Uptake of Amino-acids by Zirconium Phosphates. Part 2.† Intercalation of L-Histidine, L-Lysine, and L-Arginine by α-Zirconium Phosphate

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The uptake of L-histidine (His), L-lysine (Lys), and L-arginine (Arg) by α-zirconium phosphate has been studied at 25 °C. The curves of amino-acid uptake against amino-acid added for His and Arg show only one plateau, while that for Lys exhibits two plateaux indicating that its uptake proceeds in two steps. Each amino-acid forms two intercalated phases at low uptakes in which the interlayer space is probably occupied by a monolayer of the guest molecules placed nearly horizontal to the sheet of the host crystal or in slightly different orientations. The intercalated solids with His and Arg are completely decrystallized at higher loadings becoming gels formed of lath-shaped particles, with the amino-acid uptake capacity being 4.0 mmol g^{-1}. The Lys intercalate formed initially at the second uptake stage is highly disordered but maintains a certain degree of order in the host lattice. At higher levels of Lys, the second uptake stage results in the formation of a phase $Zr(HPO_4)_2(Lys)_{1.85}\cdot H_2O$ with an interlayer spacing of 23.1 Å in which the guest molecules are intercalated probably as a bimolecular layer. The striking contrast between the intercalation behaviour of Lys and the other amino-acids is explained in terms of the structural and functional characteristics of the individual acids.

Zirconium phosphate is known to be an inorganic ion exchanger with a layered structure and behaves as an intercalating agent of polar organic substances.[1,2] In particular, basic molecules are easily taken up by the phosphate, due to its acidic properties. It has also been applied as an adsorbent to remove ammonium ions produced by the enzymatic decomposition of urea.[3]

It is of interest to investigate the affinities of biologically related organic compounds for zirconium phosphate. In the previous paper [4] we examined the adsorption characteristics of some neutral amino-acids for α-zirconium phosphate $Zr(HPO_4)_2\cdot H_2O$ with an interlayer spacing of 7.6 Å and revealed that the adsorption takes place in two steps on the surface of the phosphate crystal. This paper reports the intercalation behaviour towards three representative amino-acids with different basicities or isoelectric points (pI), *i.e.* L-histidine (His; pI 7.47), L-lysine (Lys; pI 9.74), and L-arginine (Arg; pI 10.76).

Experimental

The sample used, $Zr(HPO_4)_2\cdot H_2O$, was the same as that referred to in ref. 4. L-Histidine, L-lysine, and L-arginine were reagent grade and used without further purification.

The phosphate sample (0.1 g) was suspended in distilled water and a precalculated amount of 0.2 or 0.4 mol dm^{-3} amino-acid solution was added to keep the volume to solid ratio constant at 40 cm^3 g^{-1}. The mixtures were shaken at 25 ± 0.5 °C for 4 d, after which the solids were separated by centrifugation at 15 000 *g* for 10 min and stored in a desiccator over calcium nitrate solution (relative humidity 0.51) at 25 °C. The instruments and procedures used for measuring the concentration and pH of the supernatants were described previously.[4] The amounts of amino-acid taken up by the exchanger were determined as differences between the initial and final concentrations. The amounts of phosphate released to the solution were obtained by the molybdate colorimetric method using *p*-methylaminophenol as reductant.[5]

X-Ray diffraction patterns were measured with a Toshiba Gigerflex at a rate of 1° min^{-1} using Cu-K_α rays, and electron micrographs and electron-diffraction patterns were taken using a Hitachi H-500 electron microscope. Infrared transmission measurements were made by the KBr-pellet method using a Hitachi model EPI-E3 spectrometer. In order to determine the water content in the resulting solids, thermogravimetric measurements were carried out on a Shinkuriko thermogravimetric analyser with a heating rate of 10 °C min^{-1}.

For a comparative study, a similar procedure was used to prepare the phosphate complexes with histamine, ethylenediamine, and n-propylamine and their structural and morphological properties were examined.

