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Comparative study on classical biochemical determination and supramolecular sensing of creatine in urine sample

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I. Introduction

Creatine is a breakdown product of creatine phosphate from muscle and protein metabolism in human. It is released at a constant rate by the body, and an approximately half of creatine originates from food. The results of the creatine tests can provide a very strong indication of how well the kidneys are functioning. The normal ranges for a creatine test are: 0.5 to 1.1. Milligrams (mg) per deciliter (DL) in women, 0.6 to 1.3 mg/DL in men and 0.5 to 1.0 mg/DL for children ages 3 to 18 years. If the level of creatine increased it could be the symptoms of Chronic or acute kidney disease, Congestive heart failure Diabetes Hyperthyroidism (overactive thyroid) Urinary tract obstruction, Muscular dystrophy and other muscle diseases. If your creatine test results are low, you may have: Severe liver disease Protein malnutrition Muscle wasting, etc. Lafolie P (1991) reported the importance of estimation of creatine in urine samples when screening for abused drugs. The Jaffe method remains the default method for estimation of creatine (B D Toora (2002). But this method has been shown to interfere with antibiotics like streptomycin with creatine either positively or negatively with the determination of creatine at various concentrations by the Jaffé reaction (Syal K 2013). W. J. Birdsall (2009) reported the estimation of creatine by Cu II/CuI redox couple complexes. Sebastian Haaf (2019) studied the role of crown ether as Secondary Coordination Spheres for the Manipulation of Ligand–Metal intramolecular Electron Transfer in Copper–Guanidine Complexes in the determination of guanidine and Ca ions simultaneously. Along list of interfering substances of Jaffe method that affects creatine estimation, but the method is popular perhaps for its inherent simplicity but in the clinical context of drugs like aminoglycosides, cisplatin, phenytoin, deriphyllin or levofloxacin, alkaline picrate method may not be made redundant due to false chromogenic formation. We, hereby, stress on the need of innovation in the field of diagnosis of kidney damage, improvement in methods of creatine estimation and recommend a boost in research to study potential interfering agents of alkaline picrate method used for creatine estimation in the scenario of rapidly developing new drug molecules.

II. Methodology

Synthesis of Crown ether catenated copper II Neocuproine

$CuCl_2$ solution, 1.01M, was prepared by dissolving 0.426 g $CuCl_2$.$2H_2O$ in water, and diluting to 250 mL. Ammonium acetate buffer at pH 7.0, 1.0 M, was prepared by dissolving 19.27 g NH_4Ac in water and diluted to 250 mL.Neocuproine (Nc) solution, 7.51 M, was prepared by dissolving 0.039 g of Nc in 96% ethanol, and diluted to 25 mL with ethanol. 3ml of alcoholic solution of crown ether was treated with this complex compound.

Estimation of Creatine by absorption spectrophotometer

Standard Creatine stock solution was prepared. Dilution factor was applied as 1:100. Standard working solution was also prepared. Working solutions were taken in different ml ranges as 0.5, 1,1.5, 2, 2.5 and water as taken as 2.5, 2, 1.5, 0.5 as shown in Table-1. A Water sample was taken as blank to find absorbance in Colorimeter. Urine sample(3 ml) was diluted to 50 ml. These were treated with Crown ether catenated copper II neocuproinecompound as the main redox probe in identifying creatine absorption as green colour to yellow colourand the absorbance values were noted. Standard creatine solution was prepared and the absorption values were noted as shown in the Table-1. Unknown creatine level in urine sample was calculated from the following equation

Urinary Creatine/ mg/24 hrs = Creatine/100* Volume of Urine mL/24 hrs

5/100*3=0.15 mg

III. Results And Discussion

Crown ether catenated copper complexes Cu (II)/Cu(I) were used to determine the concentration of creatine in urine samples of humans. Previous research showed that the Picrate derivative of creatine consisted of some other interfering elements. Hence accurate results could not be derived. But in this novel CUPRAC method elimination of interfering radicals was achieved by suppression of their reactivity with the crown ether.It has the capacity to dissolve both hydrophilic and hydrophobic ions. Copper II complex of neocuproine showed green colour , after the addition of creatine the coordination point changed and the end point became deep yellow attributed to donor -acceptor Pi-Pi interaction of N- ligands with metal Cu/I. The cavity in Crown ether could not be tailor made for radius of Cu atom.

