



Infrared Studies on Surgically Removed Urinary Stones

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Surgically removed urinary stones were procured from the local nursing homes. The stones were analyzed qualitatively for their mineral constituents. Thirty five stone samples, containing oxalate or phosphate or both, were selected for study. Infrared spectra of the selected stone samples were recorded in the range of 4000-500 cm^{-1} in KBr phase. Phosphate and oxalate bands were studied. Results revealed the presence of bridging and coordinating mode of oxalate and coordinating mode of phosphate in the stones. Water molecules in the stones have also been evidenced to be in the coordinated mode. Bonding of phosphate and oxalate groups in the urinary stones has been discussed in the light of the infrared data.

Key Words: Urolithiasis, Urinary stones, Urolithology, Infrared bands of urinary stones, Bonding in urinary stones.

INTRODUCTION

Urinary stones contain colloid as well as crystalloid portions. The later one is the main stubborn portion responsible for stone formation and related urolithology. The colloid portion mostly serves as the matrix. It is mainly organic in composition¹. It serves as a nidus, over which the stone grows by the deposition of the crystalloid material. The crystalloid material mostly consists from among a relatively few materials only. These are calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid and cystine. A state of super saturation of these materials in the urinary tract result in their slow accumulation over the nidus (matrix). The mode of embedment of crystalloid into the stone is an important problem to study. This is because the stones are usually very hard and difficult to crumble. The mode of chemical bonding in the stone, within the crystalloid as well as in between the crystalloids, would greatly influence the hardness of the stone. A high order of cementation would make the stone hard and difficult to crumble. It might make lithotripsy a difficult job. A study of intra-crystalline bonding through infrared spectroscopy, would be of academic as well as applied interest for urologist. Infrared bands of the groups of the crystal in the stone might give vital clues regarding the mode of bonding. With these views in mind, we have presently studied the infrared spectra of some surgically removed urinary stones containing phosphate or oxalate or both. The main aim of present studies is to analyze the phosphate and oxalate bands and to study the mode of bonding of phosphate and oxalate groups in the urinary stones.

EXPERIMENTAL

Surgically removed urinary stones were collected from the patients admitted to the different local nursing homes, where they were operated for the stone retrieval. Data concerning patient details and anatomical location of stone were also collected from the nursing homes. The stones were powdered. A portion of the powdered stone samples were preserved separately in sterilized neutral glass amples for infrared study. Remaining portion of the powders were subjected to qualitative chemical analysis for finding the mineral constituents by adopting standard methods². All chemicals used were of analytical reagent grade. Double distilled water was used for making all solutions. Carbonate was tested by the effervescence produced upon treatment of stone powder with cold 1N-nitric acid. Calcium and oxalate were detected by their precipitation from solution of stone powder (in 1N-nitric acid) with ammonium oxalate and calcium chloride reagents, respectively, at pH 5. phosphate was detected by ammonium molybdate test. Magnesium was detected by potassium dihydrogen phosphate test. Samples were also tested for uric acid and cystine by Folin's uric acid reagent and sodium nitroprusside solution, respectively. Thirty five stone samples, containing oxalate or phosphate or both, were only selected for study. Infrared spectra (FTIR) of the selected stone samples were recorded in the range of 4000-500 cm^{-1} in KBr phase on a Shimadzu 8201 PC infrared spectrophotometer.

RESULTS AND DISCUSSION

The patient details, anatomical location of stone and their mineral constituents are recorded in Table-1. Selected infrared bands of urinary stones are mentioned in Table-2.

