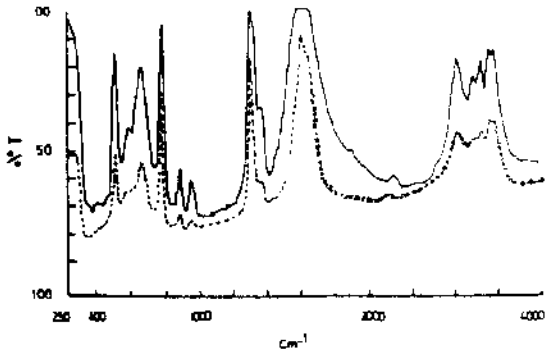

Fig.1: Infrared spectra of calcium oxalate (monohydrate) ——standard ------calculus

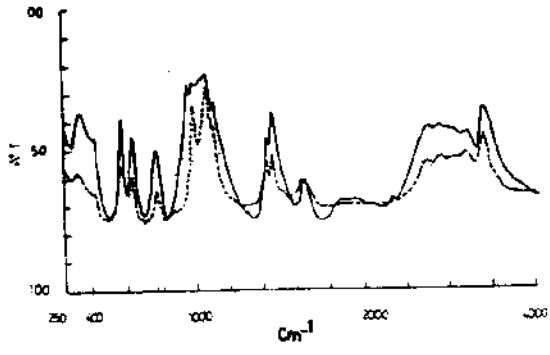

Fig.2: Infrared spectra of magnesium ammonium phosphate (hexahydrate) ——standard ------ calculus

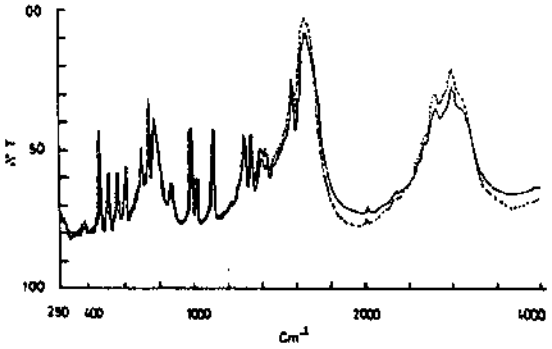

Fig.3: Infrared spectra of uric acid ——— standard - - - - calculus

Table-2: Percentage occurrence of single and mixed-component calculi.

Number of crystalline components	Number of stone	Percentage of toal stone
1	31	16.5
2	101	53.7
3	46	24.5
4	10	5.3

All the infrared spectra of urinary stone samples were carefully scrutinized to detect the presence of absorption maxima exhibited by the standard spectra of L-cystine, L-aspartic acid, L-glutamic acid, xanthine and albumin but none could be identified. This suggests that these organic compounds play no and/or very negligible role in the composition of urinary stones of this area.

Initially, Oliver and Sweet's [9] scheme was followed for the interpretation of IR spectra of mixed urinary stones but, due to technical difficulties in getting the similar shape and position of the peaks, the reference spectra of the known synthetic mixtures corresponding to the components present in the calculi, were also recorded (for instance see figs. 4 to 6).

As urinary calculi are most often consisted of oxalates, phosphates and/or uric acid/urates, the presence of these components could be detected by analysing the spectra at 1320-1315 cm^{-1} for oxalates, 1100-980 cm^{-1} for phosphates and 745-40 cm^{-1} for uric acid/urates. But, when uric acid/urate occurred as major component with oxalate, uric acid was found to interfere the oxalate peak at 1320 $cm.^{-1}$ Oxalate in such cases was identified by a relative

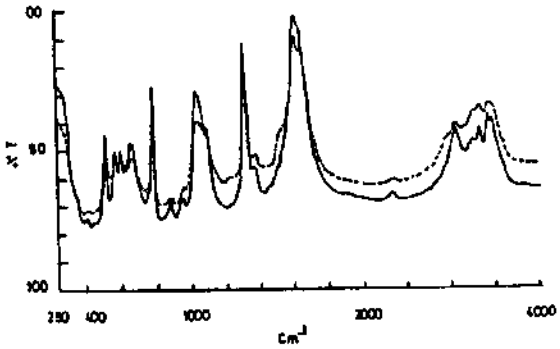

Fig.4: Infrared spectra of a calcium oxalate monohydrate and hydroxypatite mixture ——— standard ------ calculus

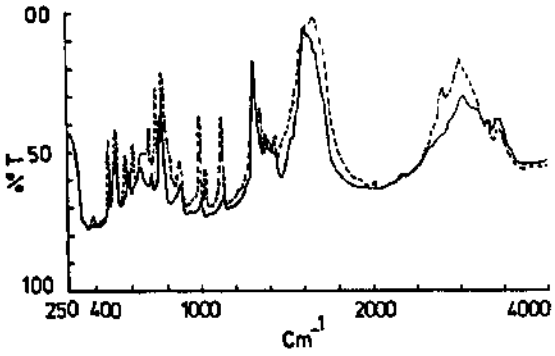

Fig. 5: Comparative infrared spectra of uric acid and calcium oxalate monohydrate (UA.COM); ammonium acid urate and calcium oxalate monohydrate (AU. COM) mixtures.
———UA.COM -----AU.COM

