

**Author(s):** Pacheco, R (Pacheco, Rita); Karmali, A (Karmali, Amin); Serralheiro, MLM (Serralheiro, M. Luisa M.); Haris, PI (Haris, Parvez I.)

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**Abstract:** The interaction of a variety of substrates with *Pseudomonas aeruginosa* native amidase (E.C. 3.5.1.4), overproduced in an *Escherichia coli* strain, was investigated using difference FTIR spectroscopy. The amides used as substrates showed an increase in hydrogen bonding upon association in multimers, which was not seen with esters. Evidence for an overall reduction or weakening of hydrogen bonding while amide and ester substrates are interacting with the enzyme is presented. The results describe a spectroscopic approach for analysis of substrate-amidase interaction and in situ monitoring of the hydrolysis and transferase reaction when amides or esters are used as substrates.

**Addresses:** [Pacheco, Rita] Inst Super Engn Lisboa, Dept Engn Quim, Ctr Invest Engn Quim & Biotecnol, P-1949014 Lisbon, Portugal; [Serralheiro, M. Luisa M.] Univ Nova Lisboa, Fac Ciencias, Ctr Quim & Bioquim, P-1200 Lisbon, Portugal; [Haris, Parvez I.] DeMontfort Univ, Dept Biol Sci, Leicester, Leics, England

**Reprint Address:** Pacheco, R, Inst Super Engn Lisboa, Dept Engn Quim, Ctr Invest Engn Quim & Biotecnol, Rua Conselheiro Emidio Navarro 1, P-1949014 Lisbon, Portugal.

**E-mail Address:** rpacheco@deq.isel.ipl.pt

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