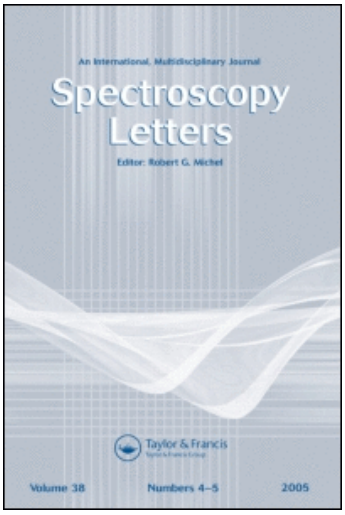

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Vibrational and SERS Spectra of Spermine Phosphate Hexahydrate

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VIBRATIONAL AND SERS SPECTRA OF SPERMINE PHOSPHATE HEXAHYDRATE

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ABSTRACT

FTIR, Raman and SERS spectra of spermine phosphate hexahydrate have been recorded and analysed. Vibrational spectra show the protonation of amino and imino groups indicated by the presence of HPO_4^{2-} ion. The molecule is found to be adsorbed to the metal surface through nitrogen and oxygen atoms of the molecules. Distortion of the HPO_4^{2-} ion, change of symmetry of the molecule due to chemisorption and the enhancement in intensity of the amino group vibrations are discussed.

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INTRODUCTION

Polyamines are aliphatic, nitrogenous, non protein bases having low molecular weight. They are present in cells in large quantities possessing the capability of substituting for cations such as K^+ or Mg^{++} that may be in short supply [1]. It has been reported that the urine of patients with solid tumours and lukaemia contains relativley higher amount of polyamines than that of healthy persons [2]. Study of polyamines are important as they stabilise DNA, bridging the two poly nucleotide strands of double helix via hydrogen bonds with the phosphate group across the grooves [2 - 5]. Spermine is a polyamine widely distributed in animal and micro organism related biological materials. Its concentration is found to be very high in pancreas, prostrate of mammels and human seman [6]. Studies on the effect of spermine on various enzyme activites and on the relationship to nucleic acids have revealed many interesting physiological and pharmacological phenomena which are still not well understood at the molecular level.

Spermine ($C_{10}H_{26}N_4$) being a strong base highly soluble in water, addition of phosphoric acid (H_3PO_4) to its aqueous solution results in the precipitation of the salt spermine phosphate hexahydrate ($C_{10}H_{26}N_4 \cdot 2H_3PO_4 \cdot 6H_2O$), hereinafter referred to as SP, immediately. In this paper the vibrational and SERS investigations of spermine phosphate hexahydrate is carried out to obtain information on the binding mechanism, geometry, conformation and orientation of the adsorbed molecule, which will be important in understanding the biological phenomena of the compound.

EXPERIMENTAL

Commercially available (Aldrich Co. Ltd 99.99% pure) spermine phosphate hexahydrate was used for the investigation. Silver colloid was prepared from sodium borohydride and silver nitrate.

Raman spectra (Fig. 1) were recorded on a Dilor Z 24 spectrometer with 300 mW laser power of a Spectra Physics model 165 argon ion laser (514.5nm). The FT-IR spectra (Figs. 2 and 3) were obtained using Nicolet - 510P (4000-400cm^{-1}) and Bruker IFS 66V (500 - 50 cm^{-1}) spectrometers.

SERS spectra (Figs. 4, 5, and 6) were recorded for concentrations 10^{-4}, 10^{-5} and 10^{-6} M in the stable colloid prepared by the method described by Creighton et al. [7]. To record the SERS spectra drops of 10^{-4} / 10^{-5} / 10^{-6} M spermine phosphate hexahydrate solution was added to 2 ml. of the silver colloid.

FACTOR GROUP ANALYSIS

SP belongs to the monoclinic system with a = 7.955, b = 23.216 and c = 6.870 Å. The space group is $P2_1/a$ $\left(C_{2h}^5\right)$ and there are two units in the crystallogrphic unit cell [6].

