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# pH characterization of urease using Raman Spectroscopy

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**Abstract:** Jack bean urease is a complex biochemical enzyme with the unique monomeric structure of polypeptides with an active site containing 2 nickel ions. Its pH-based characterization is spectroscopically investigated in an acidic (Hydrochloric acid) environment for understanding its limit of detection.

**OCIS codes:** (000.0000) General; (300.0300) Spectroscopy; (170.5660) Raman spectroscopy

## 1. Introduction

Urease or *Canavalia ensiformis* is a complex biochemical enzyme which is synthesized by various bacterial species, plants, nano-pathogens and fungi [1]. It occurs widely in leguminous seeds and plays an important role in seed germination and its chemical defense [2]. Being a complex nickel-dependent protein, its structure comprises of a monomeric chain of a single kind of polypeptide to form a chain of 840 amino acid residues (like Lys, Cys etc.) [3]. This subunit chain comprises of an active site with two nickel ions (Ni<sup>2+</sup>) which is shown in Figure 1.

A set of bridging hydroxide and water molecule complete the coordination sphere of these nickel ions. Another water molecule completes the tetrahedral cluster of solvent molecules. Given the complex hexameric structure of urease due to extensive intermolecular interactions, there are 6 monomer chain in fully loaded enzyme molecule. This gives it 6 active sites with 12 nickel ions per molecule. Figure 1 Schematic representation of Urease active site [3, 4] showing bi-nickel center. One Ni ion is connected to residues of two 'His' and one 'Lys'. The second Ni ion is again connected to two residues 'His', one 'Asp' and one 'Lys' respectively. It is seen that one of the 'Lys' sites bridge the residue between both the nickel ions. Whether urease is synthesized from plant or bacteria; its basic nickel-dependent structure, three-dimensional structural sequence and catalytic mechanism of hydrolyzing natural urea to ammonia and CO<sub>2</sub> ((NH<sub>2</sub>)<sub>2</sub>CO + H<sub>2</sub>O → CO<sub>2</sub> + 2NH<sub>3</sub>) remains same [6]. Despite this, the metabolic activity of plant and bacterial urease are different where plant urease plays a crucial role in nitrogen cycle in nature and bacterial urease (ex: from *H.pylori*) causes gastric cancer [6]. Both the processes involve dependence on pH of the environment, like hydrolysis of urea with Jack bean urease is ideally studied in a pH range of 5.80~7.49 [7] and bacterial urease neutralizes the acidic pH of stomach (2~4) [6]. The following work presents the characterization of Jack bean urease at pH 2, 3, 4 and 7 for the above hydrolyses reaction through Raman spectroscopic technique because it provides the symmetric and anti-symmetric molecular vibration. The authors presented a similar work [8] but pH-based characterization will provide the vital information on limit of detection of urease in low to high pH environments, in the presence of Ni<sup>2+</sup>-ions.

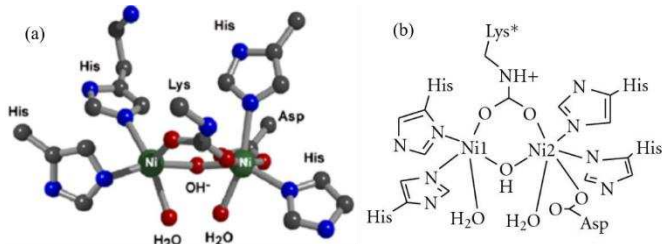


Figure 1: Schematic representation of Urease

## 2. Experimental Method

The experiment was performed using 4M urea and 0.00018M urease (procured Sigma Aldrich, Gillingham, UK) in 10ml of de-ionized water in order to simulate natural biological conditions. These quantities were calculated through empirical experimentation with different quantities of urea (MW: 60.06g/mol) and urease (MW: 48kDa). With optimum pH of 7.4, vibrational spectrum was captured for water, urea and urease solution at pH=7. In order to understand the hydrolysis reaction over time, the spectrum was measured after 2, 5, 10, 20- and 30-min gap from time of mixing the chemicals. The above steps were repeated for pH between 1~4 in order to simulate hydrolysis with bacterial urease in 10ml water (typical gastric fluid amount in a fasting condition).