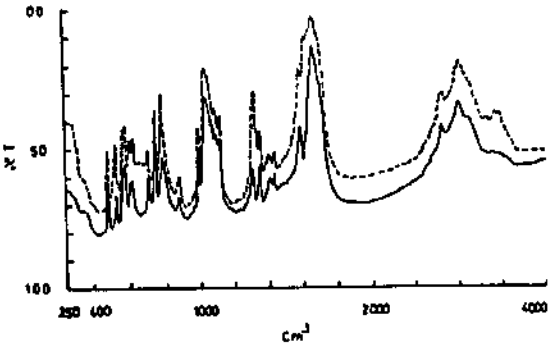

Fig.6: Infrared spectra of uric acid and hydroxyapatite (UA.HA); Uric acid, hydroxyapatite and calcium oxalate monohydrate (UA.HA.COM) mixtures ——— UA.HA ----------UA.HA.COM

enhancement in the peak intensities of uric acid bands at 1305 and 780 cm $^{-1}$ than at 1350 and 740 cm^{-1}, with a slight shift towards higher wave numbers (Fig. 7). The presence of oxalate could also be detected by a shoulder near 3400 cm.$^{-1}$

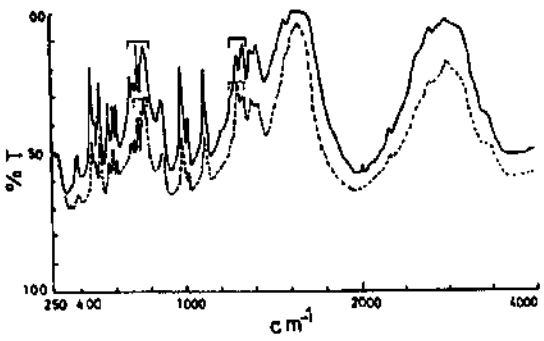

Fig.7: Comparative infrared spectra of uric acid and ammonium acid urate (UA.AU); uric acid, ammonium acid urate and calcium oxalate monohydrate (UA. AU. COM) mixtures.
———UA.AU .....----------.UA.AU.COM

The present work confirms earlier reports [6-8] that IR provides a simple way to differentiate calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) in calculi, within 3500-3050 cm^{-1} and 1000-600 cm^{-1}, with sharper and stronger peaks at 780 and 520 cm^{-1} for COM than COD.

The different forms of phosphates present in the calculi as hydroxyapatite (HA), carbonateapatite (CA), calcium hydrogen phosphate (CP) and magnesium ammonium phosphate (hexahydrate, MAP) were easily identified when present individually from their characteristic IR absorption bands. The diagnostic bands for HA and MAP were observed at 1095, 1035, 600 and 3420, 1470, 980, 769, 630 cm^{-1} respectively, while CP, owing to its different absorption pattern was detected between 1390-990 cm.$^{-1}$ The spectrum of CA although comparable to that of HA, showed additional peaks at 2510 and 710 cm.$^{-1}$

However, when HA is present together with CA, it is difficult to identify each component separately. Similarly, HA/CA and MAP can be easily distinguished (Fig. 8), but it becomes impossible to identify the minor constituent in presence of excess of the other.

Similarly, uric acid (UA), ammonium acid urate (AU), and sodium acid urate (SU) can be differentiated from one another by their characteristic bands. For instance, UA shows absorptions at 3015, 1305, 1120, 1025, 705, 575, 475 cm^{-1}, AU at 1270, 635 cm^{-1} and SU at 3600, 1732, 1520, 1252, 1192, 655, 400 cm.$^{-1}$

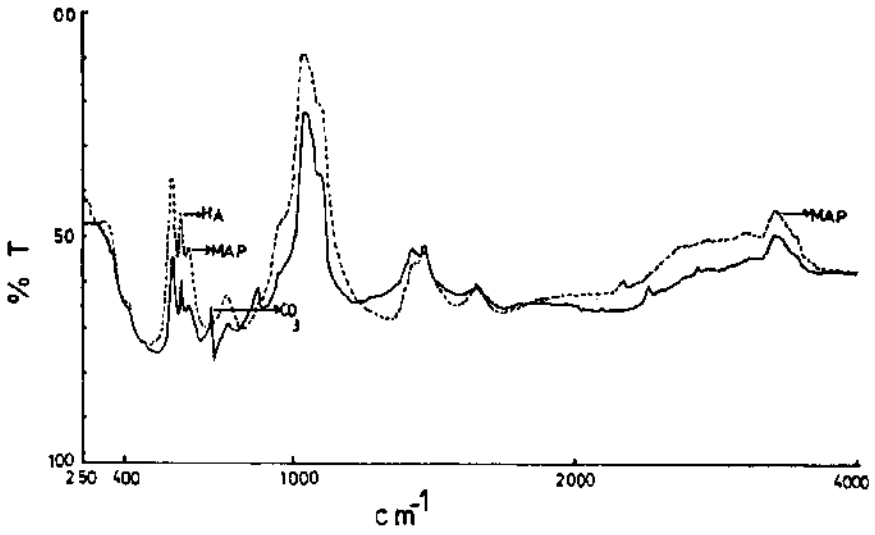

Fig. 8: Infrared spectra of carbonateapatite and magnesium ammonium phosphate (CA.MAP); hydroxyapatite and magnesium ammonium phosphate (HA.MAP) mixtures. — CA.MAP ------HA.MAP