Results

The amino-acid uptakes are plotted against the amount added in Figure 1. Figure 2 shows the variation of the pH of the equilibrated solution in the uptake process. At a level of

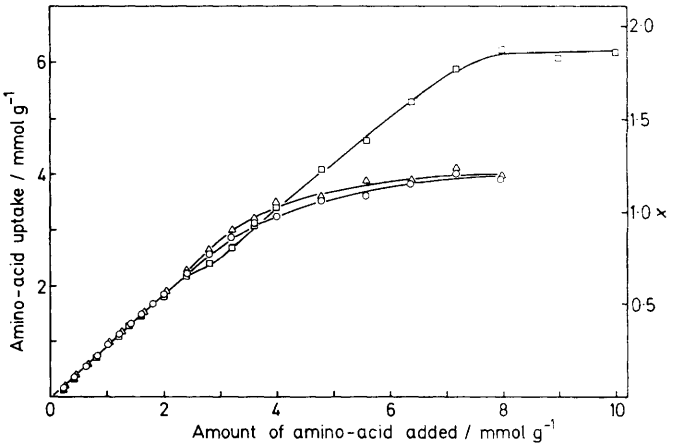


Figure 1. Amino-acid uptake by α-zirconium phosphate as a function of the amount added: ○, His; □, Lys; △, Arg. The uptake in moles per formula weight of phosphate is given on the right ordinate

† For Part 1 see ref. 4.

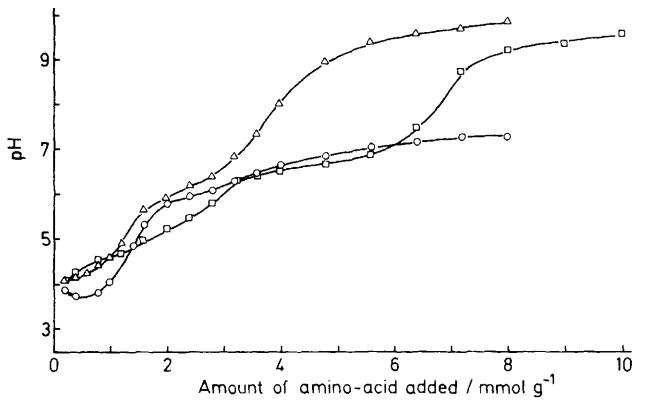


Figure 2. Potentiometric titration curves for α-zirconium phosphate with His (○), Lys (□), and Arg (△)

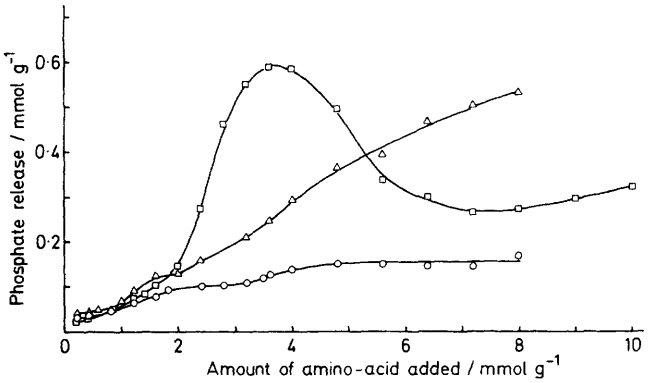


Figure 3. Millimoles of phosphate released to the solution per gram of α-zirconium phosphate. Key as in Figure 2

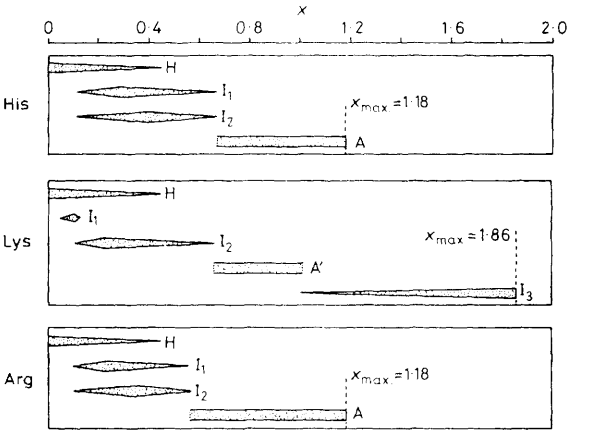


Figure 4. Phase diagram for α-zirconium phosphate complexes with His, Lys, and Arg: H = host phase; I_i (i = 1—3) = intercalated phases; A = amorphous phase; A′ = pseudo-amorphous phase (see text). Interlayer spacings (Å) of phases I_1, I_2, and I_3: His 12.0, 12.7, ——; Lys 10.9, 12.0, 23.1; Arg 12.1, 13.2, ——. The increase or decrease in the phase composition is indicated schematically by a bar of increasing or decreasing width and the maximum value of x is denoted as $x_{max.}$