Its structure consists of parallel sheets of spermine molecules separated by phosphate ions and water molecules which form a sheet of composition $HPO_4^{2-}.3H_2O$ by O - H ···O hydrogen bonds. The outstanding structural feature

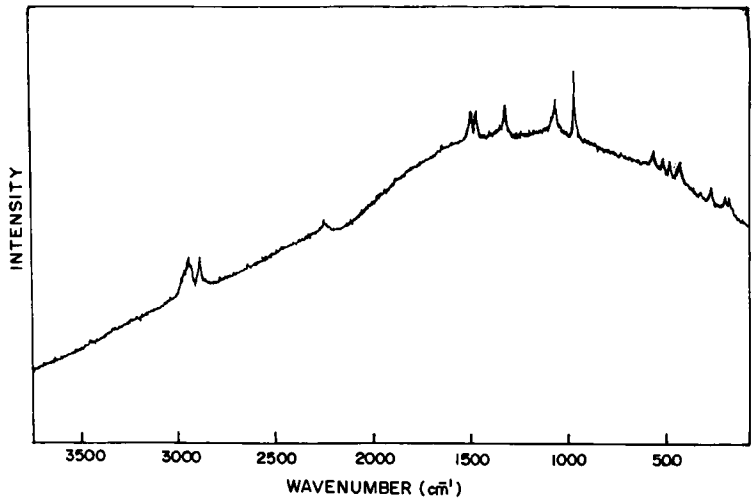

Fig. 1 Raman Spectrum of Spermine Phosphate Hexahydrate (Polycrystalline form)

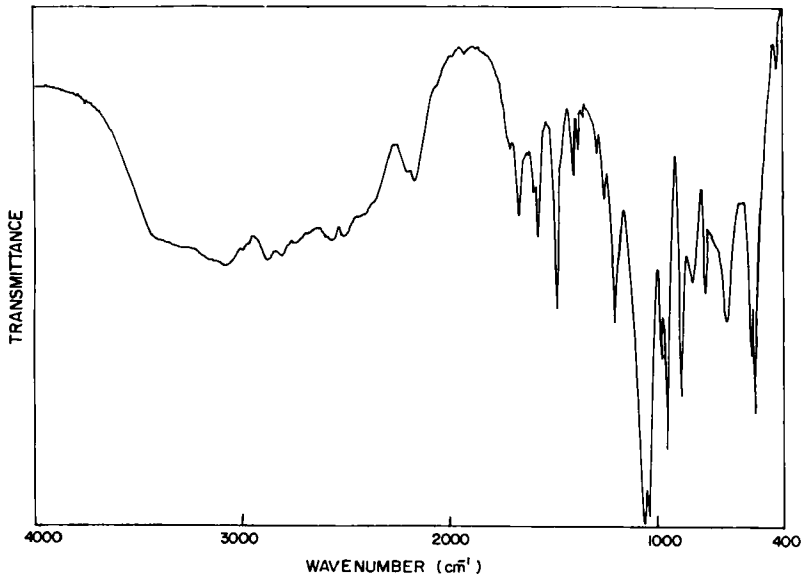

Fig. 2 FTIR Spectrum of Spermine Phosphate Hexahydrate in the region 400-4000cm^{-1}

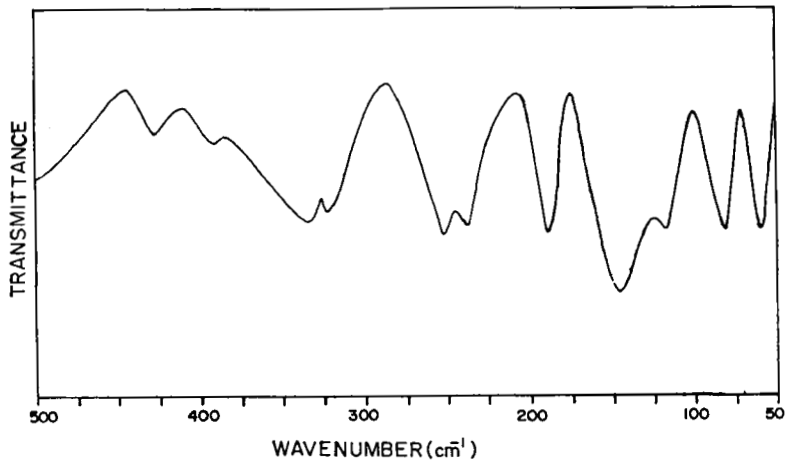

Fig. 3 FTIR Spectrum of Spermine Phosphate Hexahydrate in the region 50-500cm^{-1}

