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Studies on the Photometric Estimation of Citrate in Urine

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Abstract

A simple spectrophotometric method for the determination of citrate in urine is described. This method is based on the complexation of Cu(II) with the Citrate at an elevated pH, which results in the formation of blue colored bis-citrate-Cu(II) complex, measured at 760 nm. The method is applied to the determination of low level of citrate in the urine sample. The relative standard deviation (RSD) was more than 1.5% in the range investigated. The urinary citrate level in 100 stone formers had a median value of 0.78 mM while in the control group the median value of citrate has been 1.3 mM.

Key words: Urine, Citrate, Spectrophotometer

Introduction

Several methods are available for the determination of citrate. These include enzymatic (Moellering and Gruber, 1966; Welshman and McCambridge, 1973; Nielsen, 1976; Dagley, 1974), spectrophotometric (Millan *et al*., 1987; Taussky and Shorr, 1947; Top and Yucel, 1988) and gas chromatographic (GC) methods (Horii *et al*., 1965). Most of these methods are combresome and not amenable to adaption for rapid analysis as needed in routine clinical/pathological laboratories. Spectroscopic method (Millan *et al*., 1987; Taussky and Shorr, 1947; Top and Yucel, 1988) reported till date luck sensitivity and are not suitable where small amount citrate is to be determined in the sample. Enzymatic methods are selective and sensitive but they are expensive and from very short shelf life (Moellering and Gruber, 1966; Welshman and McCambridge, 1973; Nielsen, 1976; Dagley, 1974). It was reported that Cu(II) formed bis-complexes with siderophore at specific pH (Hughes and Poole, 1989; Farkas *et al*., 1993; Shanmukhappa and Ramappa, 1997). Citrate also belong to the class of siderophore. That is the reason, it forms stable bis-complex with Cu(II) at pH 6.5. In the present study a simple and rapid method is described for the determination of citrate in urine. The copper reacts with citrate and forms a blue complex of bis-citrate-Cu(II), with maximum absorption at 760 nm. Although Fe(II) also forms complex with citrate but this complexation takes place at a higher pH and th chances of interference with Cu-citrate at the specified pH level are minimized. The detection limit of this method was found to be 0.34 mM which was quite adequate as normal levels of citrate in urine are 0.70 mM to 3.00 mM and in case of hypocitraturea it is not less than 0.50 mM.

Materials and Methods

Equipment: An LKB Nova Spec-II NS-123 single beam visible spectrophotometer was used. All absorbance were taken three times and mean was considered as final absorbance using standared quartz cuvette with an optical path length of 1 cm.

Copper Chloride Solutions: Stock solution of copper chloride (Merck) 10 mM was prepared in distilled water. Working $CuCl_2$ solution (2 mM) was prepared by taking 20 ml of stock in 100 ml volumetric flack. The pH of stock solution was adjusted to 6.5 with 1 M succinic acid/NaOH and volume was made upto the mark with water (Sawyer *et al*., 1984).

Standard Citrate Solutions: A stock solution of citrate (5 mM) was prepared by dissolving 0.147 g of Citrate trisodium salt (MW 294) in 100 ml of water and was stored in plastic bottle. Working citrate solution (3.33 mM) was prepared by taking 66.6 ml stock standard citrate solution in 100 ml volumetric flask and volume was made up to the mark.

Calibration Curve: A 1.0, 2.0, 4.0, 6.0, 8.0 and 10 ml of working citrate solution was taken in the test tubes. Another tube was marked 'blank' and 10 ml of water was added. To each test tube, 10 ml of working copper chloride solution was added and pH was adjusted to 6.5. The total volume made up to 20 ml with water and incubated at 25°C. The standards of Citrate corresponding to 0, 5, 10, 20, 30, 40 and 50 mg citrate per 100 ml. The absorbance was recorded at 760 nm. Molar absorptivity constant (e) was 8.0×10^3.

Procedures: An aqueous solution of copper chloride (pH 6.5) was prepared. By adding 1 mg sodium potassium tartrate, in order to prevent the precipitation of cupric ions. This solution of copper chloride is then mixed with urine sample, forming a blue colored complex which was fully developed within 10 min at 25°C.

Results and Discussion

Various experimental parameters were studied in order to obtain an optimized system with maximum possible precision including reagent concentration, temperature, pH, incubation time and the presence of diverse ions. These parameters were optimized by univariate approach and optimizing one each time.

Effect of Cu(II) Concentration on Absorbance: The effect of Cu(II) concentration was noted with the increment in concentration of Cu(II) covering the range 0.25-16 mM. The results are shown in Fig. 1. The absorbance was increased gradually with increase in Cu(II) concentration upto 0.008 mM. The final concentration of 0.2 mM of Cu(II) was found to be sufficient for the complex formation in specified range.