Table. *X*-Ray diffraction pattern of the Lys intercalate separated at the stage of addition of 10 mmol Lys per gram of α-zirconium phosphate

d/Å	I/I_0	d/Å	I/I_0
23.1	76	4.27	17
11.7	100	3.99	19
7.80	11	3.90	17
4.65	34	3.66	19
4.57	37	3.33	21
4.32	16	2.68	22
4.31	16	2.65	26

less than 1 mmol g^{-1}, the amino-acid added was fully taken up by the exchanger. At higher levels the uptakes for His and Arg became increasingly less than the amount added until they reached a constant value of *ca.* 4.0 mmol g^{-1} or $x_{max.} \approx 1.18$. In contrast, the uptake curve for Lys showed two plateaux, indicating that the uptake proceeds in two steps with a boundary concentration of *ca.* 3 mmol g^{-1} and a maximum uptake of 6.15 mmol g^{-1} or $x_{max.} = 1.86$.

The uptake of amino-acid was accompanied by the release of phosphate due to the hydrolysis of the exchanger, as shown in Figure 3. At levels of greater than 2 mmol g^{-1} amino-acid the phosphate release, particularly for Lys and Arg with high basicities, showed a remarkable increase with increasing amount added. Only the phosphate-release curve for Lys had a maximum at a level of about 4 mmol g^{-1} amino-acid.

The amino-acid complexes separated at various levels of amino-acid were characterized by *X*-ray diffraction analysis, and the results are summarized in Figure 4. Each amino-acid formed two intercalated phases (I_1 and I_2) at low uptakes whose interlayer spacings are 12.0 and 12.7 Å for His, 10.9 and 12.0 Å for Lys, and 12.1 and 13.2 Å for Arg. The two phases for His and those for Arg coexisted in the ranges $0.1 \lesssim x \lesssim 0.67$ and $0.1 \lesssim x \lesssim 0.56$, respectively, and the ratio between the contents of each two phases varied with increasing level of amino-acid addition. The 10.9-Å phase for Lys was formed only when $0.05 \lesssim x \lesssim 0.1$, followed by formation of the second intercalated phase in the range $0.1 \lesssim x \lesssim 0.66$. When $x \lesssim 0.45$ the I_1 and/or I_2 phases in turn coexisted with the host phosphate phase. Parallel to intercalation, however, the intercalated phases decrystallized to form gels until when $x > 0.67$ for His and $x > 0.56$ for Arg the intercalated solids were entirely amorphous. As exemplified in Figure 5, the gels thus produced are formed of lath-shaped particles which exhibited a tendency to become thinner and less regular in shape and flocculate into amoebiform aggregates with an increase in the amino-acid loading. In the range $0.67 < x \lesssim 1.0$ for Lys a gelatinous precipitate was formed and its *X*-ray photograph showed no reflections other than that for $d = 2.65$ Å which corresponds to the (020) reflection of α-zirconium phosphate, while when $x > 1.0$ the precipitated particles again displayed a tendency to become regular in shape and a new intercalated phase with 23.1 Å spacing was observed. In conjunction with the thermal analysis of the solid separated at the stage of addition of 10 mmol Lys per gram of phosphate, the composition of the third intercalate (I_3) was determined to be $Zr(HPO_4)_2(Lys)_{1.85}\cdot H_2O$. The *X*-ray diffraction pattern of this phase is shown in the Table. The remarkable decrease of phosphate release at high levels of Lys could be explained in terms of the formation of the third crystalline phase.

In the previous study [4] it was proposed that the adsorption of L-asparagine and L-alanine on the surface of α-zirconium phosphate crystals is accompanied by neutralization of the carboxyl groups. This was supported by the i.r. absorption of the neutral amino-acids adsorbed, the band attributable to the C=O stretching mode of the COOH group [6] being observed at 1 740 cm^{-1}.[7] On the other hand, a different tendency was noticed for the phosphate complexes with basic amino-acids, as shown in Figure 6. In the spectra of the complexes separated from the solution of low pH values weak bands