Table-1 Estimation of creatine by CUPRAC method

Sl.No	Volume of standard creatine ml	Volume of Water ml	Volme of Crown ether ml	Volume of Buffer ml	Incubation time mts	Absorbance value %
1	0.5	2.5	2ml	2ml	10mts	30
2	1.0	2	2ml	2ml	10mts	44
3	1.5	1.5	2ml	2ml	10mts	53
4	2	1	2ml	2ml	10mts	68
5	2.5	0.5	2ml	2ml	10mts	80
6	1 Urine sample		2ml	2ml	10mts	63

This method provided a good sensitivity and is comparable to standard Jaffe's method with comparatively less interference from foreign substances. ''mechanically chelating'' ligands inherently place metal ions in an unusually satirically shielded and conformational restricted environment, and this can have significant consequences for their properties as shown in figure -3. Secondly, endotopic coordination within the binding pocket of catenane was shown to alter the redox chemistry of the bound metal ion. Sauvage and co-workers found that catenanehas capable of binding a range of metal cations (Li^{+}, Ag^{+} , Zn^{2+}, $Cd^{2+,}$ $Co^{2+,}$ $Ni^{2+,}$ Fe^{2+}, and Pd^{2+}) other than Cu (I) .During the colorchangefrom green to yellow Ligand –Metal electronic charge moved as L(•)-M-L(•), may give rise to corresponding ligand-to-ligand interaction phenomena (charge transfer, electron hopping, and spin-spin coupling) and to redox-induced electron transfer with counterintuitive oxidation-state changes. But the results showed that the values were correlated as shown in Table-2.

It is perhaps surprising that, although the catenane effect and the modification of redox behavior of metal ions were two of the first concrete examples of the effect of mechanical bonding on the chemistry of metal complexes, both remain largely unused as a method for generating metal complexes with tailored properties.

The following schemes 1-3 explained the reaction mechanism. Quantity of creatine in the working solution showed the different absorbance for various concentrations in the spectrophotometer. Urine sample showed within the range of working solutions. From the graph the concentration was calculated. The quantity obtained from this method was accurate and reliable.

Statistical tools to find the accuracy

Pearson correlation table -2 explained the properties of all the absorption and creatine values with the urine sample.T-test showed the accuracy of the results. High positive correlation of 0.29 & 0.988 at 95% with covariance 11.7 denoted the urine (unknown) creatine has absorbance value within the absorbance range of standard working solution of creatine.

Pearson correlation Table-2

Pearson Product Moment Correlation - Ungrouped Data		
Statistic	Variable X	Variable Y
Mean	1.41666666666667	56.3333333333333
Biased Variance	0.451388888888889	266.222222222222
Biased Standard Deviation	0.671854812358212	16.3163176673606
Covariance	11.7333333333333	
Correlation	0.891954248205158	
Determination	0.795582380891229	
T-Test	3.94560323621119	
p-value (2 sided)	0.0168801699088157	
p-value (1 sided)	0.00844008495440783	
95% CI of Correlation	[0.291147574530602, 0.988189400582655]	
Degrees of Freedom	4	
Number of Observations	6	

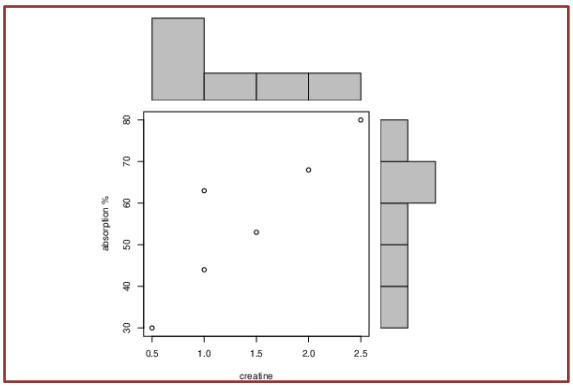


Figure-1 Pearson correlation plot

Box-Cox linear plot explained the correlation among the working solution and unknown sample of creatine. High correlation at 0.9 with0.4 Lamda max confirmed the accuracy of the results.

