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Quantitation of urea in urine by Fourier transforms infrared spectroscopy

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ABSTRACT

The objective of the present works is to quantitate the amount of Urea in Urine by using FT-IR Spectroscopy. The normal urine collected from the healthy male volunteers, different concentrations in the range of 1.25, 2.5, 5 and 10µg/mL were prepared by adding Urea. Then FT-IR spectra were recorded at the region 1500 -700cm^{-1} . The overall spectra of urine samples are dominated by urea. The spectra reveals that the primary peak at 3400cm^{-1} and a secondary peak at 1641cm^{-1} it is due to presence of urea. A graph between concentration of urea and intensity of absorption shows a linear relationship. There is an increase in the intensity of absorption at wave number 1641cm$^{-1.}$ This further confirms the specific peak for Urea

Keywords: FT-IR Spectroscopy, Urea, Glomerular Filtration and Urine Formation

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INTRODUCTION

A normal adult male subject has a GFR of approximately 125 mL/min. About 180 L of fluid per day are filtered through the kidneys. In spite of this large filtration volume, the average urine volume is 1–1.5 L. Up to 99% of the fluid volume filtered at the glomerulus is reabsorbed. The driving force for filtration through the glomerulus is the hydrostatic pressure of the blood flowing in the capillaries. Out of the 25% of cardiac output or 1.2 litres of blood/min that goes to the kidneys via renal artery, only 10% or 120 to 130 ml/min is filtered through the glomeruli, the rate being called as the glomerular filtration rate (GFR). Though some 180 litres of protein and cell free ultrafiltrate pass through the glomeruli each day, only about 1.5 litres is excreted as urine, the remainder being reabsorbed from the tubules. Besides fluid regulation, the kidney also regulates the retention or excretion of various solutes and electrolytes (). With the exception of proteins and protein-bound substances, most small molecules are filtered through the glomerulus from the plasma. The filtrate contains some ions, glucose, and essential nutrients as well as waste products, such as urea, phosphate, sulfate, and other substances. The essential nutrients and water are reabsorbed at various sites, including the proximal tubule, loops of Henle, and distal tubules. Both active reabsorption and secretion mechanisms are involved. The urine volume is reduced, and the urine generally contains a high concentration of metabolic wastes and eliminated.

The glomerulus filtrate from the Bowman's capsule contains waste products like urea, electrolytes, amino acids and glucose. The urine urea nitrogen is a measure of protein breakdown in the body. Besides waste nitrogen carrier, the urea also play an important role in the counter current exchange system at loop of henle of the nephrons that allows

the re absorption of critical ions and water from the excreted urine. Urea is reabsorbed at the collecting duct of the nephron, but some amount flows back in to the ascending limb of loop of henle through the collecting duct and then in to the urine. This process is controlled by ADH and allows the body to create hyper osmatic urine which has large concentration of dissolved substance than the blood plasma. The amount of urea in urine ranges from 15-25 gm/24 hour collections. For normal human being it is from 60 to 100 mg/dl in random collection. The urine urea nitrogen test is performed to measure protein breakdown in the body to find the protein intake and also find the kidney function. The low level of urea excretion by kidney indicates the kidney problem and malnutrition. The elevated value of urea is an indication of too much protein intake and protein breakdown. Urea is also measured in the blood as Blood Urea Nitrogen (BUN) test. The increased value of BUN is called uremia occurs in both acute and chronic renal failure, congestive heart failure, where there exist a faulty urine formation and excretion. Where there is low urea in the urine and large BUN.

