International Journal of Applied
and Natural Sciences (IJANS)
ISSN (P): 2319–4014; ISSN (E): 2319–4022
Vol. 8, Issue 4, Jun–Jul 2019; 171–180

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

ISOLATION AND SPECTROSCOPIC CHARACTERIZATION OF UREA FROM HUMAN URINE

Gongden J.J, Uduah O.V, Gurumyen K.K & Lohdip Y.N

Research Scholar, Department of Chemistry, University of Jos, P.M.B 2084, Jos, Nigeria

ABSTRACT

Urea (UC) becomes isolated per litre of human urine by evaporation/concentration, nitration, precipitation, crystallization and recrystallization. It is then characterized by ^{1}H-NMR, ^{13}C-NMR, UV-vis and FT-IR, melting point analysis, refractometry, pH and conductivity measurements. The percentage yields of urea obtained is 61.3% (~5.7g/L). The characterization of UC showed chemical shift of ^{1}H-NMR, and ^{13}C-NMR at 2.75ppm and 163.78ppm respectively while UV-vis was 212.80nm and FT-IR result showed symmetric and asymmetric frequencies peaks of V_s (NH_2) and V_{as} (NH_2), a combination band of V_s (NH) and V_{as} (NH), C=O and V (C-N) all stretched. It is a white needle-like structure, very soluble in water, but slightly soluble in ethanol and methanol. Other results of the physical properties of UC are pH (27^{o}C) = 5.70, refractive index (n) =1.321, melting point = 135 – 141 (^{o}C) and electric conductivity = 1.9 x 10^{-4}. The XRD showed the crystal to be amorphous. The results obtained revealed that useful substance, urea, can be isolated using simple methods for possible use as a ligand for synthesis of urea-based complexes and for the production of urea-based fertilizer, where pure urea is not available or costly which complement Green chemistry.

***KEYWORDS**: Urea, Human Urine, Spectroscopic, Characterization*

Article History

Received: 23 May 2019 | Revised: 17 Jun 2019 | Accepted: 31 Jul 2019

INTRODUCTION

Most animals, especially mammals discharges liquid wastes from their body, either in form of sweat or/and urine. Over four billion people are discharging untreated human excreta into the environment without any prior treatment (Jenna *et al.*, 2018). If properly managed and utilized, urine can serve as a fertilizer because of its nutrient contents. Urine can be collected separately and dehydrated in an alkaline bed producing a nutrient rich fertilizer (Senecal et al., 2018). Urine is usually rich in nitrogen and contains urea, as its main solid component. Primarily, it consists of water (91 to 96%), with organic solutes including urea, creatinine, uric acid, and trace amounts of enzymes, carbohydrates, hormones, fatty acids, pigments and mucins. Also, present are inorganic ions such as sodium (Na^+), potassium (K^+), chloride (Cl^-), magnesium (Mg^{2+}), calcium (Ca^{2+}), ammonium (NH_4^+), sulfates (SO_4^{2-}) and phosphates (e.g. PO_4^{3-}) (Anne, 2017). Urea (CO $(NH_2)_2$), an organic compound dissolves readily in water.

Urea is a colorless, odorless crystalline or pellets powdered or granulated organic substance that melts at 132.7°C-135^{o}C and decomposes before boiling. Its density is 1.32 g/cm^3 and it is highly soluble in water and ethanol, but insoluble in ether and practically non-toxic (LD_{50} is 15 g/kg for rats). Urea was the first organic compound to be synthesized from inorganic reagents (Fairall, 1996). Urea has been known since 1773 to be a major component of mammalian urine, by

combining cyanic acid and ammonium in vitro (Ekinne-Saffran, 1999). The formation of urea in animals occurs in the liver. It is an endogenous product of protein and amino acid metabolism (Thomas, 1991). It is usually formed via enzymatic deamination. The amino group ($-NH_2$) is removed as ammonia and excreted, either unchanged or as urea or uric acid. Approximately 20–35 g of urea is excreted in human urine per day containing 9.3g/L, depending on several factors (quantity of protein consumed, health, water intake etc).