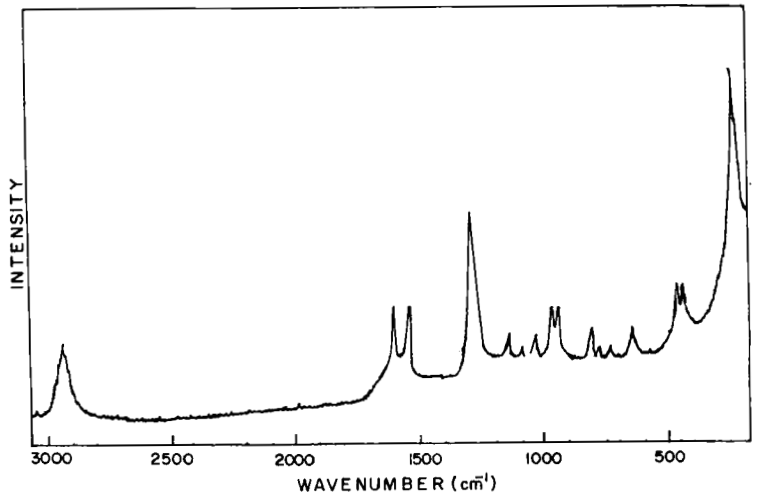

Fig. 4 SERS Spectrum of 10^{-4}M Spermine Phosphate Hexahydrate in silver sol

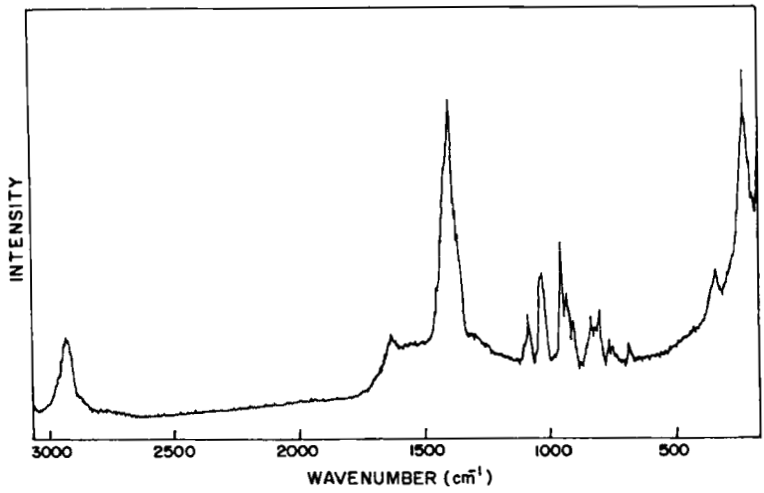

Fig. 5 SERS Spectrum of 10^{-5}M Spermine Phosphate Hexahydrate in silver sol

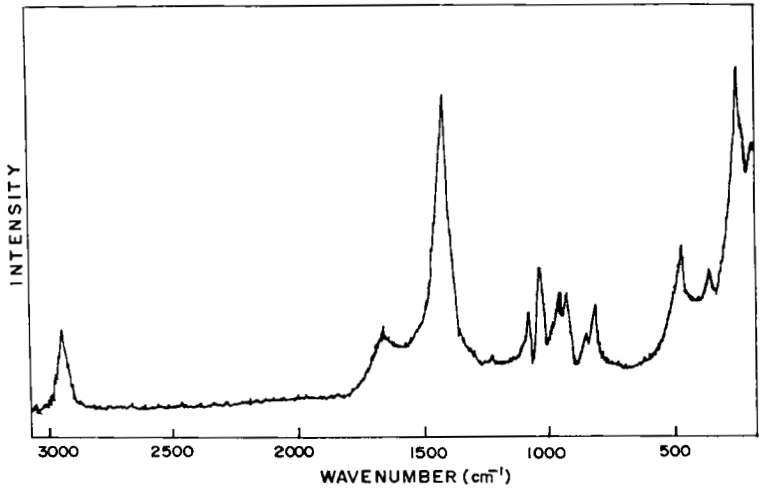

Fig. 6 SERS Spectrum of 10^{-6}M Spermine Phosphate Hexahydrate in silver sol

is the presence of two kinds of sheets. These two kinds are stacked alternately parallel to the (001) plane, being held together by N - H ···O hydrogen bonds.

