Measurement and analysis of creatinine, creatinine clearance and its effect on kidneys using interference filters

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Abstract : *This paper deals with the introduction about renal system of human body and concentrated upon effects of renal system under non clearance of creatinine .It is also discussed in this paper that various methods used for the measurement of clearance. This paper can give clear idea about glomerular filtration rate an creatinine clearance. Further discussed about analytical methodologies used in measuring the same such as End point and kinetic reaction methods. The chief functions of the kidneys are to filter the blood, which regulates the concentration of water and sodium, to reabsorb water, glucose and amino acids, and to excrete the waste as urine. The typical adult produces 1 – 2 liters of urine per day dependent upon such factors as hydration and activity levels, environmental factors, weight, and the individual's state of health. Thin tubes, called ureters, transport small amounts of urine from the kidneys to the bladder about every 10 – 15 seconds. A healthy bladder can hold up to 2 cups of urine comfortably for two to five hours [1].*

Keywords*: Creatinine, glomerular filtration rate (GFR), End point and kinetic reaction methods.*

1. **Introduction**: Creatinine is the breakdown product of 'creatinine phosphate' in muscle produced at constant rate by the body and filtered out of blood by kidneys. Creatinine is an anhydride of creatine [2]. Creatine is present in tissues (muscle, brain, blood etc.) as the high energy compound, phosphocreatine and as free creatine. Three amino acids glycine, arginine and methionine are required for creatine formation. The poor filtration of urine results in rise in creatinine level, hence the calculation of creatinine clearance is required in urine and blood.

Fig:1 explains chemical structure of creatinine The urinary system, also known as the renal system, consists of two kidneys, two ureters, the bladder and the urethra. The urinary system works in conjunction with the lungs, skin and intestines to excrete waste and keep body chemicals and water in balance [1].

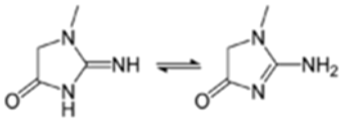


Fig:1 explains chemical structure of creatinine

The urinary system is regulated by blood pressure, the nervous system, and hormones produced by the endocrine system. The primary function of this organ system is to produce, store and eliminate urine. However, the urinary system has other important functions: Table 1.indicates various 1stages of chronic kidney disease

Table 1. stages of chronic kidney diseases Creatinine is an important clinical analyte for the diagnosis of renal and muscular dysfunction. It is a dehydrogenated form of creatinine (i.e. metabolic byproduct of amino acid) that provides energy to muscles tissue. The normal clinical range of creatinine in the human blood is ranging from 44 to 106 µM. However, it can exceed up to 1000µM during nephrons malfunction. Therefore, precise monitoring of creatinine in the blood is compulsory during routine checkup. Fig 2.depicts different stages of chronic kidney disease.

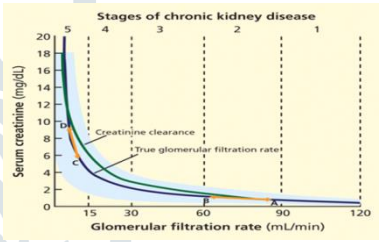


Fig 2: Depicts different stages of chronic kidney disease.

Table 1: stages of chronic kidney disease

S.no	GFR(mL/min)	Stage of Chronic kidney disease	Condition of Renal system/Precautions
1	Above or equal to 90-120	1 st	Normal kidney function
2	Between 60-89	2 nd	Normal kidney function, but urine abnormalities exists
3	Between 30-59	3 rd	Middle stage of kidney functioning.
4	Between 15-29	4 th	Severe malfunction of kidneys
5	Below or equal to14	5 th	Very severe or end stage

Regulation of electrolyte balance such as sodium, potassium, calcium and magnesium which affect and regulate hydration of the body as well as blood pH. These electrolytes are essential for nerve and muscle function [20].Regulation of acid-based homeostasis which keeps the tightly controlled and highly sensitive body pH in balance. A normal body pH measures between 7.38 and 7.42 leaving little room for deviation [1, 2].Control of blood volume and maintenance of blood pressure. Blood volume is regulated by the kidneys and generally equates to between 4.7 and 5 liters of blood circulating in an average adult [1].There are three processes involved in kidney functioning: Glomerular filtration, Tubular reabsorption and tubular secretion.

a) Glomerular filtration: Involves the ultra-filtration of plasma in glomerulus. The filtration Collects in the urinary space of Bowman's capsule and then flows downstream through the tubule men, where tubular activity alters its composition and volume. b) Tubular reabsorption: This involves the transport of substances out of tubular urine. These substances are then returned to the capillary blood, which surrounds the kidney tubules. Reabsorbed substances include many important ions (e.g., Na+, K+, Ca2+, Mg2+, Cl-, HCO3-, and phosphate).c) Tubular secretion: This involves the transport of substances into the tubular urine. Many organic anions and cations are taken up by the tubular epithelium from the blood surrounding the tubules and added to the tubular urine. Some substances (e.g., H+, ammonia) are produced in the tubular cells and secreted into the tubular urine. The terms reabsorption and secretion indicates, the "movement out of" and "into tubular urine", respectively. Tubular transport (reabsorption, secretion) may be either active or passive. d) Excretion: refers to elimination via the urine. In general, the amount excreted is expressed by the following equation:

Excreted=Filtered-Reabsorbed + Secreted

The chief functions of the kidneys are to filter the blood, which regulates the concentration of water and sodium, to reabsorb water, glucose and amino acids, and to excrete the waste as urine. The typical adult produces 1 – 2 liters of urine per day dependent upon such factors as hydration and activity levels, environmental factors, weight, and the individual's state of health. Thin tubes, called ureters, transport small amounts of urine from the kidneys to the bladder about every 10 – 15 seconds. A healthy bladder can hold up to 2 cups of urine comfortably for two to five hours [1].