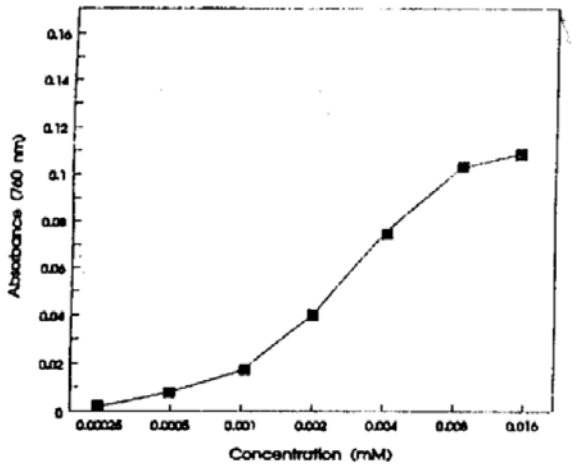

Fig. 1: Effect of concentration of Cu^{++}

Effect of pH on Absorbance: The effect of pH (2-10) of reation mixture on absorbance are shown in Fig. 2. The absorbance increases with pH up to 6.5 and decreases thereafter. Above pH 6.5, less bis-citrate-Cu(II) complex formation took place which caused a decreased in absorbance and therefore, pH 6.5 was selected for subsequent studies. The effect of incubation time of the reaction mixture was also observed. The color fully developed after 10 min at 25°C with maximum absorbance. To observe the stability of the colored complex, the absorbance was recorded after various times intervals and found that the color was stable to upto 30 minutes after which little decrease in absorbance was observed. The effect of temperature was observed by incubating the reactant mixture at different temperatures using a water bath maintained at variable temperature (25-60°C). It was observed that at 60°C the absorbance was maximum within a minute. Further increase in temperature resulted in constant absorbance.

Effect of Diverse Ions and Organic Matter: Possible interferences was recorded by measuring a known amount of citrate (1.7 mM) in the presence of different anions and cations. Possible interferences of organic matter present in urine in large amount are also recorded. The results obtained are summarized in Table 1.

Application of Established Method to Normal and Pathological Urine Sample: The proposed method was applied for the determination of Citrate in urine sample. First morning voided urine (spot urine) of 100 stone formers, (70 male age in between 25-65 years and female age in between 20-60 years) and 42 normal control, (25 male age in between 25-60 years and 17 females age in between 20-55 years) was used. Using the described method, the morning urinary citrate level in stone formers had a median value 0.78 mM in the range of 0.42-1.78. While in control group the median value of citrate is 1.3 mM in the range of 0.75-1.88. This method was also applied to investigate urinary citrate in population based data in 200 children in 24 hrs urine samples. The 24 hrs urinary citrate had the median value 1.4 per 24 hrs in the range of 0.67-1.185. The $FeCl_3$ method (Top and Yucel, 1988), at concentration of citrate less that 1.5 mM was unable to accurately detect the ferric citrate produced, owing to the large reagent and urine blank absorbance. This method therefore lacks the sensitivity to accurately identify low-citrate excreters. At a concentration of citrate amount greater than 1.5 mM the two methods correlated well.

Table 1: Effect of diversed ions and organic matter present in urine on the absorbance

Anions/ cations	Concentration (ppm)	Citrate (mg/dl)	Error (%)
None	---	50	---
CH_3COO^-	1000	49.8	-0.4
NO_3^-	1000	49.7	-0.6
NO_2^-	100	50.1	+0.2
SO_4^{--}	1000	47.5	-5.0
Cl^-	1000	45.8	-8.4
Co_2^{--}	1000	48.7	-2.6
PO_4^{---}	1000	48.3	-3.4
Ca^{++}	1000	50.1	-0.2
NH_4^+	1000	47.5	-5.0
	Organic matters		
None	–	50.0	--
Urea	16.2 g/L	47.5	-5.0
Creatinine	1.09 g/L	49.7	-0.6
Uric Acid	0.40 g/L	48.5	-3.0
Glucose	0.06 g/L	49.2	-0.6
Oxalates	0.01 g/L	50.2	+0.4

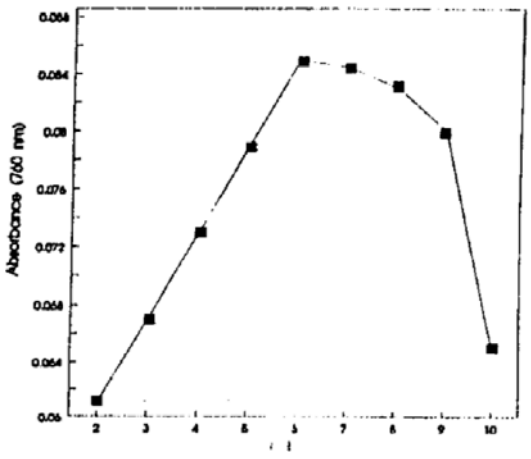

Fig. 2: Effect of pH

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