

4 Interactions of HEDP with HA

4.1 Introduction

Chapter 3 was concerned mainly with HEDP, its protonated forms and their conformations in the solid state and in solution. An understanding of the interaction of HEDP in solution with the bone mineral interface is also of importance to appreciate and compare the potencies of various bisphosphonates in the future. This chapter discusses the use of Raman spectroscopy to probe the nature of this solid-solution interface.

4.2 The interaction of HEDP with various calcium phosphates

Raman spectroscopy has been successfully used to characterise bone [1], HA as a model of bone [2, 3] and bisphosphonates [4–10]. The interaction of HEDP with HA has also widely been studied using theoretical [11] and experimental methods [12]. Thus far, it seems that only one published Raman spectroscopic study has attempted to investigate the interaction of a bisphosphonate with HA [13]. The main reason for this is probably the fluorescence that is observed when using FT-Raman with 1 064 nm excitation. This fluorescence is confirmed by an FT-Raman spectroscopic study of fluoroapatite, heated HA, calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, calcium hydrogenphosphate dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, calcium hydroxide and calcium carbonate, which showed strong fluorescence bands in the Raman spectra of these compounds [14]. It was found to be due to the impurities present, such as rare earth minerals contained in the structure of the

compounds. It was therefore decided to in this study use an excitation line, such as the 514.5 nm (green) line of an Ar-ion laser, to ensure that the HEDP-solid interaction could be investigated without the interference of fluorescence.

As mentioned in Section 1.4, HA can be used as a simplified model of bone because the Raman spectra of the mineral phase of bone and HA match, as shown in Figure 3-25. CaHPO_4 , unlike its dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ [15], does not occur in normal or pathological calcifications but it showed similar reactivity towards HEDP. In a previous study [16] an ‘adhesion-decalcification concept’ was proposed in which HPO_4^{2-} is initially formed during the action of carboxylic acids on HA and the subsequent decalcification thereof, allowing $\text{Ca}^{2+}(\text{aq})$ to be available for complex formation. A similar process might be involved in the reaction of bisphosphonic acids with HA, and thus CaHPO_4 was included as a source of aqueous HPO_4^{2-} for comparison with the reactions of HEDP with bone and HA. As Raman spectra were obtained at the solution/solid reaction interface, one would not be able to distinguish by Raman spectroscopy chemically between CaHPO_4 and $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ as the source of $\text{HPO}_4^{2-}(\text{aq})$.

The interaction of HEDP was investigated at both low (0.005 M) and high (0.5 M) concentrations of HEDP, and it appears that it interacts with bone, HA and CaHPO_4 in the same manner, forming needle-like fused spheres, as can be seen in Figure 4-1.

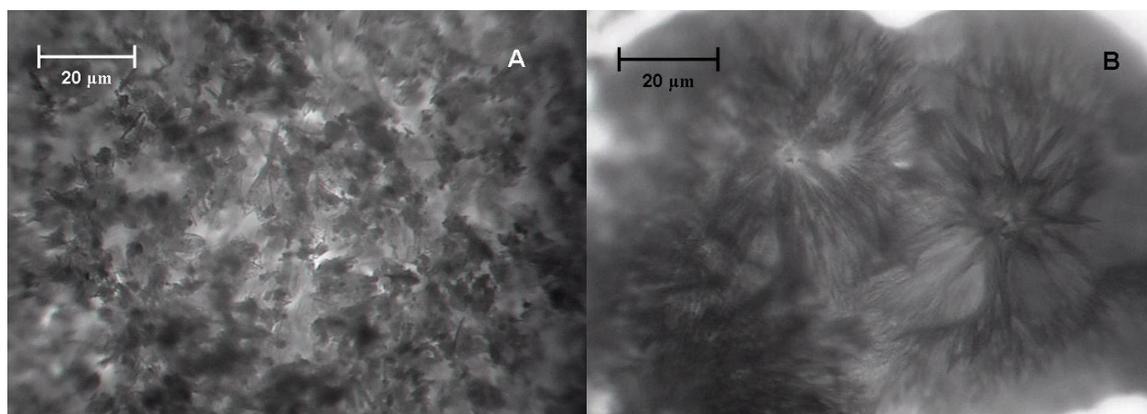


Figure 4-1. Micrographs of HA crystals A. before addition and B. after addition of 0.5 M HEDP