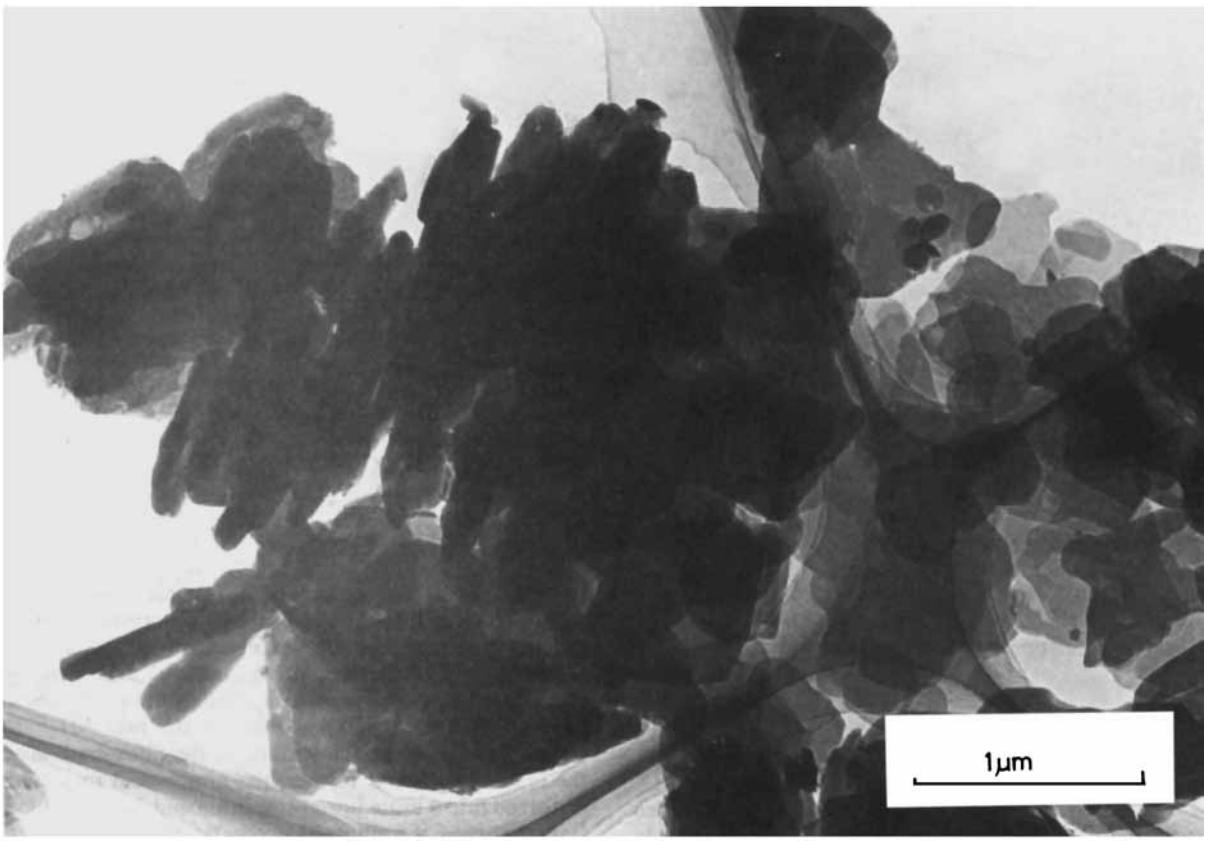


Figure 5. Electron micrograph of the His intercalate containing 0.4 mmol of His per gram of phosphate