Pure urea was first isolated from urine in 1727 by the Dutch scientist Herman Boerhaave (Higgins, 2016). Urea is the highest solute constituent by mass contained in the human urine. Apart from the natural urea obtained from human urine, urea has also been discovered artificially from inorganic materials. Over a hundred million tons of urea fertilizer is frequently been synthesized industrially every year (Guillou, 2013). Friedrich Wohler (1800 - 1882), a German physician and chemist by training, in 1828, carried out several reactions that resulted in the production of synthetic urea [$(NH_2)_2CO$] (Steven, 2018).

Urea can be used as starting materials in the chemical industries for preparing formaldehyde-Urearesin (plastics), barbiturates and fertilizers (Adel *et al.,* 2014). Urea is also extensively used as organic ligand, which can undergo both mono-dentate and bi-dentate coordination with d-block transition metals to form organo metallic complexes. Complex of urea has been used to promote healing in infected wounds and many other applications in the field of medicine. When used as fertilizer, it is advantageous, because: it has the highest nitrogen content than any other nitrogenous fertilizer in the market; its cost of production may be low; it can be used for any type of crop; and causes no harm to the soil. In this research, modified Boerhaave's method is being used to isolate and characterized urea from human urine for different possible uses.

EXPERIMENTALS

Urine Sampling

Figure. 1: Urine Sample.

Four litres of early morning urine samples were collected from four donors (one litre each) and homogenised into a 4L plastic (fig. 1) rubber as described by Karim (2018). The sample was stored at room temperature (25^oC). A 6M solution of HNO_3 (70.0%, 63.01 g/mol, sp.gr. 1.42; 15.8 M) was prepared by dissolving 190 mL in 500 mL of deionized water, in a 500 mL volumetric flask at room temperature. 1M solution of Potassium carbonate, K_2CO_3 was also prepared by dissolving 69.1 g of the salt in 500 mL of deionized water in a 500 mL volumetric flask at room temperature. All the chemicals used in this study were of analytical reagent grade and used without further purification. The isolation of urea from urine involves both the physical and chemical methods and processes. Boerhaave (1727) and Ben (2016) methods

were modified and used to isolate urea crystals from the human urine sample. About 63% of Urea becomes isolated per liter of urine by evaporation/concentration, nitration, precipitation, crystallization and recrystallization and decolorization.

Evaporation / Concentration

The sample was evaporated to a volume of 250 ml and in a syrup form. "Urine-syrup" was allowed to cool at room temperature and vacuum-filtered. Urea nitrate, a crystalline compound was formed according to equation (1) and purified by repeated recrystallization.

$$(NH_2)_2CO\ (aq) + HNO_3\ (aq) \rightarrow (NH_2)_2COHNO_3\ (s) \quad (1)$$

Regeneration of Urea

Urea was regenerated from the urea nitrate by reacting the urea nitrate with 100 ml of 6M potassium carbonate (K_2CO_3) in a clean 1000 ml beaker according to equation (2).

$$(NH_2)_2COHNO_3 + K_2CO_3 \rightarrow (NH_2)_2CO + KNO_3 + H_20 + CO_2 \quad (2)$$

Solubility difference of urea and potassium nitrate in hot ethanol was used to separate the products. The ethanolic solution of the urea was evaporated to thick syrup. It was then recrystallized with a mixture of water and ethanol (1:3). This was heated again to evaporate the water and ethanol and allowed to cool at room temperature. Confirmed urea crystals were formed and further left overnight in a freezer with more crystals being formed. The crystals were dried at room temperature and stored for further analysis.

Characterization of the Isolated Urea Crystals

Analysis for the physical properties of the isolated urea crystals, IUC, was carried out as preliminary tests. The results of the color, solubility, melting point etc., were determined and the results compared with those of a pure urea (PU) sample of analytical grade, which was used as a control. In addition to the colour, solubility and melting point tests, standard spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, UV-vis and FT-IR), refract meter, pH and conductivity measurements were also carried out to characterize the isolated urea crystals.