The standard group theoretical analysis [8] gives the distribution of irreducible representations at k = 0 (excluding 3 acoustic modes) as follows (Table 1)

$$SP = 111A_g + 111B_g + 110A_u + 109B_u$$

where the 'g' modes are Raman active, and 'u' modes are IR active. The symmetry of the phosphate ions is lowered from T_d to C_1 in SP. The correlation schemes for its internal modes are shown in tables 2 to 7. Corresponding frequencies are also given in brackets.

INTERNAL VIBRATIONS OF SPERMINE MOLECULE

The spermine molecule occupies a general position in the crystal lattice and hence all the 237 fundamental vibrations are Raman and IR active. However, as the unit cell contains large number of CH_2 groups, the phonon energies will be closely spaced and all the predicted modes cannot be identified. The crystal structure determination [6] reveals the possibility for the protonation of amino and imino nitrogens at the expense of the phosphate group. In that case NH_3^+ and NH_2^+ vibrations are the ones expected in the spectrum. Identification and interpretation of the vibrational frequencies can reveal information regarding the presence of NH_3^+, NH_2^+ and deprotanation of phosphate group.

The stretching modes of CH_2 group usually occur in the region 2800 - 3100 cm^{-1}. In the present case it is observed at 2505, 2558, 2873 and 3072 cm^{-1}.

Table 1

Factor group analysis of Spermine phosphate hexahydrate (Space group $P\,2_1/a$ ==> C_{2h}^5 ; $Z^B = 2$).

Factor group species is C_{2h}	NH_3^+ (C_1 site)		CH_2 (C_1 site)		NH_2^+ (C_1 site)		HPO_4 (C_1 site)		H_2O (C_1 site)		Optical modes	Acoustic modes	Activity	
	Internal modes	External modes	Internal Modes	External modes	Internal modes	External modes	Internal modes	External modes	Internal modes	External modes			Raman	IR
A_g	6	3T, 3R	15	15T, 15R	3	3T, 3R	12	3T, 3R	9	9T, 9R	111		a	f
B_g	6	3T, 3R	15	15T, 15R	3	3T, 3R	12	3T, 3R	9	9T, 9R	111		a	f
A_u	6	3T, 3R	15	15T, 15R	3	3T, 3R	12	3T, 3R	9	9T, 9R	111	-1	f	a
B_u	6	3T, 3R	15	15T, 15R	3	3T, 3R	12	3T, 3R	9	9T, 9R	111	-2	f	a
	24	12T, 12R	60	60T, 60R	12	12T, 12R	48	12T, 12R	36	36T, 36R	444			

T - Translations; R - librations; a - active; f - forbidden

Table 2

Correlation of the internal vibrational modes for PO_4 units in SP.