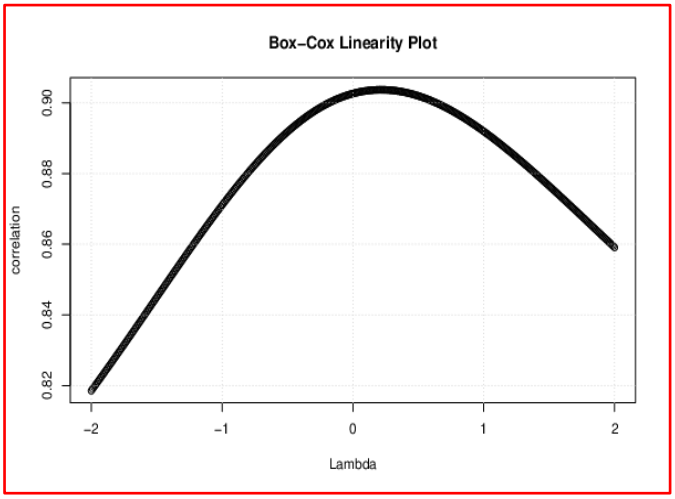


Figure- - 2 Box-Cox lambda max correlation

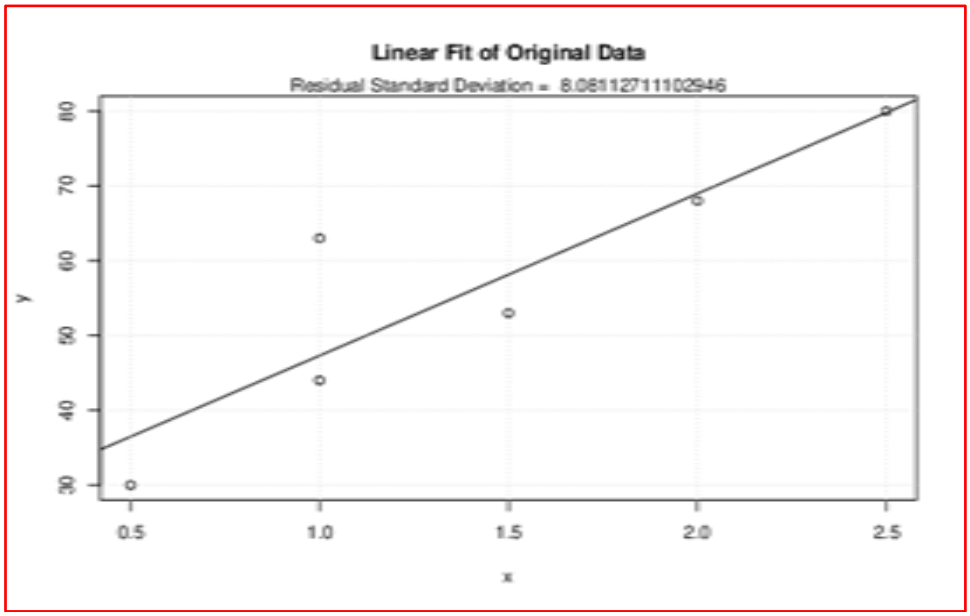
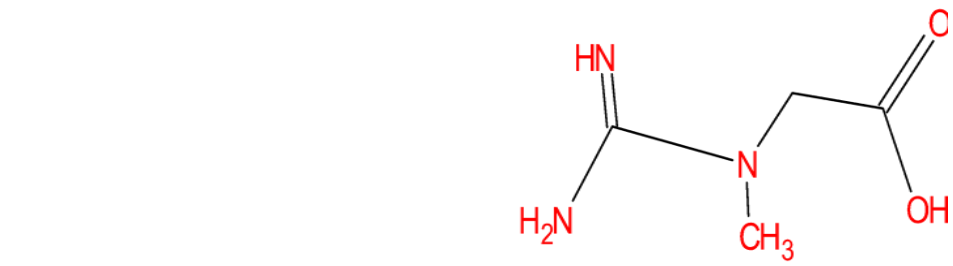
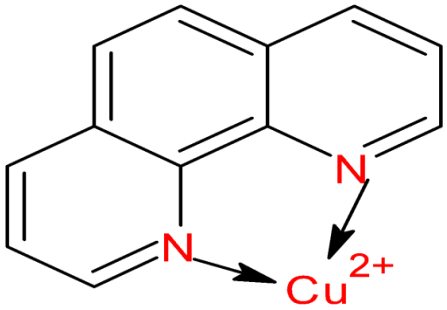