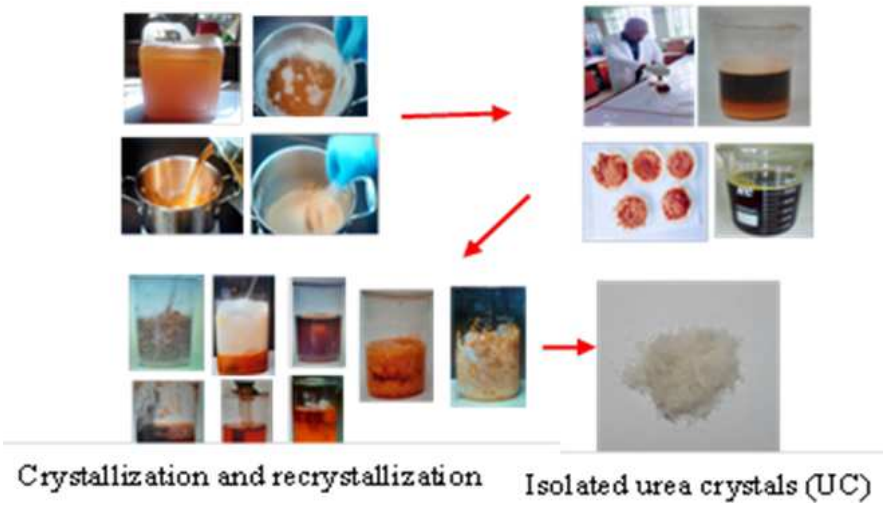

Figure 4: Isolation of Urea from Urine.

RESULTS AND DISCUSION

Table 2: Physical Properties of Isolated Urea IUC and Pure Urea, PU

Parameter	Standard PU(Control)	Sample IUC
Water (27^{o}C)	+++	+++
Ethanol (27^{o}C)	+++	++
Methanol (27^{o}C)	+++	++
pH at (27^{o}C)	7.05	5.70
Refractive Index (n)	1.491	1.321
Melting point (^{o}C)	132-136	135-141
Electric Conductivity (S/cm) (27^{o}C and atm pressure)	1.5×10^{-4}	1.9×10^{-4}
Colour and particulate nature	White Needle-like	White Powder

Key: PU = Pure Urea, IUC = Isolated Urea Crystals (sample)

Percentage Yield of IUC

Figure 4, shows the isolated urea (IUC) (fig 4b), side product (SP) (fig. 4c) and the pure urea standard (PU) (fig. 4a) respectively. The yield of PU and IUC were calculated using the formula below. IUC yield was calculated with respect to 9.3g of theoretical quantity of urea contained in one litre of the urine sample, as determined quantitatively according to the formula:

$$\% \text{ yield of UC} = \frac{\text{Mass of UC in g/l of urine}}{\text{Theoretical mass of 9.3g/l of urine}} \times 100$$

5.7g of the crystals was isolated per litre of the urea sample. This corresponds to approximately 61.3% yield. The 5.7g of urea per litre of urine sample is about 66.3% yield when compared with the 8.6g contained in the urine sample, which was determined quantitatively. However, the low yield may be attributed to metabolic factors of the sample donor, the method of isolation being applied, or some environmental factors. Furthermore, it is also possible that SP (fig. 4c) could contain some urea, which may not have been completely converted to urea crystals.

Plate 1: Isolated Urea Crystals, UC

Plate 2: Standard UreaCrystals

The results of the color, solubility, melting point, pH-value, refractometry and conductivity determined are shown in Table 2.