Figure 4-2 shows the Raman spectra of the reaction products formed during the HEDP(aq)-solid interaction. From this, it can be seen that HEDP reacts with the Ca^{2+} ion found in all three solids, forming similar Ca complexes. The three non-overlapping bands identified in Section 3.5.2 for $\text{CaH}_2\text{L}\cdot 2\text{H}_2\text{O}$ and the unknown Ca-HEDP complex can be used to identify which of these compounds has formed. Bands at 640, 963 and 1072 cm^{-1} are assigned to the $\text{CaH}_2\text{L}\cdot 2\text{H}_2\text{O}$ complex and bands at 658, 946 and 1088 cm^{-1} to the unknown Ca-HEDP complex. It can be seen that the Raman spectra of bone and HA are very similar, and judging from the relative intensities of the Raman bands of the two Ca complexes, it seems that the $\text{CaH}_2\text{L}\cdot 2\text{H}_2\text{O}$ complex is dominant in the bone and HA cases, but the unknown Ca-HEDP complex is favoured in the CaHPO_4 interaction. This is proof that HA can be substituted for bone when studying the interaction of HEDP by means of Raman spectroscopy at the solid-solution interface.

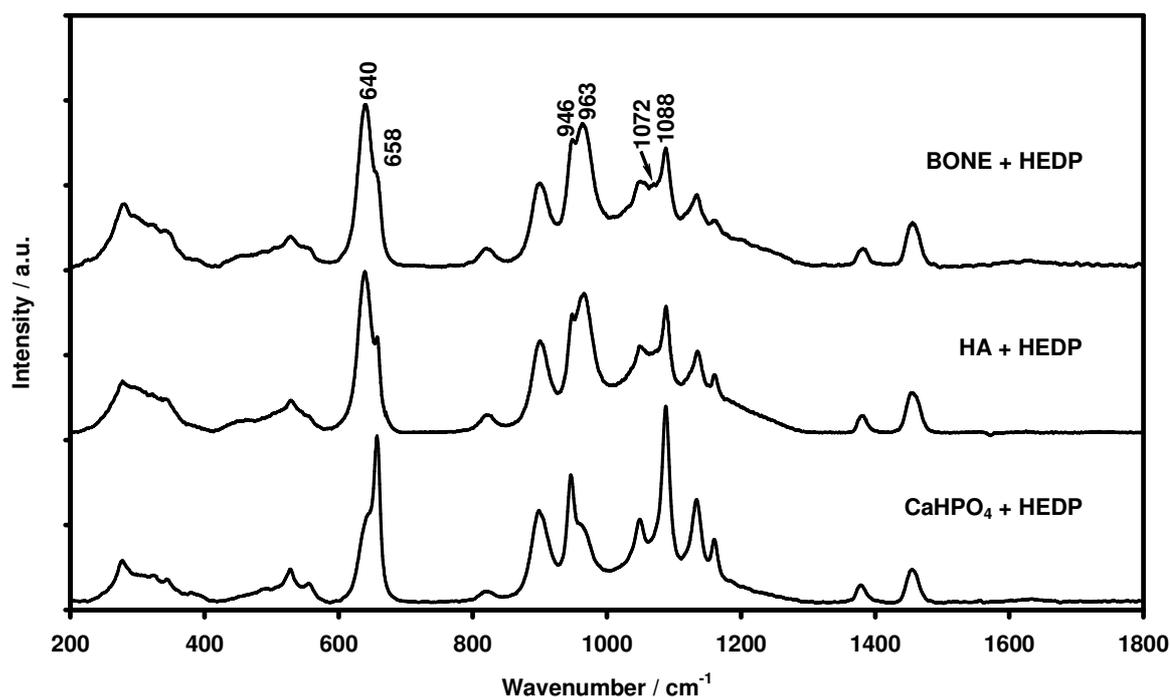


Figure 4-2. Raman spectra of products formed during the interaction of HEDP with bone, HA and CaHPO_4