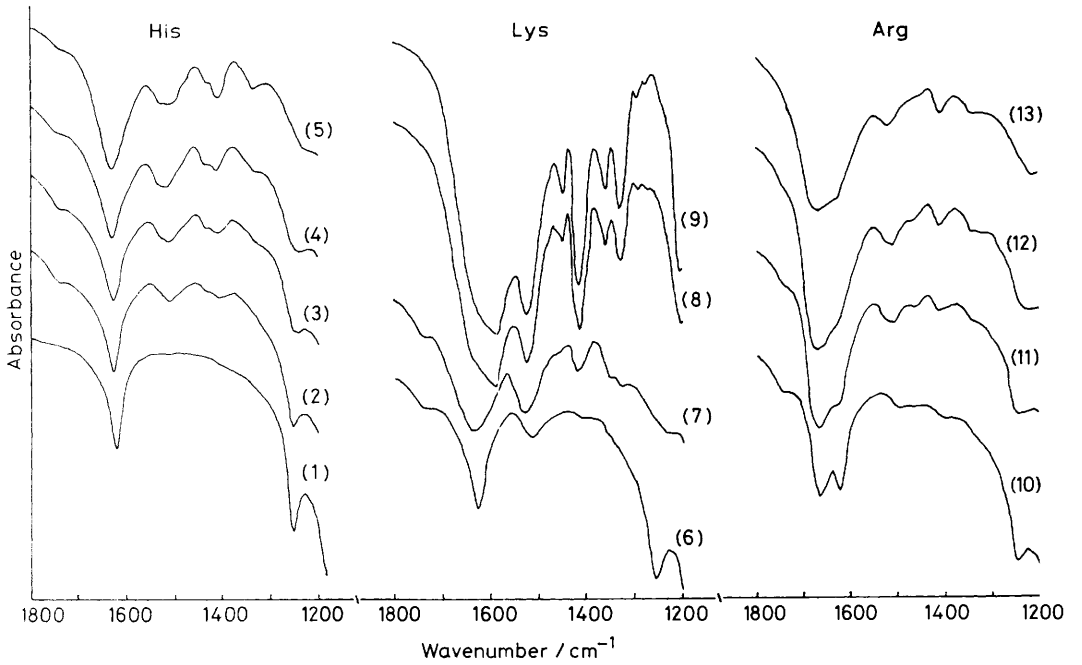
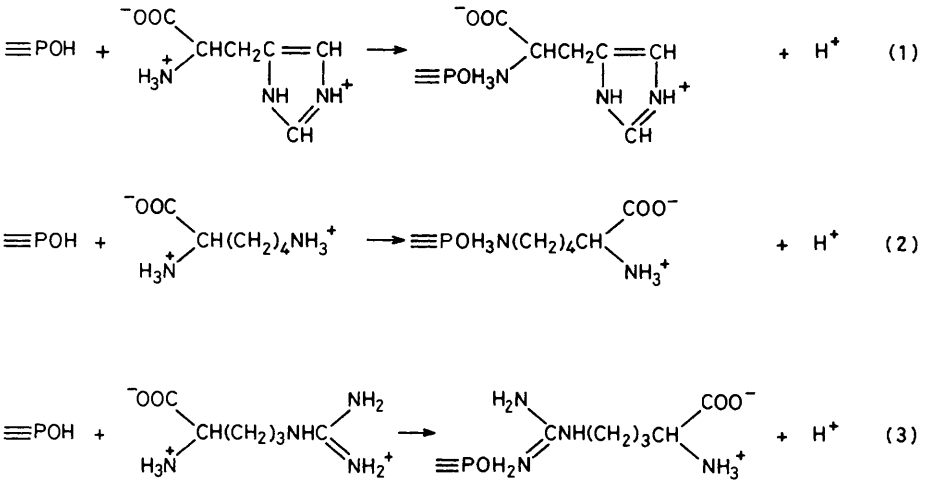


Figure 6. Infrared spectra of α-zirconium phosphate (1) and its complexes with His, Lys, and Arg. Amino-acid addition levels (mmol g^{-1}): 0.6 (2), 1.0 (3), 1.6 (4), 2.4 (5), 0.4 (6), 2.4 (7), 5.6 (8), 10.0 (9), 0.4 (10), 1.0 (11), 2.0 (12), and 2.8 (13)



occur near 1 740 cm^{-1}. For the complexes obtained at higher pH, however, this band shows no appreciable increase in intensity (His) or disappears completely (Lys and Arg).

Discussion

The molecules of His, Lys, and Arg in solution over the pH range used exist predominantly as $NH_3^+CH(COOH)RH^+$ near pH 4 or below but as $NH_3^+CH(COO^-)RH^+$ at higher pH. The previous study [4] revealed that neutral amino-acids existing as zwitterions $NH_3^+CH(COO^-)R$ adsorb on the surface of α-zirconium phosphate crystals by the replacement of surface exchangeable protons by the terminal NH_3^+ cations. The intercalation of basic amino-acids by the phosphate will also proceed by a similar ion-exchange reaction through the α-NH_3^+ groups or the protonated termini of the basic side groups. The i.r. data reported above and the dissociation properties of amino-acids indicate that the carboxyl groups of the amino-acid molecules intercalated have the same ionized or un-ionized form as in the equilibrated solution. Further, it is most probable that the larger the acid dissociation constant pK_a the greater is the availability of the functional group through which an amino-acid molecule binds to the interlayer POH sites of the phosphate crystals. According to this criterion, the tendency to bond formation will be guanidino in Arg ($pK_a \approx 13.2$) > ε-amino in Lys ($pK_a = 10.3$) > α-amino ($pK_a \approx 9.0$) > imidazole ($pK_a = 6.0$) in His. This suggests that the molecules of His, Lys, and Arg are likely to anchor through the protonated α-amino, ε-amino, and guanidino-groups to the POH sites, respectively. On the basis of these considerations, as a working hypothesis the reactions (1), (2), and (3) are suggested for the intercalation of His, Lys, and Arg (with the COO^- groups) respectively by α-zirconium phosphate. The protons produced by the reactions would be consumed by neutralization of the hydroxide ions generated by the protonation of the basic side groups.