Urea has a melting point of 133°C. It is soluble in water and ethanol, but insoluble in ether (Omar, 2012). The result of the physical properties of the IUC given in Table 2 is in agreement with those of the PU and literature. However, there is a slight observable variation in the melting point, pH-value, particulate appearance and refractive index. The

melting point of IUC was found to be 139^{o}C ±5^{o}C as against the 134^{o}C ± 5^{o}C of PU. The slight difference may be attributed to the presence of some impurity, such as unconverted/remnant of protein materials from the urine sample. The pH-value, 5.7 of IUC shows that it is slightly acidic, while that of PU is 7.9 just slightly above neutral point (i.e., pH = 7.0). This result may be ascribed to some traces of concentrated trioxonitrate (V) acid, which may not have been completely converted to the expected "urea-nitrate".

The refractive index of PU is 1.491, which is slightly higher than that of IUC, 1.321. As light passes through the interface between two mediums of different refractive indices, it bends; besides, PU had a brighter appearance compared to IUC, which may be as a result of the "yellow pigment" or colouration of urine, which may have interfered with the absorption of light and the reflected incident rays by the two substances. The refractive index of IUC is in agreement with the refractive index of urea from human urine, as reported by Savarimuthu & Nagaraj 2016 (table 1). Both IUC and PU crystals have white coloration. Although PU is powder, while IUC has a needle-like crystalline appearance. Zing et al., (1996) have reported that urea crystals exhibit a needle – like shape. The electrical conductivity of IUC and PU showed that they are both non-electrolyte. The needle-like structure of IUC is a characteristic structure or form of urea that is crystallized via water medium.

UV–Vis Spectra Result of UC and PU

The result of the UV-vis spectrum of IUC is slightly different from that of the PU (Fig. 3). There were absorptions at wave lengths 236.80, 206 and 204 nm respectively (i.e., 1, 2 and 3). The peaks, 2 and 3, may be an essential parameter for frequency doubling purposes. The wave lengths 236nm and 212.8 nm of PU and IUC respectively are in agreement with the absorption wave length of urea according to the spectrum recorded in NIST Chemistry WebBook (Fig. 6).

Table 3: UV-vis Spectra Results of PU and IUC

No.		PU	IUC
1.	Wavelength (nm)	236.80	212.80
2.	Absorbance	0.276	3.095

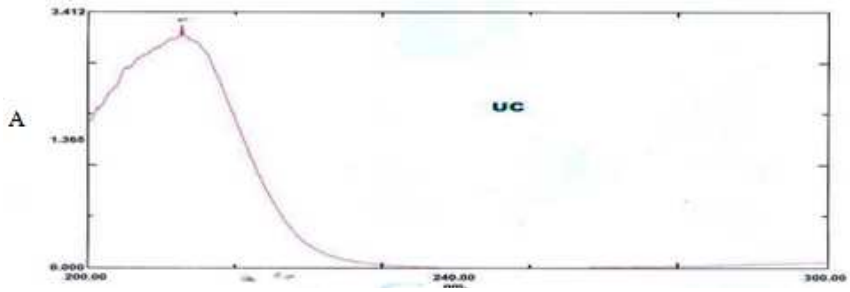

Figure. 3: UV-vis Spectrum Isolated Urea Crystal.

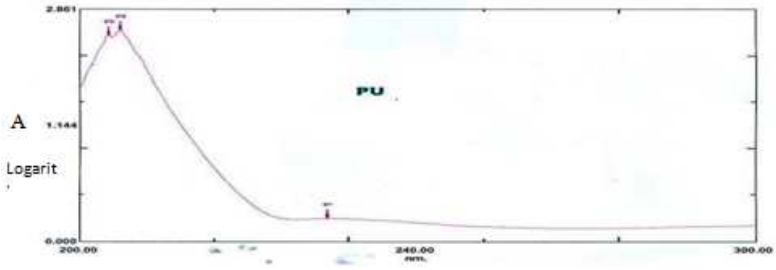

Figure. 2: UV-vis Spectrum of Pure Urea.