Figure- 3 Box-Cox Linearity plot

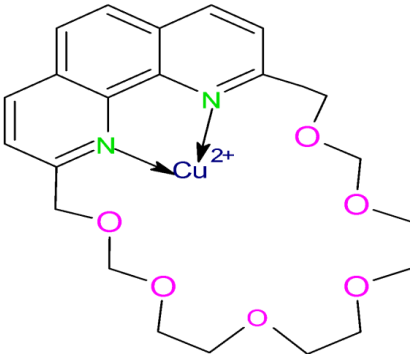
Box-Cox Linearity plot explained the unknown sample was highly matched with standard 1 ml solution.

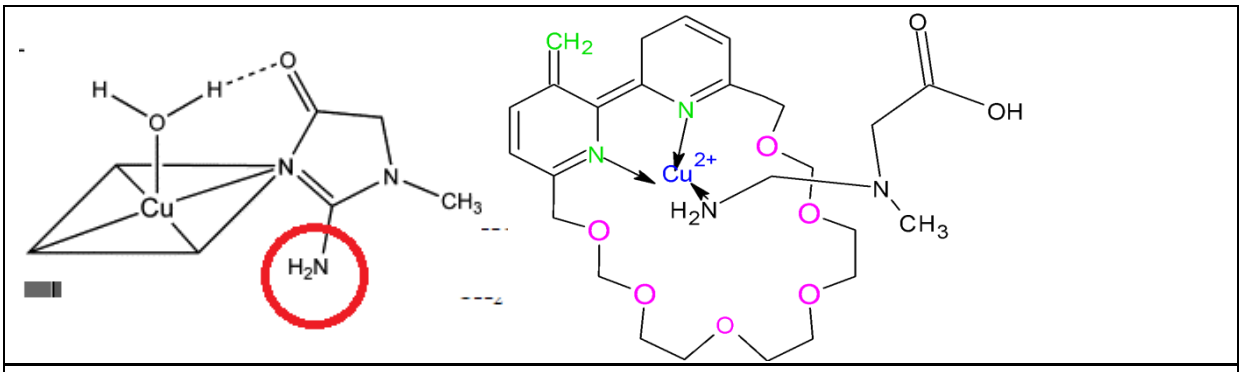




Scheme-I - Crown ether + Creatine + Copper II Neocuproine

Scheme -2 Creatine react with Chromogenic copper crown ether complex





Scheme-3 CopperII / Copper I crown ether catenated complex with creatine

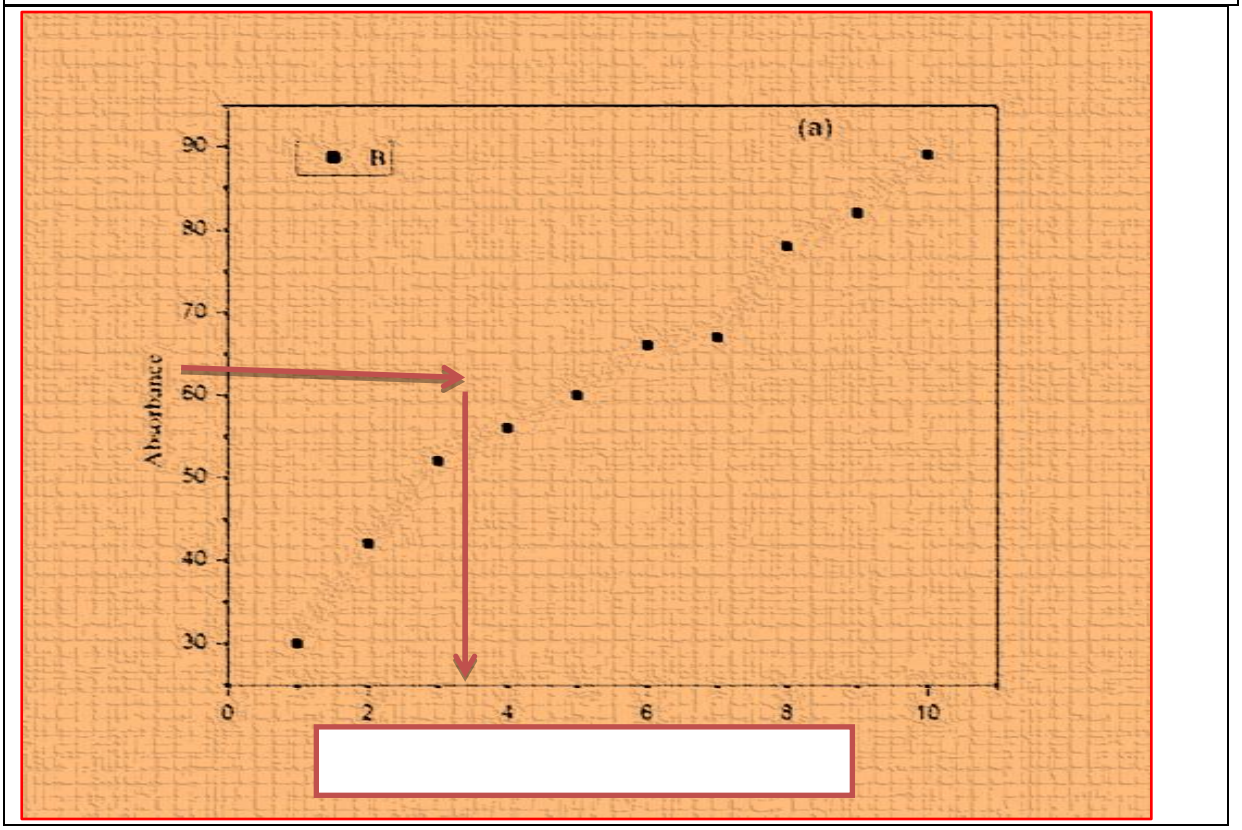


Figure-4 Determination of creatine

Scheme 1-3 explained the reactivity and colour change for the detection of creatine in the urine sample. Figure 3 explained the correlation between absorbance % with the concentration of working solution and the unknown urine sample .Each detection of creatine reflected the colour change and absorbance in colorimeter

In Picrate method, false quantity may be possible because of interfering ions along with creatine. But in this CUPRAC method Crown Ether Functions as Secondary Coordination Spheres for the Manipulation of Ligand–Metal Intermolecular Electron Transfer in Copper– creatineComplexes as previously reported for guanidine. In this work crown ether function centers were attached as secondary coordination spheres to a redox-active Cu-ligand and the effect of metal encapsulation into the crown ether functions on the electronic structure of copper complexes. The use of secondary coordination sphere modifies to extensively change the electronic structure of a coordination compound opens up the possibility for a sophisticated control of the properties and chemical reactivity of creatine and changed the colour from green to yellow.

IV. Conclusion

This was the comparative study between picrate and CUPRAC methods to identify the accurate quantity of creatine in the urine sample. A Cu (II) -neocuproine proved to be a suitable reagent for the determination of creatine in pure form. The high molar absorptivity of the proposed method is decisive

advantages since the interference from associated ions were not observed. The proposed method is simple, time saving and reproducible. Thus, the CUPRAC method can be used as an alternative for rapid and routine in the determination of creatine.

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