Calcium carbonate (CC) absorption at 1420 cm^{-1} is fairly characteristic, but becomes difficult to recognize in the presence of MAP, due to ammonium ion absorption in the same region. The carbonate was therefore, identified at 710 $cm.^{-1}$

When compared (Table 3), the results obtained by IR and wet chemical tests were found to be in good agreement. The object of this comparison was to assess the accuracy and reliability of wet chemical tests, performed. Although, some discrepancies were noted in the detection of phosphate, ammonium and magnesium, the tests for calcium, oxalate, uric acid/urate and carbonate proved to be highly reliable. Phosphate identified by molybdate test in 21 calculi was not detectable by IR analysis. This probably could be due to the molybdate reagent which is more sensitive for phosphate than IR method [10], or an error in discriminating between the yellow precipitates and the yellowish brown turbidity of molybdate reagent due to moderately large amounts of oxalate [11].

Table-3: Number of analyses, where the qualitative method agreed or disagreed with IR method (n = 188).

Component	Ageement	Disagreement
Calcium	170	-
Magnesium	16	9
Ammonium	53	15
Oxakate	145	-
Phosphate	94	21
Uric acid/Urate	96	4
Carbonate	18	1

Conversely, ammonium ion found to be associated with urate in the central nuclei of 15 calculi could not be detected by wet chemical test which might be due to dilution factor as wet chemical tests were performed with whole stone powder.

Conclusion

With reference to analysis of urinary calculi, wet chemical and infrared spectroscopy are the two major techniques which have been most widely used. However, the cost of IR instrument greatly influences the availability of this technique to investigators in this part of the world. Since, wet chemical tests identify only the ions or radicals in calculus in solution, the sensitivity of these tests in case of mixed uroliths vary strongly. Thus, it becomes difficult to identify the compounds present in the mixed calculi, as a cation is always associated with several anions. Secondly, it seems impossible to differentiate hydrates of calcium oxalate and uric acid and different forms of calcium phosphate by wet chemical tests which also require larger amount (greater than 10 mg) of the material, hence are not suitable for layerwise study of uroliths. Whereas, infrared spectroscopy can be ranked as a reliable and accurate method. Therefore, the availability of IR

spectrophotometer in the range of 4000-250 cm^{-1} provides the facility of determining a comprehensive chemical composition of urinary calculi.

Acknowledgement

The authors are grateful to Drs. Jan Muhammad Memon (Professor and Surgeon, Liaquat Medical College Hospital Jamshoro) and Gul Hassan Kazi (Professor and Director, Centre of Excellence in Analytical Chemistry) for providing urinary stones and infrared analysis facilities respectively.

References

1. D.E. Beischer, *J.Urol.*, **73**, 653 (1955).
2. M. Weissman, B. Klein and J.Berkowitz, *Anal.Chem.*, **31**, 1334 (1959).
3. B. Klein, M. Weissman and J. Berkowitz, *Clin.Chem.*, **6**, 453 (1960).
4. Y.C Tsay, *J.Urol.*, **86**, 838 (1961).
5. E. Takasaki, *Calc. Tiss.Res.*, **7**, 232 (1971).
6. M. Modlin and P.J. Davies, S. Afr. *Med.J.*, **59**, 337 (1981).
7. J. Bellanato, L.C. Delatte, A. Hidalgo and M. Santos, *Int.Symp.Renal Stone Res.*, Madrid, **237** (1972) (Karger, Basel 1973).
8. R.M.H. Bollain, C.A. Cimadevila, P.J.F. Alonso and M.F. Bermejo, *Acta Quimica Compostelana*, **4**, 44 (1980).
9. L.K. Oliver and R.V. Sweet, *Clin.Chim.Acta.*, **72**, 17 (1976).
10. M.H. Gault, M. Ahmed, J. Kalra, I. Senciall, W. Cohen and D. Churchill, *Clin.Chim.Acta.*, **104**, 349 (1980).
11. C.M. Corns, *Ann.Clin.Biochem.*, **20**, 20 (1983).
12. M. Berthelot, G. Cornu, M. Daudon, M. Helbert and C. Laurence, *Clin. Chem.*, **33**, 2070 (1987).
13. F.D. Khand, M.S. Memon, A.F. Ansari and J.M. Memon, *J. Pak. Med. Assoc.*, **36**, 300 (1986).
14. A. Hesse and K. Molt, *J.Clin.Chem.Clin.Biochem.*, **20**, 861 (1982).