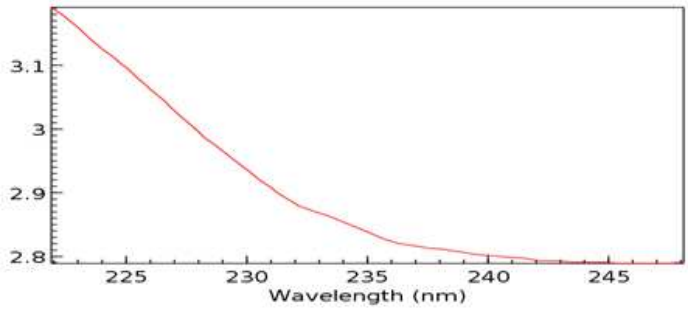

Figure. 6: Urea UV-vis Spectrum (Literature)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry).

FT-IR Spectra Analysis of IUC and PU

The result of the FTIR spectra of the isolated urea, IUC was compared with that of PU (standard sample). Both were recorded on FTIR-ATR (Shimadzu 8400S model) utilizing KBr disc process and all samples were scanned over a range of 750-4000 cm^{-1}. The FT-IR spectra results of PU and IUC are shown in figure 7 and figure 8. The vibrational mode of the N-H group in the isolated urea is of a secondary amide, which is in agreement with, Shweta, (2003). The band corresponds to $-NH_2$ stretching of PU and IUC 3309.96 and 3394.83 respectively (Table 5).

The characteristic vibrational frequencies of urea (PU and IUC) are reported in the literature by (Web; shodhganga, retrieved 2019). An intense sharp peak occurred each, at 1519.96 and 1527.67 for PU and IUC respectively. The peaks are attributed to the presence of the carbonyl functional group. The peak corresponding to v C-N is observed at 1033.88 for both PU and IUC. These frequencies are indications that there is an n*-σ transition of the double bond of C=O. The identified functional groups in PU and IUC crystals are systematically assigned with the corresponding wave number, in table 4.

Table 5: Characteristic FT-IR Frequencies and Assignments of Urea PU and IUC

Reference	PU (cm^{-1})	Intensity (%T)	IUC (cm^{-1})	Intensity (%T)	Band Assignments
3422–3320 (m)	3309.96 3063.06	87.631 90.103	3487.42 3394.83	84.893 85.431	V_s (NH_2) V_{as} (NH_2) } stretch
3000 – 2800 (sh)	Combination band				V_s (NH) V_{as} (NH) } stretch
1700–1510 (s)	1519.96	97.487	1527.67	96.389	C=O stretch
1250–1020 (m)	1033.88	97.462	1033.88	98.347	V (C-N) stretch

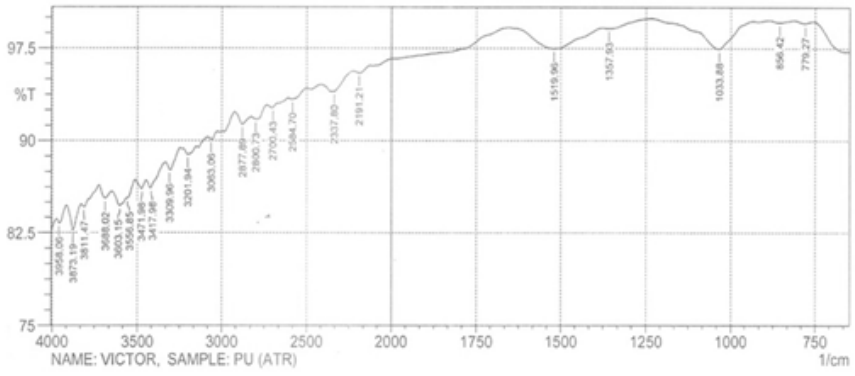

Figure. 7: FT-IR Spectrum of PU (ATR).