TABLE-1
PATIENT DETAILS, ANATOMICAL LOCATION AND MINERAL CONSTITUENTS OF SELECTED URINARY STONE SAMPLES

Sample	Sex*	Age (year)	Anatomical location	Mineral constituents
S1	F	45	Renal Pelvis	Oxalate & Phosphate
S2	F	38	Renal Pelvis	Oxalate
S3	F	21	Kidney	Oxalate & Phosphate
S4	M	6	Bladder	Oxalate & Phosphate
S5	M	48	Bladder	Oxalate & Phosphate
S6	M	42	Ureter	Oxalate & Phosphate
S7	F	43	Renal Pelvis	Oxalate & Phosphate
S8	M	27	Rt. Vesico Ureter	Oxalate & Phosphate
S9	M	39	Ureter	Oxalate & Phosphate
S10	F	26	Renal Pelvis	Oxalate
S11	F	42	Kidney	Oxalate
S12	M	37	Ureter	Oxalate
S13	F	42	Renal Pelvis	Oxalate & Phosphate
S14	M	21	Pelvis	Oxalate & Phosphate
S15	M	32	Ureter	Oxalate & Phosphate
S16	M	30	Ureter	Oxalate & Phosphate
S17	M	2.5	Bladder	Oxalate & Phosphate
S18	M	28	Bladder	Oxalate & Phosphate
S19	M	33	Renal Pelvis	Oxalate & Phosphate
S20	F	44	Renal Pelvis	Oxalate & Phosphate
S21	M	52	Bladder	Oxalate & Phosphate
S22	M	25	Ureter	Oxalate & Phosphate
S23	M	36	Pelvis	Oxalate & Phosphate
S24	F	55	Kidney	Oxalate & Phosphate
S25	F	53	Bladder	Oxalate & Phosphate
S26	F	45	Ureter	Oxalate & Phosphate
S27	M	32	Bladder	Phosphate
S28	M	45	Ureter	Phosphate
S29	F	27	Upper Calyceal System	Phosphate
S30	M	46	Kidney	Oxalate & Phosphate
S31	M	17	Ureter	Oxalate
S32	M	20	Bladder	Oxalate
S33	F	35	Pelvis	Phosphate
S34	M	45	Bladder	Phosphate
S35	M	39	Kidney	Oxalate & Phosphate

*F = Female; M = Male

The relative importance of the crystalloid and colloid phases of the urinary calculi is not yet clear. In fact, the role of colloid phase (matrix) in the initiation of urinary stone is also not yet clearly resolved³. The prerequisite to resolve these aspects is to have a better understanding of the ultrastructure of the calculi. A number of physicochemical techniques like X-ray diffraction, electron optical studies, infrared studies might give a better information about the crystalline component. The bonding mode of various groups in the crystalline structure also becomes very important in unfolding the intra-crystalline forces. These factors, in turn, throw light on the hardness of the stone. The inorganic crystalline components of urinary stones are virtually limited to the oxalates and phosphates of calcium and magnesium. Studies on the nature of phosphate and oxalate bands in the infrared spectra of urinary stones would be helpful in unraveling the mode of bonding of these (phosphate and oxalate) minerals.

Phosphate in the urinary stones mostly remains as calcium hydroxyl apatite, calcium hydrogen phosphate (brushite) or magnesium ammonium phosphate (struvite). The phosphate ion has a tetrahedral symmetry. It normally shows four IR absorption modes. In a non-equivalent force field around the phosphate ion, however, there occurs distortion from the tetrahedral symmetry^{4,5}. In the case of ionic phosphate the totally symmetric P-O stretching mode (ν_1) is Raman active. However, in the case of coordinated phosphate, this band (ν_1) becomes IR active⁶. The position of ν_3 band gradually shifts to higher frequencies as the basicity of phosphate decreases from tri to mono. The intensity and broadness of the band also decreases in the same order. In case of coordinated phosphates, the ν_3 band splits into two when unidentate and into three when bidentate⁶.

Presently, the phosphate-containing urinary stones showed ν_1 (non-degenerate P-O stretching mode), ν_3 (asymmetric P-O stretching mode) and ν_4 (O-P-O bending mode) bands in the region 987-944, 1135-1003 and 672-512 cm^{-1} , respectively. The ν_3 band has mostly shown rather low, compared to its usual position. In some cases it showed as doubly split band. The ν_4 band, however, has invariably been found to be split into two, one band showing rather down (526-512) and the other showing slightly up (672-626), compared to its usual position. The intensities of ν_3 bands have also been observed to be mostly weak. This decreased intensities of ν_3 and ν_4 , their low position as well as their split (particularly ν_4) suggest that the phosphate in the stones is coordinated to the metal and is, probably, unidentate⁶⁻⁹.