It was seen that low HEDP concentrations (i.e. adding multiple drops of the 0.005 M solution (pH ~2.2) or a drop of 0.5 M HEDP (pH ~0.8) to the HA surface)

at first favoured the formation of the unknown Ca-HEDP complex identified by its Raman bands at 658, 946 and 1088 cm^{-1} . Further addition of a sufficient amount of HEDP resulted in $\text{CaH}_2\text{L}\cdot 2\text{H}_2\text{O}$ starting to form, as evidenced by the appearance of its Raman bands at 641, 963 and 1072 cm^{-1} , and both complexes were then present. Similar results were obtained for the addition of HEDP solutions to CaHPO_4 . When a small volume of 0.5 M HEDP was added to bone, both complexes formed simultaneously. These observations suggest that the formation of the different complexes is dependent on the pH at the solid-solution interface where the CaH_2L complex forms under more acidic conditions. This is in good agreement with the reported successive, pH-dependent formation of several calcium complexes in solution [17], as well as with the formation of Ca complexes, as thoroughly discussed in Section 3.5.2 where it was postulated that the unknown Ca-HEDP complex was probably CaHL^- and would likely precipitate as $\text{Ca}_3(\text{HL})_2\cdot n\text{H}_2\text{O}$.

All the above studies were done at conditions (low pH, high HEDP concentrations) not found at the biological bone surface, and the Ca^{2+} was liberated during an initial acid-leaching process. Two 0.5 M HEDP solutions were adjusted with NaOH to a pH of 5.0 and 7.4 respectively. At pH 5.0 the dominant protonated form of HEDP is H_2L^{2-} , whereas the human physiological pH is slightly basic at 7.4 [18]. Trying to record spectra at the solid-solution interface for these pH values proved unsuccessful. Both solutions were then placed in contact with HA for seven days and the solutions as well as the solid remaining after the solvent had evaporated were analysed using Raman spectroscopy.

Figure 4-3 shows the spectra of the two solutions. The experimentally generated species distribution diagram has HEDP present as 90% HL^{3-} and 10% H_2L^{2-} at pH 7.4. The bands at 970 and 1064 cm^{-1} in the spectrum obtained for the solution with pH 7.4 are assigned to the HL^{3-} form of HEDP, as seen in Table 3-14. The shoulder at ~ 1058 cm^{-1} may be a result of the most intense band of H_2L^{2-} . The bands observed at 931 and 1062 cm^{-1} in the spectrum of the solution at pH 5.0 are assigned to the H_2L^{2-} form of HEDP. Reference Raman spectra were obtained of solids before HA was brought into contact with the solution. These spectra represent the $\text{Na}_x\text{H}_y\text{L}_z\cdot n\text{H}_2\text{O}$ salts present in the solution.

If any change were to occur due to the interaction of the HEDP solution with HA, it would only be possible to observe this indirectly from the change in the spectra associated with these salts as a function of pH, as the solution composition changes. It will probably not be possible to observe directly with Raman spectroscopy any Ca-HEDP complexes that could have formed in this manner as such complexes are highly insoluble and would therefore not be present in significant quantities upon evaporation of the aqueous medium.