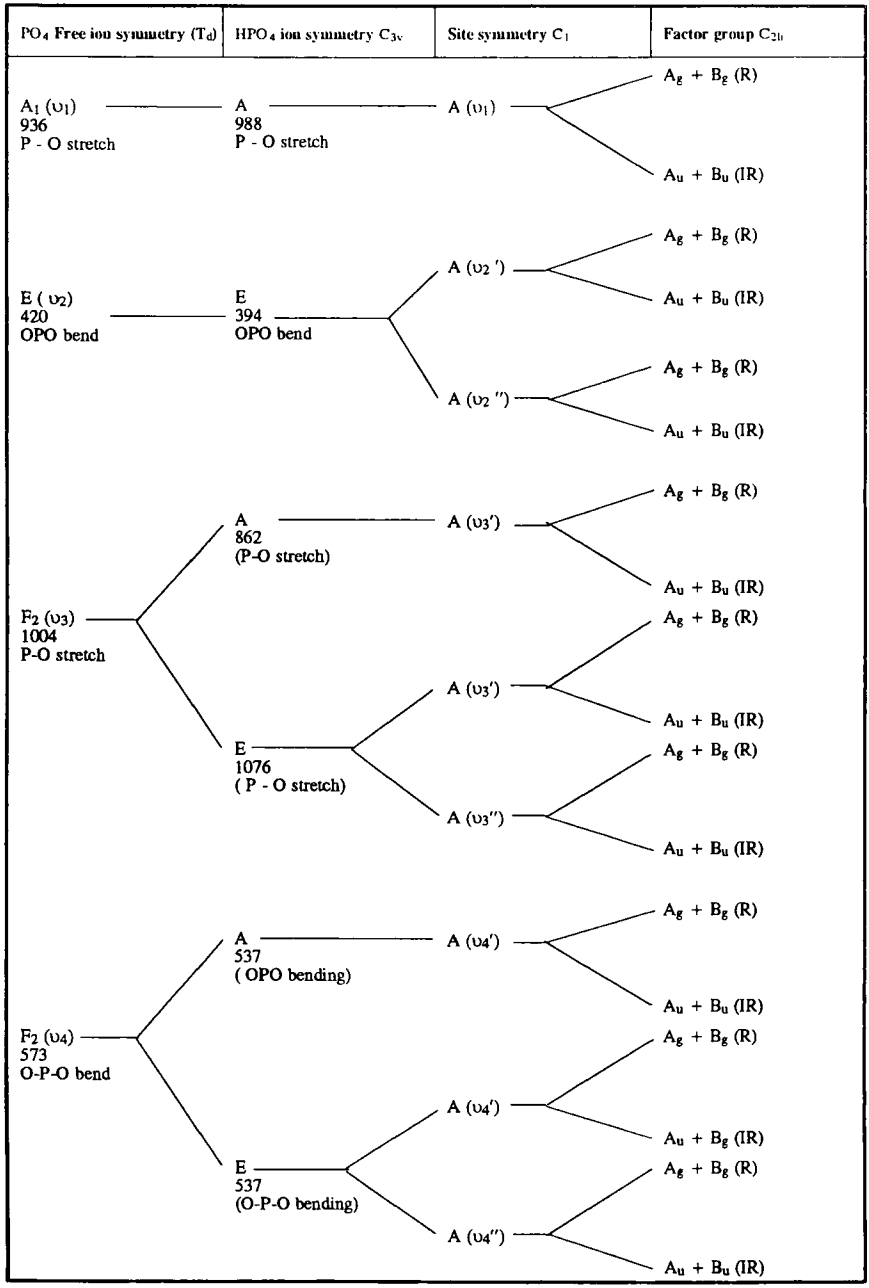

Table 3

Correlation scheme for the internal vibrations of HPO_4 ion in SP.

f^{γ}	HPO_4 ion symmetry C_{2v}	Site symmetry C_1	Factor group symmetry C_{2h}
PO_4 modes			
4	A_1	A	$9A_g$ (R)
8	E		$9B_g$ (R)
4	A_1		$9A_u$ (IR)
8	E		$9B_u$ (IR)
4	A_1		
8	E		
H modes			
4	A_2	A	$3A_g$ (R)
8	E		$3B_g$ (R)
			$3A_u$ (IR)
			$3B_u$ (IR)

Table 4

Correlation scheme for the internal vibrations of NH_3^+ group Spermine molecule in SP.

f^γ	Free ion symmetry C_{3v}	Site symmetry C_1	Factor group symmetry C_{2h}
8	A_1	A	$6A_g$, $6B_g$, $6A_u$, $6B_u$
16	E		

Table 5

Correlation scheme for the internal vibrations of NH_2^+ group in SP.

f^γ	Free ion symmetry C_{2v}	Site symmetry C_1	Factor group symmetry C_{2h}
4	A_1	A	$3A_g$, $3B_g$, $3A_u$, $3B_u$
4	A_1		
4	B_1		

Table 6

Correlation scheme for the internal vibrations of CH_2 group in SP.

f^{γ}	Free ion symmetry C_{2v}	Site symmetry C_1	Factor group symmetry C_{2h}
20	A_1	A	$15A_g$
20	A_1		$15B_g$
20	B_1		$15A_u$
			$15B_u$

The lowering of these frequecies to 2500 cm^{-1} must be due to the bonding of the CH_2 group to a nitrogen atom [2]. The wagging, twisting and rocking modes of CH_2 are also identified. The complete assignments are given in Table 8.