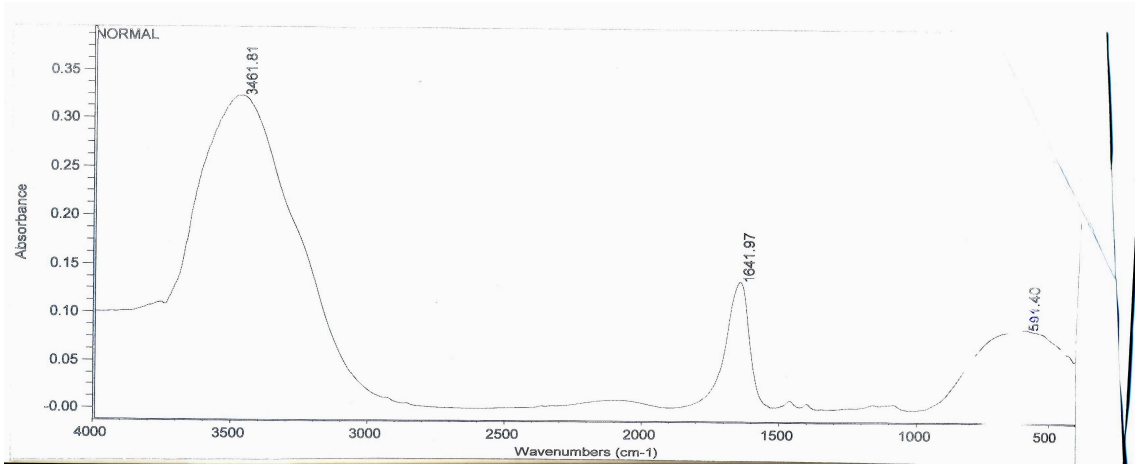
Spectroscopy is the interaction of matter and electromagnetic radiation which falls on it and as well as with the particle radiation. The spectroscopy is the measurement of absorption, scattering and emission of electromagnetic radiations by atoms or molecules. When atoms or molecules absorb the electromagnetic energy then they are transferred to higher energy levels. The electrons are promoted to higher orbital by visible or ultra violet radiations, vibrations are excited by infrared radiation and rotations are excited by microwaves.

The FT-IR in tissue diagnostics, the investigation of body fluids has been gaining importance. The mid–IR region is very useful in the identification of disease patterns using the FT-IR spectrum of human urine. Hence FT-IR technique is employed in the present work to analyze the urea from the chemical compositions of urine and to quantitative the urea at different temperatures.

MATERIALS AND METHODS

Materials and Methods

The urine samples were collected from a healthy male in the age group of 28-35years and treated with Urea of research grade (Sd fine chem., Mumbai,India) in the concentrations range of 1.25, 2.5, 5 and 10 µg/mL**.** The FT-IR spectra were recorded with Thermo Nicolet Nexus 670. The table top Thermo Nicolet Nexus 670 calibrated and checked with polysterene film. The sample filled in the liquid cell of 1mm thickness with a micro syringe. The liquid cell was placed in the sample compartment. The resolution was kept at $4cm^{-1}$ and scanning time was fixed at 38Sec. A total number of 32 scans were carried out on each sample. The scanning range fixed from 4000 – $400cm^{-1}$ for each sample. And also the ranges 2000-$1400cm^{-1}$,1400–$600cm^{-1}$ and 1200–$1000cm^{-1}$ were carried out.