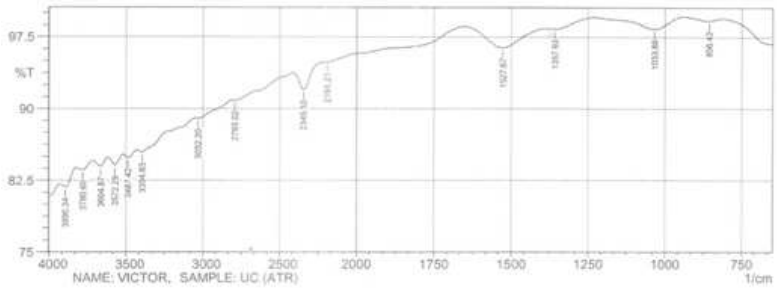

Figure. 8: FT-IR Spectrum of IUC.

Nuclear Magnetic Resonance Spectra results of IUC (^{13}C-NMR and H^{1}NMR)

The ^{1}H-NMR and ^{13}C-NMR results of IUC shows chemical shifts at 2.75ppm and 163.78ppm respectively (fig. 9 and 11). ^{13}C-NMR spectrum of the IUC has only one peak at 163.78ppm, which corresponds to the 165-170ppm chemical shift range of C=O carbon of amide in literature. The presence of one single peak in the ^{13}C-NMR spectrum (fig. 10) is an indication of single carbon atom in the molecule of urea, $(NH_2)_2CO$. The quaternary carbons (CN or C=O) functional group in the urea molecule, is clearly detected at 163.78ppm in the ^{13}C-NMR spectrum. This characteristic chemical shift of C-N or C=O in the urea molecule is in agreement with William Reusch (2013) range of 160-170ppm ^{13}C-NMR shift for amides. The chemical shift range of proton in a nitrogen (amine or amide) environment is 0.5+ or -5 (Laurie, retrieved 2019). The chemical shift of UC, 2.75 (fig. 9) corresponds with the chemical shift of urea in the spectrum of the standard (fig. 11).

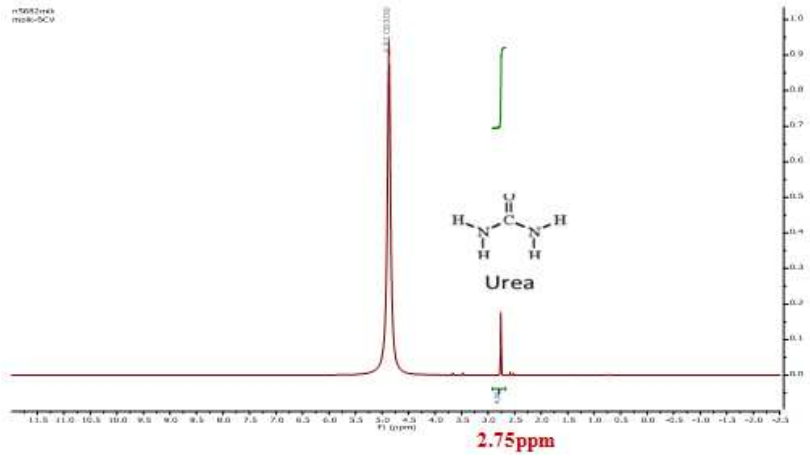

Figure. 9: ^{1} H-NMR spectrum of IUC.

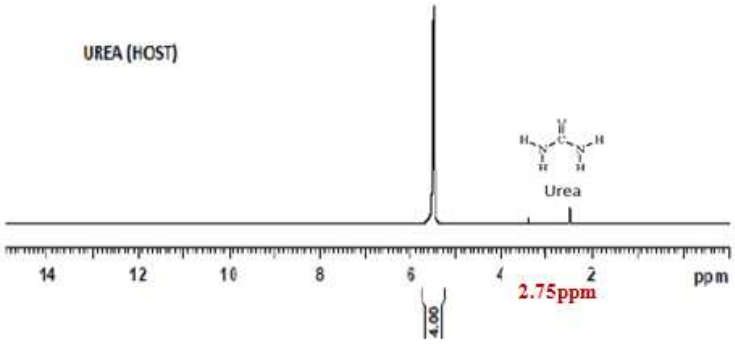

Figure. 10: ^{1} H-NMR Spectrum of Pure Urea (Literature).