Metal oxalates of ionic nature show $\nu(\text{C}=\text{O})$ bands in the region *ca.* 1700 cm^{-1} . In this case the C=O groups are terminal. In bridged oxalates this band usually show down to *ca.* 1600 cm^{-1} region¹⁰. Transition metal oxalates like that of cobalt, nickel, iron, *etc.*, are usually in the polymeric form with bridging C=O groups¹¹. The C=O bands are usually found at much lower position compared to the terminal ones. Crystal structure determination of nickel squarate dihydrate also showed a three dimensional polymeric network of octahedrally coordinated nickel(II) with bridging C=O groups¹². In case of trivalent metal oxalates as well as of Pt(II), Pd(II) and Be(II), on the other hand, a monomeric structure exists with $\nu(\text{C}=\text{O})$ frequency lying above 1700 cm^{-1} , suggesting a truly covalent metal-oxygen bonding¹⁰. All C=O groups are terminal ones. The bridged oxalate groups of Ni(II), Co(II) and Fe(II) oxalates show $\nu(\text{C}=\text{O})$ at a lower (*ca.* 1635 cm^{-1}) frequency. Earlier, these low values categorized them to be ionic oxalates. However, they are actually the covalently bonded bridging oxalates. $\nu(\text{C}=\text{O})$ values of different oxalates of transition metals are shown below¹⁰:

C=O Frequencies of oxalate complexes of transition metals:

Metal	$\nu(\text{C}=\text{O})$ (cm^{-1})
Fe(III) (monomer)	1712 (free/terminal)
Co(III) (monomer)	1707 (free/terminal)
Pd(II) (monomer)	1698 (free/terminal)
Mn(II) (polymer)	1635 (bridged)
Ni(II) (polymer)	1635 (bridged)
Co(II) (polymer)	1625 (bridged)
Fe(II) (polymer)	1620 (bridged)

TABLE-2
KEY INFRARED BANDS (cm⁻¹) OF SURGICALLY REMOVED URINARY STONES

Stone sample	v(OH) & v(O-H...O)	v(C=O)	Asym. P-O stretch (ν ₃)	Sym. P-O stretch (ν ₁)	O-P-O bending mode (ν ₄)	δH ₂ O (coordinated)	Mineral constituent
S1	3494, 3342, 3066, 2293	1674, 1641	1108	950	660, 519	885	Oxalate & Phosphate
S2	3482, 3196, 2452	1658, 1624	–	–	–	863	Oxalate
S3	3460, 3326, 3080, 2360	1653, 1618	1105	952	662, 519	880	Oxalate & Phosphate
S4	3492, 3337, 3061, 2310	1639, 1614	1109	950	663, 516	885	Oxalate & Phosphate
S5	3490, 3372, 3068, 2342, 2368	1631	1110, 1008	953	661, 520	885	Oxalate & Phosphate
S6	3451, 3340, 2364, 2341	1627	1123, 1003	952	667, 519	883	Oxalate & Phosphate
S7	3521, 3363, 3050, 2350, 2372	1648, 1610	1096	966	660, 517	878	Oxalate & Phosphate
S8	3435, 3320, 3078, 2363,	1613	1094	980	665, 517	883	Oxalate & Phosphate
S9	3550, 3370, 3080, 2320, 2386	1649, 1616	1098	962	668, 519	872	Oxalate & Phosphate
S10	3435, 3324, 3076, 2310	1678, 1639	–	–	–	815	Oxalate
S11	3425, 3320, 3060, 2340, 2365	1679, 1628	–	–	–	880	Oxalate
S12	3430, 3290, 3080, 2368	1665, 1642	–	–	–	882	Oxalate
S13	3520, 3285, 3060, 2340, 2368	1656, 1612	1090	950	660, 518	884	Oxalate & Phosphate
S14	3437, 3341, 3066, 2364, 2342	1621	1069	950	667, 518	885	Oxalate & Phosphate
S15	3430, 3328, 3060, 2340, 2362	1628	1096	987	665, 518	882	Oxalate & Phosphate
S16	3528, 3292, 3075, 2348	1624	1130, 1005	986	668, 522	882	Oxalate & Phosphate
S17	3491, 3338, 3068, 2318	1658, 1609	1108	950	668, 516	886	Oxalate & Phosphate
S18	3500, 3380, 3052, 2340, 2370	1628	1092	978	659, 517	882	Oxalate & Phosphate
S19	3480, 3292, 2348, 2367	1632, 1614	1110	955	663, 516	834	Oxalate & Phosphate
S20	3523, 3381, 3241, 2370	1638, 1612	1108	959	662, 518	849	Oxalate & Phosphate
S21	3492, 3348, 3076, 2352, 2370	1631	1109, 1012	958	671, 526	860	Oxalate & Phosphate
S22	3470, 3320, 2340, 2362	1652, 1608	1102	958	664, 518	882	Oxalate & Phosphate
S23	3491, 3341, 3065, 2363, 2342	1617	1104	949	665, 517	884	Oxalate & Phosphate
S24	3500, 3236, 2348	1654, 1614	1082	955	662, 521	828	Oxalate & Phosphate
S25	3496, 3288, 3058, 2343, 2368	1636	1088	956	651, 518	882	Oxalate & Phosphate
S26	3489, 3334, 3061, 2362	1624	1135, 1004	950	663, 517	885	Oxalate & Phosphate
S27	3450, 3290, 2453, 2485	–	1084	956	669, 522	842	Phosphate
S28	3360, 3182, 2465	–	1079	948	626, 512	852	Phosphate
S29	3400, 3180, 2330, 2490	–	1125, 1018	955	672, 526	863	Phosphate
S30	3486, 3225, 2482	1632	1092	944	662, 518	858	Oxalate & Phosphate
S31	3530, 3270, 2350, 2480	1660, 1628	–	–	–	875	Oxalate
S32	3434, 3247, 2361	1674, 1632	–	–	–	882	Oxalate
S33	3420, 3262, 2352, 2476	–	1120, 1014	944	665, 522	856	Phosphate
S34	3510, 3192, 2388	–	1086	953	658, 514	849	Phosphate
S35	3472, 3176, 2332, 2485	1626	1126, 1009	946	662, 512	843	Oxalate & Phosphate