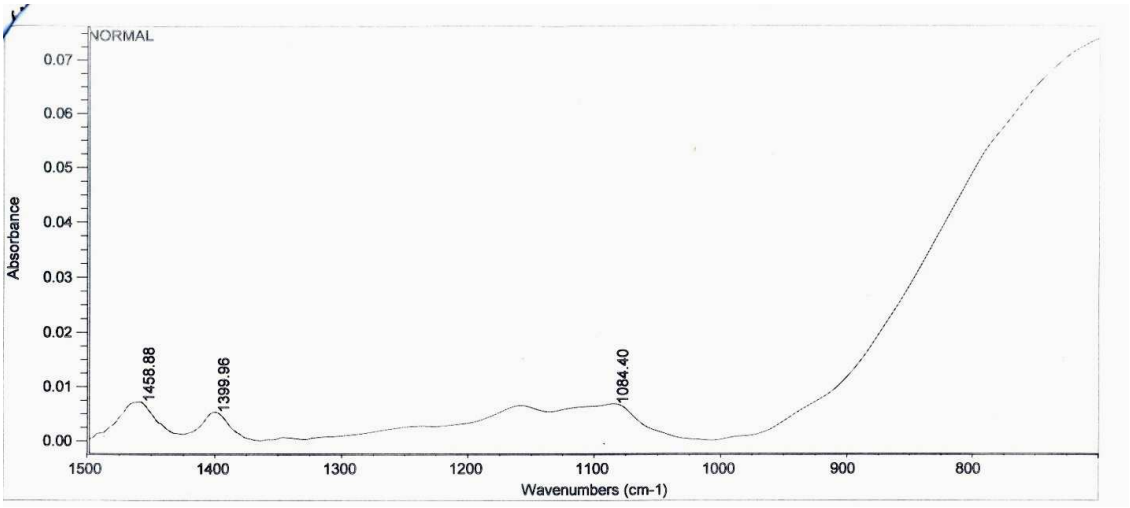
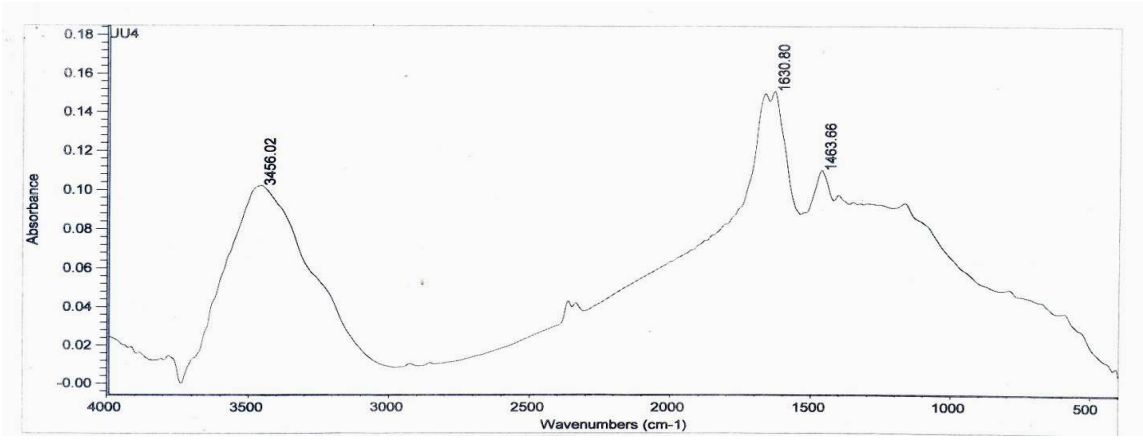


Fig 1(a) and 1(b) the FT-IR spectra of normal urine in Mid-IR region and 1500-700 cm^{-1}

The vibration spectra occur in infra red region. When infrared radiations of some frequency falls on molecules; the molecules absorbs energy and get excited to higher vibrational levels. The molecules absorb a quantum of energy give rise to characteristic based of the molecules from 50 to 12000 cm^{-1}. It is generally subdivided into three regions. Far IR 400 – 50 cm^{-1}, mid IR 4000 to 400 cm^{-1}, and near IR 12,500 to 4000 cm^{-1}. The mid IR region is the most commonly used for standard research investigations. Fig 1(a) and 1(b) shows the FT-IR spectra of normal urine in Mid-IR region and 1500-700cm^{-1} respectively. Table 1 gives the wave numbers and assigned functional groups obtained from standard FT-IR spectral library.

Fig 2(a) & 2(b) gives the FT-IR spectra of urine added with urea at concentrations 1.25 µg/mL in the Mid-IR region and 1500-700 cm^{-1} as sample code UU4. Fig 3 (a) to Fig 3 (c) presents the FT-IR spectra in Mid-IR region for urine with added urea at concentrations levels 2.5.µg/mL, 5 µg/mL and 10 µg/mL respectively. The intensity of the peak existing at 1641 cm-^{1} is increasing as the concentration of urea increases. This peak is more specific to urea. Table -1 describes the wave number and assigned functional groups for FT-IR spectra of normal urine. Table-2 represents Wave number and assigned functional group for normal urine added with Urea**.** Table - 3 shows the intensity of absorption and concentration of urea added to the normal urine. Fig 4 shows the increase in the absorption at wave number 1640 cm^{-1} with the increase of urea concentration.