2.Inulin clearance equals the GFR:

Glomerular filtration (GFR) is the rate at which the plasma is filtered by the kidney glomeruli. If we had a substance that was cleared from the plasma only by glomerular filtration, it could be used to measure GFR.

The ideal substance to measure GFR is inulin, a fructose polymer. Inulin is suitable for measuring GFR for the following reasons

i) It is freely filtered by the glomeruli.

ii) It is not reabsorbed or secreted by the kidney tubules.

iii) It is not synthesized, destroyed, or stored in the kidneys.

iv) It is nontoxic.

v) Its concentration in plasma and urine can be determined by simple analysis.

$$GFR=\frac{Uin \times V}{Pin}= Cin$$

Where, Pin=plasma inulin,Uin=Urine inulin, V=Urine flow rate, Cin=inulin clearance.

The endogenous creatinine clearance is calculated from the formula

$$C_{creatine}=\frac{(U\ creatinine\ \ X\ \ V)}{P\ creatinine}$$

However, the above tests of the creatinine are based on the nature of sample of blood, it may be plasma or serum.

3. Difference between plasma and serum

Serum is thought to be derivative words for "whey" as in "curds and whey" are the products formed when milk is allowed to clot. The whey is the liquid component whilst the curds are the solid parts. If blood is allowed to clot the liquid component is therefore called serum. If blood is prevented from clotting then the liquid component is called plasma [7]. The process of separation of serum and plasma from the blood sample are shown in fig 3

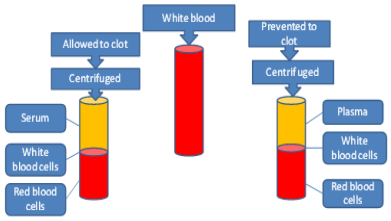


Fig 3:.Difference between plasma and serum

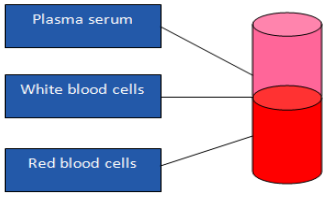


Fig 4 : Haemolysed sample

If some of the red blood cells have lysed (broken open) and their contents have now contaminated the plasma or serum sample it is called haemolysed sample. This will cause error in reporting amongst others elevated potassium, magnesium and phosphate. Some analytical methods may be able to negate the effect of the hemolysis sample [4].

Fig: 1.5 shows red blood cells and white blood cells in plasma serum which is called as haemolysed sample.

Serum creatinine is commonly measured by alkaline picrate, enzymatic, and high-performance liquid chromatography (HPLC) methods. These methods of measuring serum creatinine are standardized to the isotope dilution mass spectrometry (IDMS).

4.Crcl TEST:

In order to measure creatinine clearance (Crcl) the following methods are presently used, however these methods also suffer from some drawbacks.

1) Using concentration of serum creatinine along with sex, age, weight and race with24-hour urine collection [5].

2) Analytical: Isotope dilution mass spectrometry (IDMS) for 0.7 mg/dL [15, 17].

3) Urine Creatinine drug methods [6, 8].

5. Instrumentation:The above block diagram (fig.5) indicates different parts of the experiment. These are carried out on BS-300 chemistry analyzer, Mindray make colorimeter and RD/Hitachi 902 analyzer and the following procedural steps are implemented to accomplish the experimental investigation[9,10].(1) Collection and storing the blood sample of different patients in glass cuvettes.(2). Separation of serum and plasma from human blood samples by means of centrifuge.(3). The samples were analyzed for creatinine concentration by usual colorimetric method. The concentration of serum was measured by measuring the absorbance of the test solution and standard solution.

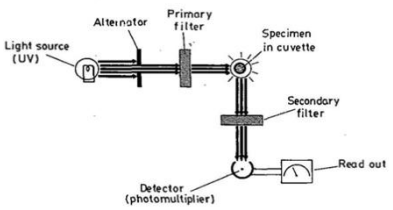


Fig.5 Instrumentation

(4). Monochromators and filters are used to obtain creatinine of creatinine was observed and calculated reaction times.(5). End-point and kinetic methods are used to analyze the concentration of creatinine.(6). These tests are also implemented using RD/Hitachi 902 analyzer and Mindray make colorimeters.(7).Concentration of creatinine was calculated using Beer's law. Obtained values of creatinine concentrations in End-point and kinetic methods are compared against colorimetric method.(8).Creatinine clearance rates are also calculated using the concentration values age, weight and gender of the patients.(9).Calculated creatinine clearance rates in End-point and kinetic methods are compared against the colorimetric method.(10).Obtained creatinine concentration was converted into creatinine clearance rate in mL per min. The renal diseases are estimated from the rate of Crcl.