In the alkali metal adducts of Ni(II)/Co(II) oxalates also the v(C=O) values were found to be invariably below 1650 cm⁻¹ suggesting bridged oxalate groups¹⁰. Thus it has been generalized that in metal oxalates, when the C=O groups are free (terminal), the frequencies are usually above 1700 cm⁻¹, whereas, when the C=O groups are bridging, the v(C=O) shows¹⁰ below 1650 cm⁻¹.

Presently, in the case of urinary stone samples containing oxalate, the C=O bands have been mostly observed at rather low position, in the range 1679-1608 cm⁻¹. More than one band has been observed mostly. In case of single band, it showed rather very low at ca. 1625 cm⁻¹. These observations suggest that the C=O of oxalate in the stone is in bridging mode^{10,13,14}. The split of the band also suggest that the group (C=O) is also coordinated to the metal (Ca²⁺). This bridging of oxalate is also perhaps responsible for strong intra-crystalline forces and stubbornness of the oxalate stones.

In most of the stones both oxalate and phosphate have been found to be present which is also the usual case. It looks from the mode of IR bands, that, oxalate and phosphate both simultaneously coordinate to the metal (Ca²⁺) to form some sort of mixed ligand complex, with oxalate assuming the bridging role between the two metal ions. In pure oxalate stones, however, the complex seems to be a single ligand bridged one.

Spectra of all the stones showed sharp intense bands in the region 3500 to 3000 cm⁻¹ suggesting the presence of water

molecules. These bands, however, may also be due to the OH group of apatite. These H₂O/OH seem to be coordinated, because, the v(OH) band has mostly been found to split into two or three. This is further substantiated by the fact that a sharp band is invariably shown at ca. 880 cm⁻¹, which may be assigned to rocking mode of coordinated water¹⁵. The H₂O/OH might also be involved in hydrogen-bonding because in almost all cases additional weak bands appear at 2290-2500 cm⁻¹ region.

Conclusion

The level of cementation in the urinary stones, in a way, is the function of intra-crystalline and inter-crystalline forces. They, in turn, depend on mode of bonding of the constituents. Present studies on infrared bands of oxalate and phosphate groups in the urinary stones throw some light on the mode of their bonding in the stones. Present observations that the phosphate in the urinary stones is mostly in the coordinated nature and that the oxalate groups are in the coordinating and bridging mode in the urinary stones explain, to some extent, the hard cementation of the later. These studies would also lead to a better understanding of the biomineralization processes.

The study of infrared bands of surgically removed urinary stones would also be useful from the clinical point of view. A better understanding of the chemical composition and type of the stones and their level of hardness would enable the surgeons

and physicians to plan a better management and selection of suitable surgical option in case of a future recurrent stone episode of the same patient.

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