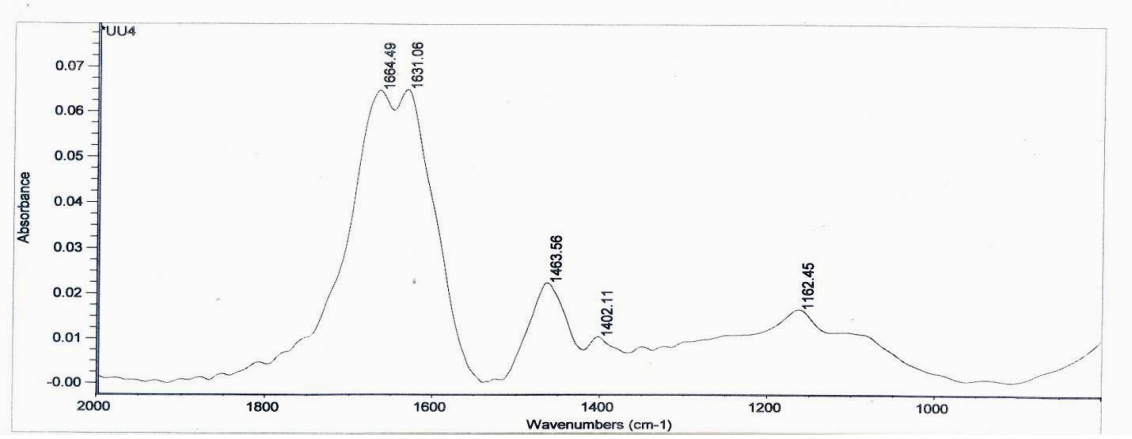


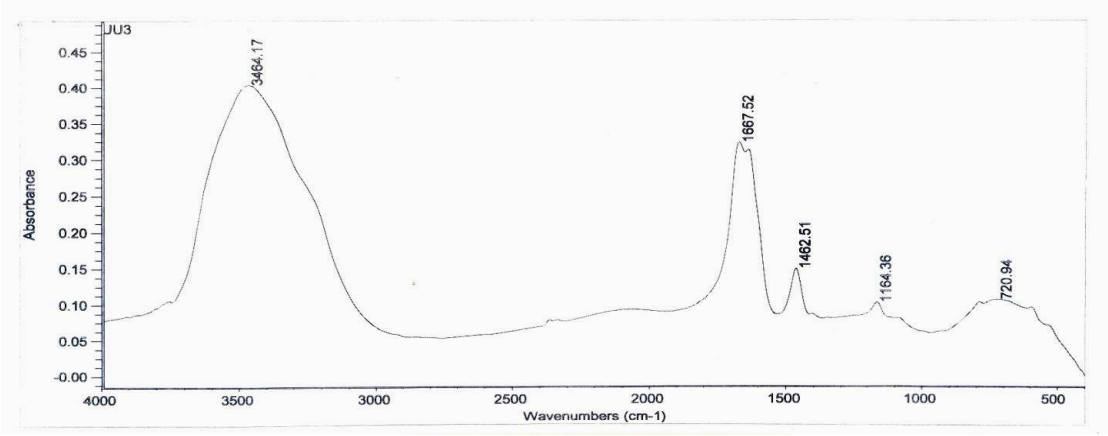
Fig 2(a) and Fig 2(b) FT-IR spectra of urine added with urea at concentrations 1.25 µg/mL in the Mid-IR region and 2000-800 cm^{-1}

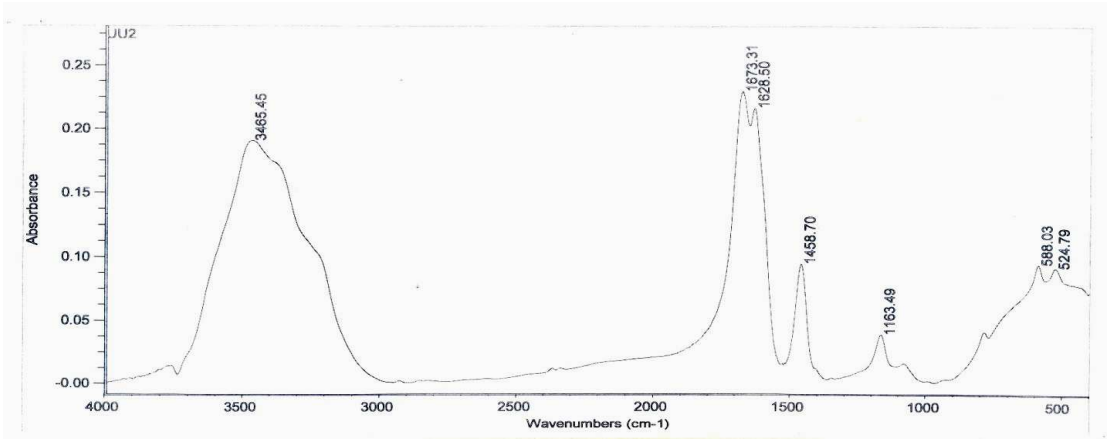
Table 1: Observed Absorption Frequency in the region of 400 – 4000 cm^{-1} and functional group for normal urine