VIBRATIONS OF HPO_4^{2-}

The protonation of the amino and imino nitrogens at the expense of H_3PO_4 results in HPO_4^{2-} ion. The phosphate tetrahedra is distorted slightly with P - O distances 1.517, 1.518, 1.529, and 1.589 Å. The acid hydrogen atom is bonded to the oxygen atom of the longest P - O bond [6]. The vibrational assignment can be done on the basis of characteristic vibrations of HPO_4 with C_{3v} symmetry. In Sp the HPO_4^{2-} ions occupy sites of C_1 of lower symmetry than

Table 7

Correlation scheme for the H_2O vibration (internal and librations) in SP.

f^Y	Free ion symmetry C_{2v}	Site symmetry C_1	Factor group symmetry C_{2h}
Internal modes			
12	A_1	A	$9A_g$
12	A_1		$9B_g$
12	B_1		$9A_u$
			$9B_u$
Librational modes			
12	A_2	A	$9A_g$
12	B_1		$9B_g$
12	B_2		$9A_u$
			$9B_u$

the free ion symmetry. This may lead to activation of inactive modes along with splitting and / or shifting of internal modes. Though the free ion approach leads to only 3 bands each for stretching ($2A_1$ + E) and bending (A_1 + 2E) modes of the PO_4 group in both IR and Raman spectra, the site symmetry approach predicts four and five bands respectively for these modes. The appearence of four stretching bands and nine bending bands for HPO_4^{2-} which

Table 8

Vibrational Spectral data (cm^{-1}) and band assignments of SP.

Powder	IR	Assignments
66	57 m	
	80 m	T PO_4 ,
	119 m	
158	145 s, br	R PO_4
189	188 m	t CH_3
257	240 m	
	251 m	
	323 m	δ_s O–P–O
	336 m	
	392 vw	
427	430 vw	δ_s PO_3, δ_{as} O–P–O , t NH_3^+
476		
512	535 s	δ_{as} PO_3
563	556 s	
	670 m, br	R H_2O
	774 m	γ P–O–H,
	835 m	t NH_2^+
	888 s	υ_s p–O(H)
953	957 vs	υ_{as} P–O(H), υ C–C
	985 m	υ_s PO_3
1050	1047 vs	υ C - N
	1068 vs, br	υ_{as} PO_3
	1202 m	δ OH
	1252 w	δ P - O - H
1311	1293 w	ρ CH_2
	1342 vvw	t CH_2
	1361 vvw	ω CH_2 , γ NH_2^+
	1404 w	δ_s CH_2
1464	1462 sh	δ_{as} CH_2
1491	1481 s	δ_s NH_3
	1574 m	NH_2 scissoring
	1592 sh	
	1664 m	δ H_2O, υ_{as} NH_3^+
	1706 w	Overtones &
	1863 vvw	combinations of
	1917 vvw	δ, ρ ω (CH_2)
	1979 vvw	&
	2049 vvw	t, ω, ρ (NH_3^+)
2248	2164 m	
	2505 s	
	2558 s	υ_s CH_3,
2878	2873 s	
2937	3072 s, br	υ_{as} CH_2, υ_s NH_3^+
	3140 s, br	υ_{as} NH_3^+,
3150	3300 s, br	υ_1, υ_3 H_3O
	3450 s, br	

is more than those predicted by factor group analysis, indicates that the effect of site symmetry in the crystal is considerable. The internal vibrations of HPO_4^{2-} group can be considered as arising from those of PO_3 and P - O (H) vibrations.

In the IR spectrum, the PO_3 asymmetric stretching mode appears as the most intense band at 1068 cm^{-1} as expected. The PO_3 symmetric stretching mode (A_1) though IR inactive is observed as a medium intense band at 985cm^{-1}. This is a consequence of site symmetry effect which confirms the lowering of the symmetry of HPO_4^{2-} ion.

The IR and Raman frequecies corresponding to υ_6 (E) P - O - H bending, υ_2 (A_1) P – O (H) stretching, υ_1 (A_1) PO_3 symmetric stretching and υ_4(E)PO_3 asymmetric stretching modes appear slightly shifted from the corresponding frequencies in the spectra of HPO_4^{2-} aqueous solution [9]. This indicates that the HPO_4^{2-} tetrahdra is slightly distorted in the crystal. Thus various frequencies observed for the phosphate group are close to the ones observed for HPO_4^{2-} ion which also substantiate the deprotonation of phosphate group.

The normal Raman spectrum has bands of relatively weak intensity probably due to excessive flourescence. Even the υ_s PO_3 which is expected to be very intense could not be observed.