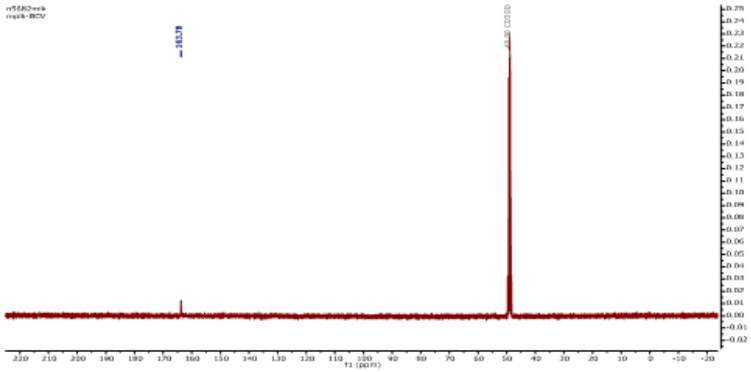

Figure. 11: ^{13}C-NMR Spectrum of CU.

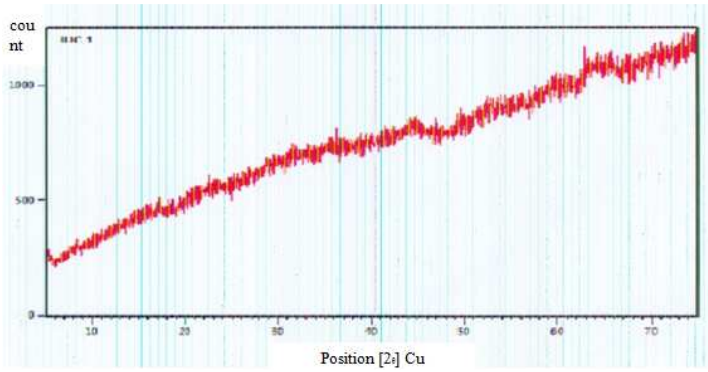

Figure. 10: XRD Spectrum of IUC.

CONCLUSIONS

About 63% of Urea becomes isolated per liter of human urine by evaporation/concentration, nitration, precipitation, crystallization and recrystallization, and decolorization. The molar conductivity data of the isolated urea in methanol KI indicates that it is non-electrolyte. The isolated urea was also very soluble in water and soluble in methanol. Other physical parameters of IUC are in agreement with those of the PU, which was used as standard for comparison. The spectroscopic data of the isolated urea: ^{1}H-NMR, ^{13}C-NMR, UV-vis and FT-IR ascertained IUC to be urea. Based on the findings of this study, it can therefore be reported that, urine is a very useful waste. It contains over three thousand chemical components of which urea is the highest after water and can actually be isolated by simple process of evaporation, nitration, crystallization and recrystallization for possible use as a ligand for synthesis of urea-based complexes and for the production of urea-based fertilizer, where pure urea is not available or costly.

REFERENCES

1. *Anne .M (2020). What is the chemical composition of Urine? Retrieved March 30, 2020 from https//www.thoughtco.com/thechemical-composition-of-urine-603883.*
2. *Adel, S.M., Omar, B.I., Abdel, M. A. A & Al-Majthoub, M. M (2014) Chromium (III), Manganese (II), Cobalt (II), Iron (III) and Nickel (II) Complexes with urea at High Temperature: Preparation and Spectroscopic Studies. Research Journal of Pharmaceutical, Biological and Chemical Science. 5 (2) 981-988*
3. *Duraton F., Jankovski .J.,Wiecek .A. Argiles A. (2016). On the discovery of Urea: Identification, Synthesis and observations that let to establishing the first uraemic retention solute. National Centre for Biotechnolgy Information, NCBI (PubMed).Retrieved February 14, 2020 from https//www.ncbi.nlm.nih.gov/PubMed/26913884.*