Wave number (cm^{-1})	Functional Group
3461	H_2O or N-H
1641	Amide I. the C=N stretching absorption for open chain compound, helical structure –NH_2.
591	Strong C-H deformation , alkynes
1458	CH_2, CH_3 bending modes. ν(N=O) symmetrical deformation.
1084	Very Weak, Sugar ring vibration
1399	Lactate, carboxylic acids and derivatives.

Table 2: Observed Absorption Frequency in the region of 400 – 4000 cm^{-1} and functional group for normal urine added with Urea

Wave number (cm^{-1})	Functional Group
3456 – 3465	HO-H or N-H
1630 – 1667	Urea, (strong) Amide I Amide I. the C=N stretching absorption for open chain compound, helical structure –NH_2, due to urea.
1457 - 1460	CH_2, CH_3 bending modes. ν(N=O) symmetrical deformation.
1400	CH_2, CH_3 bending modes of lipids. C=O symmetric stretching vibration of COO^-.
1160	C – O stretching of protein, glycogen and C-C of carbohydrates
588 - 520	S –S disulphide





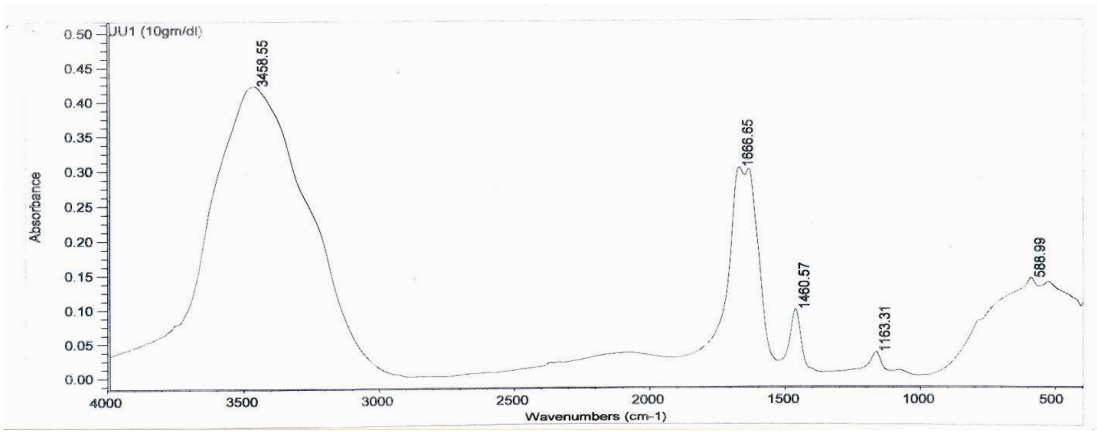


Fig 3(a) , 3(b) and Fig 3(c) FT-IR spectra in Mid IR region for urine added with urea at concentrations 2.5, 5 and 10 µg/mL

Table 3: Intensity of absorption at wave numbers for urine added with urea at different concentrations

Sl. No	Concentration of urea	Wave number (cm^{-1})
Normal	0	3461(0.323) 1641(0.133) 591(0.0854) 1458(0.0072) 1084(0.0068) 1399(0.0052)
UU4	1.25 µg/mL	1630 (0.151) 1463 (0.110) 3456 (0.102) 1631 (0.065) 1664 (0.0649) 1463 (0.0224) 1162 (0.0166) 1402 (0.0105)
UU3	2.5 µg/mL	3464 (0.403) 1667(0.323) 1462 (0.149) 720 (0.107) 1164 (0.102)
UU2	5 µg/mL	1673 (0.203) 1628 (0.217) 3465 (0.190) 1458 (0.0947) 588 (0.0944) 524 (0.0918)1163 (0.0388)
UU1	10 µg/mL	3458 (0.422) 1666 (0.303) 588 (0.143) 1460 (0.0971) 1163 (0.0339)