SERS SPECTRA

In the metal adsorbate stretching mode region two bands have been observed. The one around 225 cm^{-1} is strong in all the concentrations whereas the one around 170 cm^{-1} is medium intense and found only in two concentrations (Table 9). These are assigned to υ Ag ···· N and υ Ag·····O respectively. This observation clearly shows that the molecule is adsorbed to the metal surface through different sites - through the amino and imino nitrogens and through the oxygen of the phosphate group.

In the SERS spectrum the band observed around 1400 cm^{-1} corresponding to NH_2^+ rocking is the most intense in all the three concentrations. In the 10^{-5} M concentration it appeared as a triplet (1360, 1395 and 1410 cm^{-1}). The splitting of this NH_2^+ rocking mode can be attributed to the change of symmetry of the molecule on chemisorption and the consequent breakdown of selection rules. The twisting and scissoring modes of NH_2^+ and bending modes of NH_3^+ have also shown intensity enhancement. Stretching vibrations of NH_3^+ and asymmetric stretching vibrations of NH_2^+ are also found to be enhanced. Thus it is evident that the NH_2^+ and NH_3^+ vibrations are generally affected by adsorption of the molecule to the silver surface. It is reasonable to infer that the mechanism behind the interaction between the amino group and silver surface is the co-ordination through nitrogen lone pair electrons.

The molecule adsorbed with its plane perpendicular to the metal surface shows greater enhancement of the in plane vibrational modes [10]. According

Table 9

SERS Spectral data (cm^{-1}) and band assignments.

10^{-6} M	10^{-5} M	10^{-4} M	Assignment
171 m	167 m		υAg···O
229 s	225 s	227 s	υAg···N
342 w	339 m	451 m, br	δ PO_3, t NH_3^+
458 m, br		479 m	
	685 w	659 w	δ as PO_3
	747 w	766 vw	
804 m	801 m	807 vw	NH_2^+ twist
836 w	821 m	834 w	
	836 m		
	900 m	931 m	υ C – C
923 m	928 w		
959 m	952 m	957 m	υ_s PO_3
	986 vw		
1026 m	1027 m	1040 w	υ C – N
1074 w	1083 w	1091 vw	υ_{as} PO_3
1225 vw	1300 vw	1168 w	
	1360 sh		
1407 vs	1395 vs	1395 s	NH_2^+ rock
	1410 sh		
	1487		δ_s NH_3^+
		1583	NH_2^+ scissorring
1646 w, br	1638 m, br	1622 m	δ_{as} NH_3^+
	2200 m, br		
2929 m	2900 m	2930 m	
2995 vw	2925 m	3060 vvw	υ NH_2^+, υ_s NH_3^+
	2980 w		
3238 m, br	3247 m, br	3259 m, br	υ_{as} NH_3^+
	3389		

to the surface selection rule [11], a molecule adsorbed on the surface of small isolated metal spheres with its z axis perpendicular to the metal surface and in the plane of the molecule, the vibrations with larger polarizability component along the z axis are expected to be more enhanced.

In SP, as the out of plane bending NH_2^+ vibrations (804, 1395 cm^{-1}) are found to be more enhanced than the in plane bending vibrations (1487, 1638 cm^{-1}), the molecule is expected to be adsorbed on the metal surface through nitrogen with its plane perpendicular to the metal surface.

The υ_s PO_3 which is not observed in the normal Raman spectrum has appeared in all the three concentrations. In the spectra of 10^{-5} M concentration this mode is split into two which indicates lowering of symmetry due to adsorption. Bending modes of PO_3 also showed enhancement in the SERS spectra. Asymmetric PO_3 stretching mode is also found to be enhanced slightly. Thus enhancement has also taken place via the oxygen atoms of HPO_4^{2-} groups.

RESULTS

1. Vibrational spectral analysis shows the presence of HPO_4^{2-} ion indicating protonation of amino and imino groups. Slight distortion of the HPO_4^{2-} ion is confirmed.

2. The identification of two bands in the metal adsorbate stretching region is indicative of adsorption of the molecule to the metal surface through the two sites nitrogen and oxygen atoms of the molecule.

3. The splitting of the amino group vibrations in the SERS spectra suggests change of symmetry of the molecule due to chemisorption and the consequent breakdown of selection rules.

4. From the relative enhancement of amino group vibrations, it may be inferred that the molecule is adsorbed perpendicular to the metal surface.

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