6. Commercial optical interference filters

Fig 6 explains salient features of circular variable interference filters

Circular variable filters are narrow-pass interference filters of advanced design which are deposited on circular substrates, called segments. Film thickness, and therefore the wavelength of peak transmittance varies linearly and continuously with angular position on the segment.

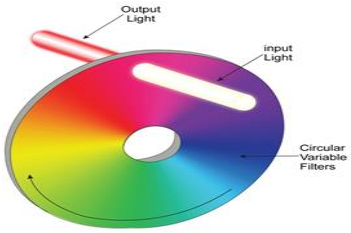


Fig.6. Circular variable interference filters

Circular Variable Filters are ideally suited as monochromators in compact, non-dispersive spectrometers, providing medium-resolution spectral radiation measurements, or when information is desired at a number of specific wavelengths in the relevant spectral range. A Circular Variable Filter can be manufactured in any wavelength range from 0.4µm in the visible region of the spectrum up to 14.3 µm in the infrared. The specific wavelength at which the radiation is transmitted by the segment is selected by appropriately positioning it on the optical beam. A Circular Variable Filter segment rotation, in a way that the beam traces a circumferential path on it, provides a continuous scan of its complete wavelength range. Circular Variable Filters are physically durable and can withstand the rigors of industrial and field environment conditions. They are resistant to abrasion and humidity, and can be cleaned by conventional optical cleaning techniques.

7 Results : Below tabulated(Table 2): results are obtained from different patients.

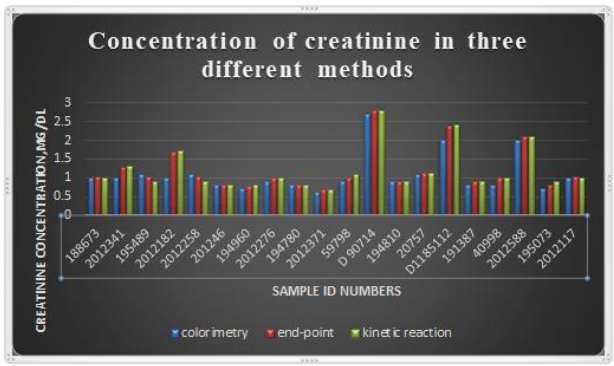
Creatinine concentrations comparison in three methods

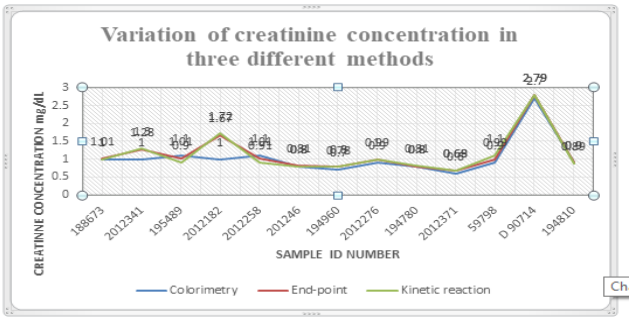
(RESULTS)Table.2

(End-point, Kinetic reaction methods with interference filters compared against Colorimetric method)

S.No	IPNo/Op No/sample ID (A.M.C/K.G.H VSKP)	Creatinine, Concentration mg/dL(Colorimetry)	Creatinine, Concentration mg/dL (Endpoint)	Creatinine, Concentration mg/dL (Kinetic reaction)
1	202114	0.8	0.98	1.01
2	76112	0.9	1.08	1.10
3	192295	1.0	1.18	1.30
4	40803	0.8	0.98	1.05
5	378461	0.9	0.98	1.04
6	201225	0.9	1.48	1.50
7	194330	0.8	0.86	0.88
8	201278	0.8	1.08	1.10
9	201256	0.8	0.78	0.81
10	58465	0.8	0.81	0.80
11	505711	0.8	0.81	0.80
12	201215	0.8	0.99	1.01
13	22916	0.7	0.87	0.91
14	1929382	0.8	0.82	0.80
15	194782	1.8	1.98	2.10
16	Op 15	0.9	0.99	1.20
17	201051175	0.9	0.98	1.08
18	195482	1.1	1.91	1.20
19	9892112	0.7	0.77	0.81
20	194829	1.0	1.08	1.10

8 Graphs: Below Graphs shows the concentration of creatinine Vs colorimetric, end-point and kinetic reaction methods.





9 Conclusions:

From the above discussions is observed that the usage of static optical interference filters yields accurate results compared to colorimetric methods. The light sources provides better monochromatic radiation to perform the experiment. The original blood samples taken to carry out the experiment are maintained at standard temperature and pressure to not alter the properties of sample. Hence the renal system functioning is also related with the

GFR ads Crcl rates, we can diagnose the condition of the patient who are under renal system disorders by testing creatinine clearance rate.

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