Table – 4 Concentration of Urea VS Absorbaces

Concentration of urea	Absorbaces (nm)
(Normal urine) 0 µg/mL	0.133
1.25 µg/mL	0.151
2.5 µg/mL	0.181
5 µg/mL	0.217
10 µg/mL	0.303

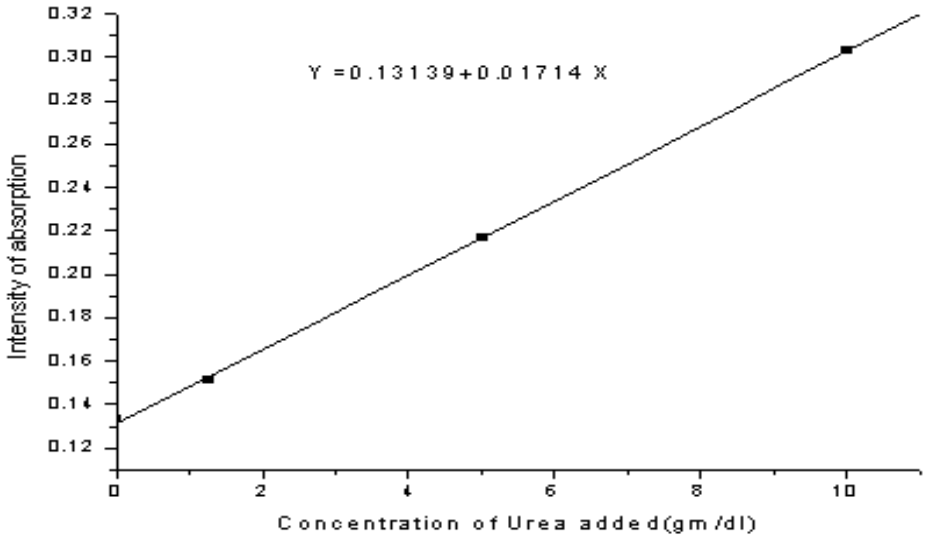


Fig: 4 Graph between intensity of absorbance of Urea and concentration

RESULTS AND DISCUSSION

The great advantage of FT-IR spectroscopy is high sensibility that permits the determination of many components, even in very small amount. The IR spectrum is like a 'finger print' of a haad of the molecular species making up of the sample. The intensities of IR spectra provide quantitative information while the absorption positions reveal qualitative characteristics about the nature of chemical bonds, their structure and their molecular environment. Specifically vibrations that have been previously used in the study of lipids are the CH_2 stretching (2850 and 2920 cm^{-1}) bending or scissoring (1450-1480 cm^{-1}) and wagging (1180-1350 cm^{-1}) modes, the lipid finger print region containing phosphate and diester stretch modes (1000-1450 cm^{-1}), and the C=0 stretching (1700-1750 cm^{-1}) vibrations[1]. The important absorption bands arise from NH, C=0, C-H and X-O bonds found in urea, carbohydrates, proteins, lipids and nucleic acids[2]. The frequency, intensity and width of the particular vibration IR spectral bands are extremely sensitive. The conformational changes and chemical vibrations in lipids, carbohydrates, urea and proteins. In the present study urea was estimated from the urine sample by FT-IR Spectroscopy[1][2].

It can be seen that an overall appearance of urine spectrum is dominated by urea, not surprisingly as urea concentration is expected to be much higher than any other urinary constituent in normal urine. The most intense bands for urea are 3400 and peaks between 1640-1620cm^{-1} and most intense absorption band in proteins is the amide-I peak, which is observed at 1652 cm^{-1}. Amide–I is mainly associated both the C=0 and C-N stretching vibration and is also related to the backbone conformation [4][5][6]. The next major absorption band is the amide-II that derives largely from the in plane N-H bending, and the C-N and C-C stretching vibrations. The present study has demonstrated that FT-IR spectrometry is a useful tool for determining concentration of multiple bio molecules in micro samples of urine and the peak at 1641 cm^{-1} can be assigned to Urea[3][4].

CONCLUSION

Application of FT-IR spectroscopic for the study of the analysis of Urea in urine. The spectra reveals that the primary peak at 3400cm^{-1} and a secondary peak at 1641cm^{-1} it is due to presence of urea. A graph between concentration of urea and intensity of absorption shows a linear relation. There is an increase in the intensity of absorption at wave number 1641$cm^{-1.}$ This further confirms the specific peak for Urea.

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