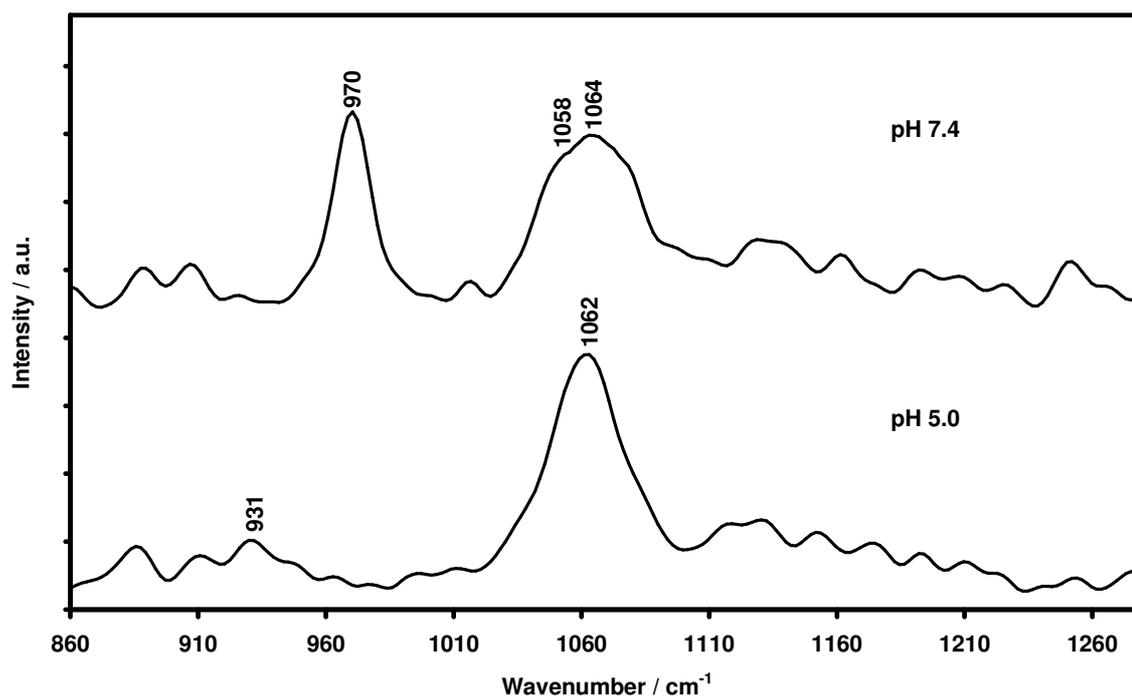


Figure 4-3. Raman spectra of HEDP solutions that were in contact with HA for seven days at pH 5.0 and 7.4

Figure 4-4 shows the Raman spectra of the solid obtained from evaporating the solution without HA interaction, as well as the solid obtained from solution brought into contact with HA at pH 5.0. The $\nu^{\text{s}}\text{C-P}$ band at 650 cm^{-1} is observed in both spectra, but an extra shoulder is observed in the spectrum at 665 cm^{-1} after HA was added. Having two $\nu^{\text{s}}\text{C-P}$ bands present indicates that a mixture of two compounds is probably present. The single band at 650 cm^{-1} is probably from the disodium HEDP salt, and the shoulder band at 665 cm^{-1} probably originates from the trisodium HEDP salt. A new band at 719 cm^{-1} is assigned to the $\nu^{\text{as}}\text{C-P}$ vibration. Changes in the region associated with $\nu\text{P-O(H)}$ bands at

1072, 1087 and 1135 cm^{-1} can also be seen, indicating that a reaction did take place.

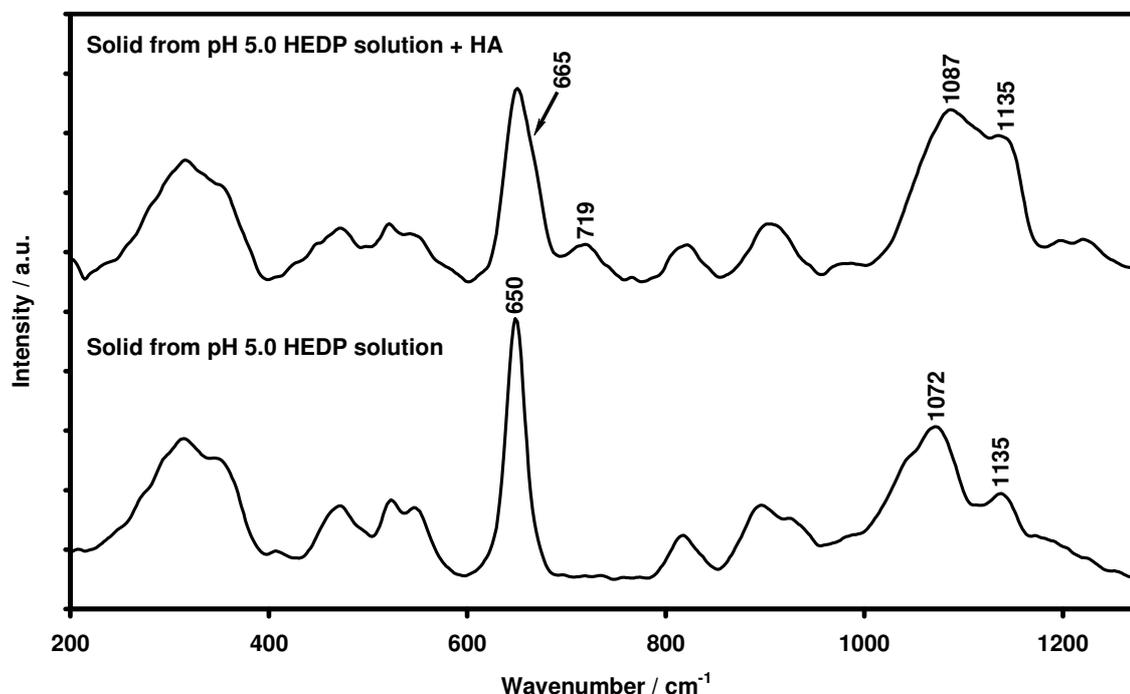


Figure 4-4. Raman spectra of solids obtained from solution before and after HEDP containing solution at pH 5.0 was exposed to HA

