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Modelling and spectroscopic studies of 1-hydroxyethylidene-1,1-diphosphonic acid and its interaction with hydroxyapatite as a model of bone

by

Werner Barnard

Submitted in partial fulfilment of the requirements for the degree

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This whole thesis is dedicated to my grandmother,

Beatrix Jeanetta Rademeyer

1918 – 2009

"And crawling on the planet's face
Some insects called 'The Human Race'
Lost in time, lost in space
and meaning…"

from the musical,
The Rocky Horror Picture Show, 1976

Abstract

Modelling and spectroscopic studies of 1-hydroxyethylidene-1,1-diphosphonic acid and its interaction with hydroxyapatite as a model of bone

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Supervisor: Prof. I. Cukrowski
Co-supervisor: Prof. P.H. van Rooyen

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The behaviour of 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP, H_4L) was studied in the aqueous medium and at the hydroxyapatite interface as a model of bone. In solution, the pH-dependency of the various protonated forms of HEDP was studied using nuclear magnetic resonance (NMR) spectroscopy of various nuclei and from this comparable pK_a values could be obtained from the ^{31}P chemical shift curve. The Raman spectra of the aqueous samples were measured and each protonated form was identified by unique vibrational bands. Multivariate curve resolution analysis was used to redetermine the species distribution diagram, as well as pure component spectra of each protonated form. Molecular modelling was employed to determine the most probable conformer present in solution and also to calculate the theoretical vibrational spectrum of each conformer. Comparison of the theoretical and experimental data allowed the assignment of the different Raman bands observed. The species present at low pH were the most problematic to analyse due to the strong inter- and intramolecular hydrogen bonding indirectly observed in the data.

The interaction of HEDP at low and high concentrations with hydroxyapatite, bovine bone and $CaHPO_4$ was investigated *in situ* by means of Raman spectroscopy and it was found that two Ca-HEDP complexes are sequentially

formed at both concentrations, and that the order of formation of these two complexes can be explained from the species distribution diagrams of Ca-HEDP complexes. One complex, $CaHEDP{\cdot}2H_2O$, was successfully isolated and characterised by means of single-crystal X-ray Diffraction (XRD) methods and Raman spectroscopy. Theoretically generated Raman spectra were used to assist in the assignment of the solid-state Raman spectrum of $CaHEDP{\cdot}2H_2O$. It is postulated that the unknown complex is the monoprotonated Ca-HEDP complex. Using the Raman spectra of the complexes and HEDP as references, it was determined that HEDP(aq) interacts similarly with hydroxyapatite, bovine bone and $CaHPO_4$ and thus hydroxyapatite can be substituted for bone in the Raman spectroscopic study of HEDP with bone. HEDP interaction was also studied at pH values of 5.0 and 7.4 to understand the nature of the interaction at the pH values at which the diprotonated (H_2L^{2-}) form is predominantly present, as well as at the pH of human blood plasma, which is slightly basic.

HEDP exists as a monohydrate at room temperature and the single-crystal structure was redetermined, during which the hydrogen positions were experimentally obtained for the first time by means of X-ray diffraction methods. The anhydrous form of HEDP exists above 70 °C and Rietveld refinement of the powder X-ray pattern of anhydrous HEDP was used to solve its crystal structure. The complexity of contributory factors allowed only for the non-hydrogen atom positions to be determined. Fourier-transform infrared (FTIR) and Raman spectroscopy were performed on both phases and there is evidence in the Raman spectrum that hydrogen bonding still plays a predominant role in the anhydrous solid state.

All these studies led to a better understanding of the nature of bisphosphonate interaction with bone and the results can therefore be applied in future medical studies for drug screening regarding bone cancer research.

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List of Related Publication/Conference Outputs

Publications

1. "Modelling and spectroscopic studies of bisphosphonate-bone interactions. The Raman, NMR and crystallographic investigations of Ca-HEDP complexes" Cukrowski I, Popović L, Barnard W, Paul SO, Van Rooyen PH, Liles DC. *Bone*, **2007**, *41*, 668.

2. "The analysis of pH dependent protonated conformers of 1-hydroxyethylidene-1,1-diphosphonic acid by means of FT-Raman Spectroscopy, multivariate curve resolution and DFT modelling" Barnard W, Paul SO, Van Rooyen PH, Cukrowski I. *Journal of Raman Spectroscopy*, **2008**, ACCEPTED, DOI 10.1002/jrs.2343.

Oral Presentations

1. "A spectroscopic comparison of the anhydrous and monohydrate form of HEDP, a bone cancer pain palliation agent" *SACI Young Chemist Symposium – Gauteng Branch*, University of Johannesburg, 27 October 2006.

2. "The investigation of 1-hydroxyethylidene-1,1-diphosphonic acid and its complexes by Raman and NMR spectroscopy" W Barnard, L Popović, I Cukrowski, PH Van Rooyen, DC Liles and SO Paul, *SACI 2007 Inorganic Conference*, Langebaan, South Africa, 8–12 July 2007.

3. "Die bestudering van 1-hidroksietilideen-1,1-difosfoniese suur en sy komplekse met behulp van Raman en KMR Spektroskopie" *SAAWK Studente Simposium*, Tshwane University of Technology, 2 October 2007.

Poster Presentations

1. “The multivariate analysis and DFT study of various protonated HEDP conformers over the pH range 1.00–13.00” W Barnard, SO Paul, PH van Rooyen and I Cukrowski, *XXI International Conference on Raman Spectroscopy*, London, UK, 17–22 August 2008.