Referring to the crystal structures of His, Lys, and Arg or their hydrochloride salts,[8-10] the van der Waals lengths along the long and the short axes of a fully extended molecule are 10.5 and 4.4 Å for His, 12.1 and 4.3 Å for Lys, and 13.1 and 4.3 Å for Arg, respectively. The first and the second intercalated phases (I_1 and I_2) have expanded their layers over that in the host phase by 4.4 and 5.1 Å for His, 3.3 and 4.4 Å for Lys, and 4.5 and 5.6 Å for Arg, respectively. These values, except that for the 10.9 Å phase containing Lys, are fairly close to the van der Waals thickness of the amino-acid molecules, indicating that the interlayer space in these intercalates would be occupied by a monolayer of the guest molecules placed nearly horizontal to the sheet of the host crystals or in slightly different orientations. The exceptionally small distances of 3.3 Å for one of the Lys intercalates might be explained by assuming that the Lys molecules with a flat configuration are held by bifurcated bonding through the two NH_3^+ termini partly dipping under the surface of the phosphate layers. As the loading increases further, the amino-acid molecules may be forced into a more upright position and form a bimolecular layer between the phosphate sheets. The intercalated layer thus formed would enlarge the interlayer space of the host crystals and also, in combination with the hydrolytic effect of the basic functions, induce disorder in the packing sequence of the phosphate layers and/or in the arrangements of the zirconium atoms and phosphate groups within each layer. The small uptake and the complete decrystallization of the intercalated solid at high levels of His and Arg could be attributed to the full development of such disorder. On the other hand, the Lys intercalate becomes highly disordered at intermediate levels where it is not completely decrystallized, that is, a certain degree of order of the host lattice remains. At high levels of Lys, the complex restores its order to form the third intercalated phase. If we assume the formation of a bimolecular layer of Lys for the third intercalate and the same geometrical relationship between the ε-amino-group and the POH site as used for the n-butylamine bilayers in α-zirconium phosphate,[11] it is necessary that the skeletal chain of Lys makes an angle of *ca.* 45° with the phosphate sheet.

The striking contrast between the intercalation behaviours of Lys and the other amino-acids may be explained as follows. Among the bonding groups through which amino-acid molecules anchor to the POH sites, the α-amino-group of His and the imine one of Arg are flanked by a carboxyl or amine group, while the ε-amino-terminus of Lys is free from such a neighbouring group. This suggests that when an interlayer POH site is occupied by a molecule of His or Arg, any other molecules approaching the neighbouring sites are subjected to steric hindrance by the initially intercalated molecule, which would decrease to a great extent the degrees of freedom of the steric or spacial configurations of the guest molecules within the interlayer space of the phosphate and accelerate the disorder of the intercalated phases at high loadings. In contrast, the freedom from such steric hindrance for Lys would serve to hamper the complete disorder of the intercalated phase at intermediate loadings and also favour the formation of the crystalline complex almost fully loaded with Lys molecules. It was

also found that α-zirconium phosphate intercalates histamine (hist) at a level of 10 mmol g^{-1} to form a phase $Zr(HPO_4)_2$-$(hist)_{1.98}\cdot 2.7H_2O$ with an interlayer spacing of 19.8 Å. This fact supports the above explanation, since hist does not possess a carboxyl group but has the same basic skeleton as His.

Ethylenediamine and n-propylamine formed crystalline complexes with the phosphate at a level of 8 mmol g^{-1} which were composed mostly of the same platelets as the host crystals, but partly of lath-shaped particles. These facts and the above observations suggest that the intercalated compounds of α-zirconium phosphate have a tendency to form lath-shaped particles. The formation of such particles may be accounted for by the curling or folding of the intercalated lamellae, as suggested by a similar observation on clay minerals, *e.g.* montmorillonite.[12] Such a deformation might be attributed to instability of the lamellar structure induced by the steric effect of guest molecules and the release of phosphate groups.

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