Figure 4-5 shows the Raman spectra of the solid obtained from evaporating the solution without HA interaction, as well as the solid obtained from solution after the solution was brought into contact with HA at pH 7.4. The new bands observed at 449 cm^{-1} are assigned to a δOPO vibration, and those at 721 cm^{-1} to the $\nu^{\text{as}}\text{C-P}$ vibration. Again, bands in the $\nu\text{P-O(H)}$ region change, as can be seen at 1076 and 1107 cm^{-1} , indicating an interaction did indeed occur.

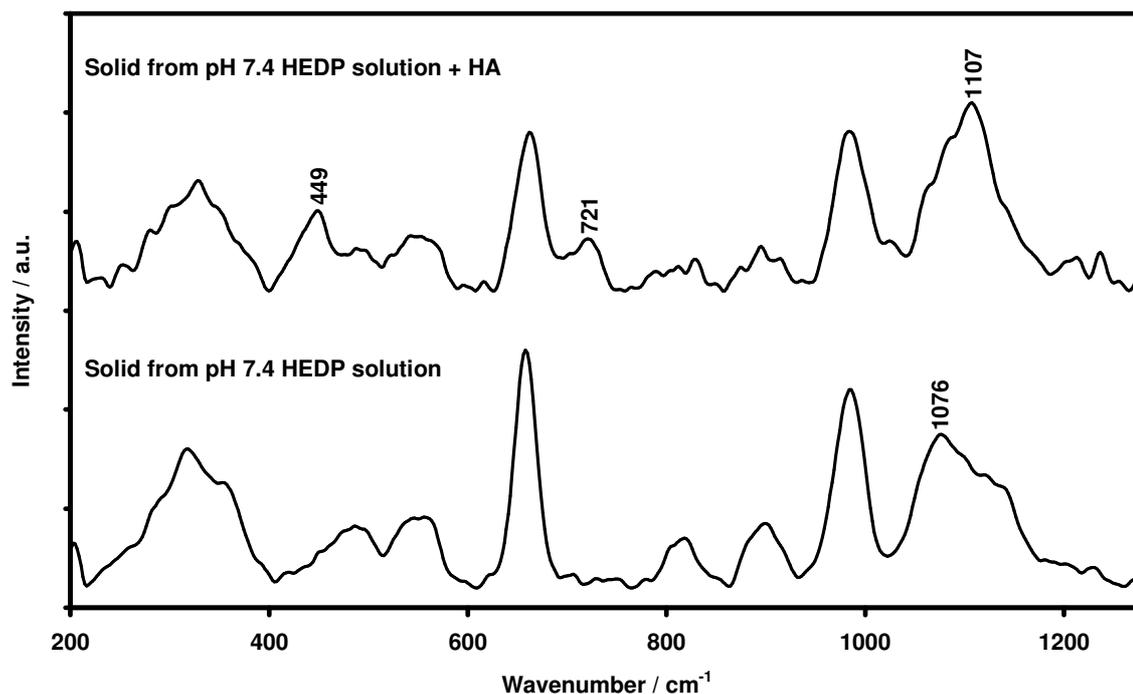


Figure 4-5. Raman spectra of solids obtained from solution before and after HEDP containing solution at pH 7.4 was exposed to HA

In the case of the solution at pH 7.4, the mechanism could not be an acid-leaching process. It is probably chemisorption of the HEDP onto the HA solid [12], and so affects the solution composition as indirectly observed in the Raman spectra of the solids obtained.

4.3 Conclusions

It is very difficult to study the solution/solid interface interaction as can be seen from the work presented in this chapter. This is especially true when trying to directly probe the solid-solution interface interaction directly using Raman spectroscopy. Still, Raman analyses of the solids obtained from the solutions successfully showed that the HEDP(aq) does interact with HA, even at pH 7.4. It would seem that after the HEDP has chemisorbed onto the HA surface at pH 7.4 the calcium is leached into solution and then exists in an equilibrium state between the solid and solution interface. This preliminary work shows that more research is required regarding spectral sampling to more efficiently study these weak interactions by means of Raman spectroscopy.