## 3. Results and Discussions

An excitation laser of 514.5nm (laser power of 25 mW at the source) was used for the experiment on the inVia™ Raman microscope. A diminished 5mW laser was focused on the above prepared samples to produce Raman spectra which were baseline corrected using Lorentz function for peak identification. As seen from the figure 2(a) the baseline spectrum of water shows the asymmetric and symmetric –OH stretch of water on the shoulder at ~3250 cm<sup>-1</sup> and ~3410 cm<sup>-1</sup>. The –OH part of water involved in hydrogen bonding can be identified at ~3630 cm<sup>-1</sup>. Urea is easily identifiable at 546 cm<sup>-1</sup>, 1014 cm<sup>-1</sup>, 1127 cm<sup>-1</sup>, 1466 cm<sup>-1</sup>, 1625 cm<sup>-1</sup> and 1650 cm<sup>-1</sup>. While Urease shows signature peak at 353 cm<sup>-1</sup>, 480 cm<sup>-1</sup>, 839 cm<sup>-1</sup>, 1078 cm<sup>-1</sup>, 2893 cm<sup>-1</sup>, 2976 cm<sup>-1</sup>.

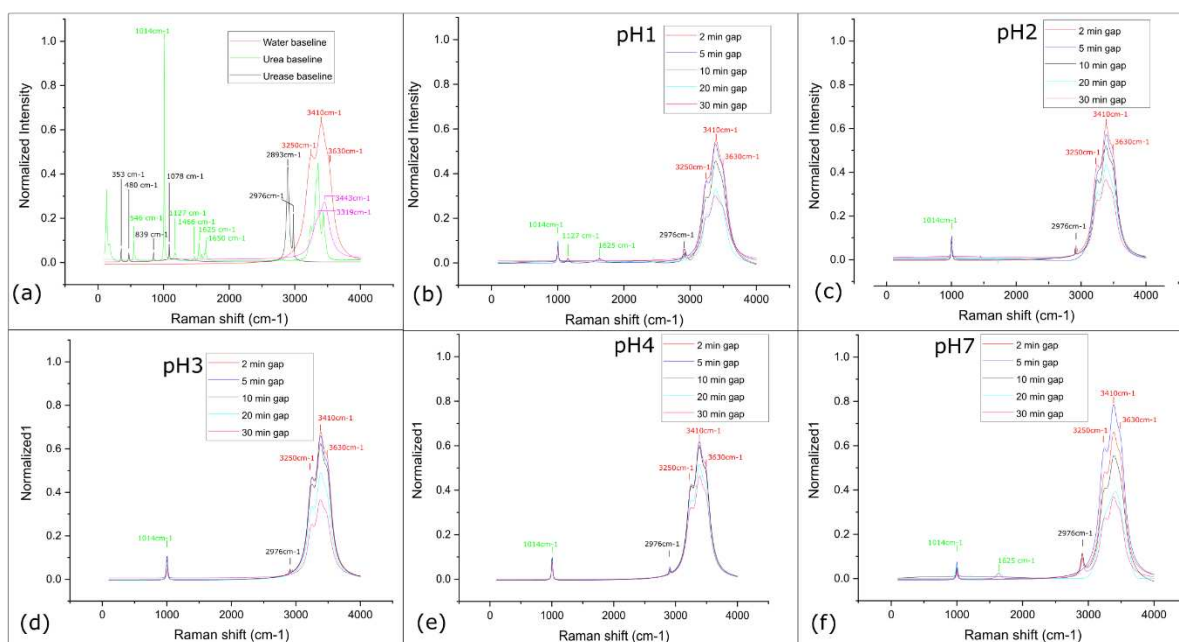


Figure 2: pHbased comparison of detection of urease

Following observations were made as the pH was increased from 1 to 7, (i) The urea peak at  $1014\text{cm}^{-1}$ , urease peak at  $2976\text{cm}^{-1}$  and the water peaks at  $3250\text{cm}^{-1}$ ,  $3410\text{cm}^{-1}$  and  $3630\text{cm}^{-1}$  was repeatable for water. The peaks for HCl were not detected as its peaks were in the water spectrum; (ii) The intensity of peaks decreased over time as the hydrolysis of urea continued; (iii) The CO and symmetric  $\text{NH}_2$  peaks at  $1127\text{cm}^{-1}$  and  $1652\text{cm}^{-1}$  respectively were not detected. This could be due to reduced acidity of the solution. The peaks from Figure 2(b)-(e) were performed with 4M and 0.00018M urea and urease respectively but in order to check the limit of detection, the Raman peaks were also obtained for 2M urea and 0.0001M urease in Fig 2(f). No prominent peaks could be obtained below this concentration.

### 3. Conclusion

Limit of detection of urea and urease compounds at different pH (1, 2, 3, 4 and 7) is demonstrated using Raman spectroscopic techniques. The minimum quantity of 0.0001M urease at 2M urea in a 10ml acidic solution was required in order to simulate for hydrolysis of urea detectable by Raman Spectroscopy. The vibrational bonds over various pH shows repeatability and reduced intensity over time indicating reduction in intensity of hydrolysis reaction.

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