4. *Environmental, Health, and Safety Guidelines Nitrogenous Fertilizer Production (2007). IFC International Financial Corporation, World Bank Group. Retrieved March 10, 2020 from https://www.ifc.org/wps/wcm/connect/b237688048855d518e94de6a6515bb18/Final+-+Nitrogenous+Fertilizers.pdf?MOD=AJPERES.*
5. *Excretion in Mammals, the Detoxification Organ, Royal Society of Chemistry. (n.a.).(n.d.), Retrieved May 15, 2019 from https://www.rsc.org/Education/Teachers/Resources/cfb/excretion.htm.*
6. *Helmenstine, Anne Marie, Ph.D. "What Is the Chemical Composition of Urine?" Thought Co, Feb. 6, 2017, thoughtco.com/the-chemical-composition-of-urine-603883.Anne Marie Helmenstine, Ph.D. Updated February 06, 2017*
7. *Jenna .S.,Annikan .N., Prithvi .S. and Bjorn .V. (2018). Hygiene aspect of treating human urine by alkaline dehydration. Water Research, 144, pp1. https//www.doi.org10.1016/j.wters.2018.07.030*
8. *Kirk Othmer Encyclopedia of Chemical Technology (1983) 3rd ed, Wiley Inter-science, Vol. 23.b. Retrieved December 20, 2019 from https://www.chm.bris.ac.uk/motm/urea/urea.html.*
9. *Laurie S. S. (n.d.). ^{1}H NMR Chemical Shifts. Organic Chemistry Lab CHM 318L California State Polytechnic University, Pomon. Retrieved May 25, 2019 from https://www.cpp.edu/~lsstarkey/courses/NMR/NMRshifts1H-general.pdf*
10. *Liji .T. (2019). Urine composition: What's Normal? Retrieved January 20, 2020 from https//www.news-medial.net/health/urine-composition-water280995-Normal.aspx.*
11. *Linda Crampton (2018) Urea in Blood, Urine, Skin Creams, and Fertilizer. Retrieved February 20, 2019 from https://owlcation.com/stem/Urea-in-the-Body-in-Skin-Creams-and-in-Fertilizers*
12. *Omar, B.I (2012). Complex of urea with Mn (II) Fe (III), Co (II) and Cu (II) Metal Ions. Pelagia. Research Library, Adv. Res, 3(6): 3522-3539. Retrieved March 12, 2019 from https://www.pelagiaresearchlibrary.com*
13. *Savarimuthu, R. & Nagaraj, D. (2016). Photonic crystal based biosensor for the detection of glucose concentration in urine. Retrieved March 14, 2019 from https://www.researchgate.net/figure/Urea-concentration-in-urine-and-its-refractive-index-resonant-wavelength-normalized_tbl5_309436030*
14. *Steven M. C. (2018). The Wohler Synthesis of Urea (1828). Retrieved April 24, 2019, from https://www.mun.ca/biology/scarr/4270_Wohler_Synthesis_of_Urea.html.*
15. *Stuart .J. Fairall (1996).Molecule of the Month-June 1996, Urea. School of Applied Science, De Montfort University. Retrieved April 20, 2020 from https//www.chm.bris.ac.uk/montm/urea/urea.html. DOI: 106084/m9.figshare.5469661.*
16. *Thomas A. Depner (1991). Urea Metabolism: Clinical Chemistry of Urea Prescribing Hemodialysis, Volume 29 ISBN: 978-1-4612-8811-4. Retrieved April 20, 2020 from https://citations.springer.com/item?doi=10.1007/978-1-4613-1509-4_2.*
17. *Wikipedia (n.d.).Urea. Retrieved April 20, 2020 from https://www.wikizero.com/en/Urea.*
18. *Zing, L., Zha, M., Arddoino, G., Franzosi, P., Zanotti, L., Zucalli, G., Paorici (1996) Solution Crystal Growth of Urea and Derivatives for nonlinear optical application. Journal of Crystal Growth 166: 528 – 532.*