4.4 References

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5 Conclusions

5.1 Summary

It has been shown in Chapters 3 and 4 how various techniques can be used to obtain different information regarding the interactions with HEDP in the solid state and in solution. These techniques also made it possible to study the various protonated forms of HEDP in solution, and their conformers and intra/intermolecular interactions in solution. Theoretical methods were used to interpret and rationalise the experimental data obtained. Also, the interaction of HEDP(aq) with hydroxyapatite as a model of bone was investigated, specifically using Raman spectroscopy. The following sections will explicitly state what each technique contributed to the understanding of the HEDP systems studied.

5.2 NMR spectroscopy

NMR spectroscopy can be a useful tool to study a bisphosphonate's structural and deprotonation processes if it is sufficiently soluble in the NMR solvent. ^1H and ^{31}P NMR are the more efficient techniques and specifically ^{31}P NMR can be used to estimate roughly the $\text{p}K_a$ values for the polyprotic acid, provided the necessary sampling resolution is achieved, as observed in Table 3-3. Also, ^{23}Na showed that $\text{Na}^+(\text{aq})$ does not interfere with the various protonated forms of HEDP in solution, and therefore will not affect the data obtained with other techniques, such as solution Raman spectroscopy.

The solution NMR spectra of HEDP and $\text{CaH}_2\text{L}\cdot 2\text{H}_2\text{O}$ are solvent-dependent, and comparison of the NMR spectra obtained in different solvents gives a better understanding of the compound's behaviour in aqueous medium, as evidenced in

Section 3.2.1. From the NMR data it was thus shown that CaH₂L does not dissociate to a large extent in solution.

5.3 X-ray diffraction methods

The determination of the solid-state structure of a compound is always of great assistance in understanding the chemistry of the compound. Determination of structures to obtain the atomic positions of the hydrogen atoms accurately is of the utmost importance in cases where the chemistry of the compound is dominated by hydrogen bond interactions, be it intra- or intermolecularly, as in the case of HEDP and practically all other bisphosphonate derivatives. Accurate determination of the crystal structure also assisted in generating theoretical vibrational spectra with more confidence for comparison with the experimental data, and thus assisted with assignment of the vibrational data.

The solving of crystal structures using powder diffraction methods is a tedious task, but highly favourable when no single crystal is available to solve the structure. The use of Rietveld refinement to solve the crystal structure of anhydrous HEDP acceptably from only a powder pattern, with no prior information regarding its structure, shows the power of the technique as well as the possible pitfalls if erroneous choices are made or if ambiguous choices arise due to inherent faults in the sampling methods. The solution of anhydrous HEDP's powder pattern would have been more difficult if a rigid-body approach had not been employed to obtain sound starting coordinates for the refinement of the independent atoms. This is because there are no heavy atoms present in this structure with which to determine accurately positions of high electron density; heavy atoms would have made the Rietveld refinement easier.

5.4 Vibrational spectroscopy

Section 3.5 shows that the use of vibrational spectroscopy, and specifically Raman spectroscopy, to monitor structural and chemical changes, or any other change that can influence chemical bonding in varied environments, is of great

value. This was well demonstrated in the solid-state Raman spectra of HEDP·H₂O and anhydrous HEDP in which the phase change that occurred in the crystal structure during the loss of hydration was observed with Raman spectroscopy; this was also done using powder X-ray diffraction.

The solution Raman spectra of HEDP allowed the discrete observation of the various protonated forms of HEDP in aqueous medium where NMR could not differentiate between discrete species. There is also strong evidence in the Raman spectra of hydrogen bond interactions in solution and this is supported by the theoretical modelling results of the various protonated conformers of HEDP. The trend of the discretely observed Raman bands was also seen to follow the chemical shift of the ³¹P NMR signal. The solution Raman spectra of H₂L²⁻, HL³⁻ and L⁴⁻ were confidently assigned with the aid of molecular modelling of the solution Raman spectra. H₄L and H₃L⁻ were problematic due to the strong hydrogen bonding interactions that occurred in solution and because the overlap of these species distribution ranges complicates the system even further.

Raman spectroscopy was also successfully employed to study HEDP(aq) which was found to interact similarly with bovine bone, HA and CaHPO₄. Thus HA can be substituted as a model of bone. Two calcium complexes formed on the solid surfaces: one was determined to be CaH₂L·2H₂O and the unknown Ca-HEDP complex is postulated to be CaHL⁻. This postulation is supported by the solution modelling of Ca-HEDP complexes in Section 3.5.2, as well as by Raman spectroscopic evidence. Also, pH and concentration dependence were observed to form each of these Ca complexes sequentially, which was explained from the modelled solution species distributions of Ca-HEDP complexes. An explanation for why CaH₂L was not determined previously is also given; this is due to the fact that the total proton concentration does not change during the formation of CaH₂L and glass electrode potentiometry would therefore not detect it.

Rigorous assignments of the Raman spectra were made for HEDP, HEDP·H₂O, CaH₂L·2H₂O and the unknown Ca-HEDP complex, aided by empirical literature data and theoretically calculated vibrational spectra. This allowed the various compounds to be compared, the structural and chemical changes occurring at

the molecular level to be ascertained, and determination of which vibrational bands are most affected by these changes and can thus be used to monitor when these changes occur.

A better understanding of which techniques can be applied successfully to investigate HEDP (either solvated or as it interacts/reacts with solid interfaces) will result in a better understanding of these processes in general for other bisphosphonates as well. It was shown, albeit at an initial stage, that two different types of interaction of HEDP could be monitored indirectly in solution in an acidic (leaching) environment and at a pH value that mimics the human physiological pH of 7.4, which is slightly basic.

5.5 Modelling techniques

Molecular modelling was used extensively, either to determine conformeric structures or to aid in the assignment of experimental Raman spectra of compounds in solution or in the solid state, as discussed in the previous chapters.

Probable conformers that are present most of the time in solution for H_3L^- , H_2L^{2-} , HL^{3-} and L^{4-} (all of which have intramolecular hydrogen bonds) were determined and it was shown that H_4L could be present as many conformers, rather than as a single major conformer. Vibrational assignment of H_2L^{2-} , HL^{3-} and L^{4-} was also done using theoretically calculated Raman spectra.

It is also reasoned that in the case of H_2L^{2-} , the predominant conformer for the uncoordinated H_2L^{2-} is different from that preferred for the Ca^{2+} coordinated form. The insolubility of $CaH_2L \cdot 2H_2O$ unfortunately did not allow experimental verification of these findings postulated from theoretical calculations.

MCR analysis of the solution Raman spectra proved invaluable in determining pure component spectra for all the various protonated forms of HEDP, but specifically for the problematic H_4L and H_3L^- species. In the case of HEDP, care

should be taken during the MCR analysis in implementing the unimodality constraint. Even though it is a valid assumption (and acceptable species distribution curves are obtained), the pure component spectra so obtained do not describe the system satisfactorily. The supposition that the strong hydrogen bonded species at low pH could be observed as a separate species was proved incorrect, but the hydrogen bonding manifests itself as anomalies in the species distribution diagram. The system can therefore be seen to be formally composed of five distinct species over the pH range, with strong hydrogen bonding interactions occurring at low pH values.

5.6 The holistic approach

In summary, each technique brings with it its own unique contribution to a fundamental understanding of the system, but each technique also has its limits. The novel possibility of using Raman spectroscopy to directly study the interaction of HEDP with HA was shown to be possible and in this way detect the formation of compounds at the solid-solution interface that cannot be detected otherwise. It is of course necessary to fully characterise all of these compounds and therefore Raman spectroscopy alone will not suffice. Using various techniques in conjunction with each other or in a supportive role, rather than as single, separate techniques, therefore gives a much better understanding of what happens at the molecular level, and what is observed with Raman spectroscopy.

5.7 Future work

The successful measurement of Raman spectra at low pH as shown in Chapter 4 shows that Raman is a very promising technique to probe interactions directly on a fundamental, molecular level at the solid-solution interface for bisphosphonates and HA. The unsuccessfulness of measuring Raman spectra at the solid-solution interface at pH 5.0 and 7.4 clearly indicates that more work is required to probe the interactions under these different conditions. This could be with reference to choice of instrument, another experimental setup under the laser objective, or an unknown problem not yet identified. Taking into consideration that this was a first



attempt to holistically attempt this, it is clear that the whole is greater than the sum of the parts for research into bisphosphonates, especially when trying to gain a fundamental understanding of what occurs at the solid-solution interface. It is therefore important to first understand these interactions with reference to simpler models (for example substituting HA for bone) before they are implemented or extended to more complex systems for final tailoring and designing of drugs for the treatment of degenerative bone diseases.