Absorption Studies of Creatinine using End-Point Method by Optical Interference Wavelength Filter

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Abstract- Accurate measurement of Creatinine is very essential in order to pre diagnosis the renal functioning of human body.In this paper we introduced the measurement of creatinine by means of studying its equivalent absorption with the interaction of wavelength of light using endpointmethod with the help of photometer.

Index Terms- absorption creatinine endpoint photometer

I. Introduction

Creatinine reacts with Picric Acid in an alkaline medium to form an Orange coloured complex[1-5]. The rate of formation of this complex is measured by reading the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of Creatinine. The reaction time and the concentration of Picric Acid and Sodium Hydroxide have been optimized to avoid interference from keto acids.

Creatinine + Picric Acid Orange coloured complex

Breakdown product of 'creatinine phosphate' in muscle produces at constant rate by the body and filtered out of blood by kidneys poor filteration of urine results rise in creatininelevel Calculation of creatinine clearance is required in the urine and blood.

II. REAGENTS

COMPOSITION (TABLE 1)

Reagent No.	Reagent	Composition	Concentration
1	Picrate Reagent	Picric Acid Preservative	40 mM/L qs
2	Sodium Hydroxide	Sodium Hydroxide	200 mM/L
3	Creatinine Standard	Creatinine Stabiliser	2 mg/dl qs

III. REAGENT STORAGE AND STABILITY

Prior to reconstitution

Unopened Reagents 1,2 are stable at Room Temperature (15 – 30^{0}c) and reagent 3 is stable at 2-8 C until the expiry date mentioned on the container label.

After reconstitution

The "Working Reagent" is stable for 7 days at 2-8^0 c.

Experiment Technical specifications and instrumentation

Sample volume**:** 70µl serum;1 1 precision µl
Reaction temperature: 37 ˚C
Cuvettes size: 5X6X25; 5mm opticl length;
Reaction volume: 180-500 µl
Photometric system: interference filters of with static –fibre optics
Lamp: tungsten-halogen lamp
Environment requirements: 15 ˚C-30 ˚C
Humidity: 35%-80%
Atm.pressure: 800hPa-1060 hPa

a)Light source : Tungsten halogen lamp

b)Interference filter[6-7]:it relies on optical interference to provide narrow band of radiation. some times these are also called as fabry perot filters. The interference filters are available for the ultraviolet, visible and well into the infrared region. An interference filter consists of a transparent dielectric(frequently calcium fluoride or magnesium fluoride)that occupies between two semi transparent materials. The thickness of the dielectrical layer is carefully controlled and determines wavelength of the transmitted radiation. when a perpendicular beam of collimated radiation strikes this array, a fraction passes through the first metallic layer and the remainder is reflected. The portion that is passed undergoes a similar partion when it strikes the second metallic film. if the reflected portion of this second interaction is of the proper wavelength, it is partially reflected from the inner side of the first layer in phase with the incoming light of the same wavelength. The result is that this particular wavelength is rein forced, and most other wavelengths, being out of phase, undergo destructive interference.

d)lens system and photometer: This system consists of 9 optical paths with interference filters

Wavelengths:340 nm,405nm,450nm,510nm,546nm,578nm,630nm,670nm,700nm
Half band width:≤12nm Measurement range:0.1-4.0 Abs
Lamp: 12 V 50 VA tungsten-halogen

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IV. METHODOLOGY

It is purely based [1-2]on end point reaction. the reaction reaches equilibrium after certain period. Because the equilibrium constant is very large, it can be considered that all substrates have changed into products, and the absorbance of the reacting liquid does not change any more. The absorbance change is proportional to the amount of the product present.

In this experiment the time when the reagent is added is t1=22 sec the time when the sample is added is t2=24 sec

The reaction starts when they are mixed. At t3= 49 sec the reaction reaches the equilibrium and the absorbance reading is taken. The reaction time is t3-t2= 49-24=25 sec

The end point reaction is not subject to such condition changes as enzyme concentration, pH value and temperature, as long as the changes are not significant enough to affect the reaction time.

Absorbance

when a[6-7] parallel monochromatic light beam whose intensity is Io goes through a flow cell(whose length is L) containing a solution (whose concentration is C),some photons are absorbed, and the intensity is attenuated from Io to I t,so the absorbance A of this solution is:

A=-Log It/I0

Where, It/I0= transmittivity

The analyzer measures the light intensity through the photo-electric conversion, linear amplification and A/D conversion.

V. CALCULATING THE RESPONSE OF THE END-POINT REACTION SINGLE –REAGENT AND SINGLE WAVELENGTH

For the analyzer, the response(R)is defined as the absorbance change before and after the reaction, or the absorbance change rate during the reaction process.

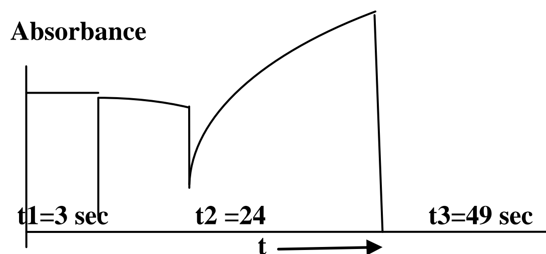


Fig 1:Endpoint reaction with single-regent and single-wavelength

As shown in the above figure 1, t1 is the time when the reagent(volume:V) is added.t2 is the time when the sample(volume:S)is added.[10]The reaction starts when they are mixed. At t3 the reaction reaches the equilibrium and the absorbance reading is taken.t3-t2 is the reaction time. So $R=R_s-R_{SMPB,}$ Both $R_{s,}$and R_{SMPB} are calculated with the formula R=At3-(K1 X A t2-1).

Where,

R_s=Response of reaction mixture,R_{SMPB} =response of sample blank. If the sample blank has not been requested,

R_{SMPB} =0

At3=Absorbance at t3=-63

A t2-1=Absorbance at the previous point before t2=-384.,K1=Single reagent test volume coefficient, If the start time of reaction is 0,K1=V/(V+S);otherwise,K1=1;If K1=1 since experiment was started at time t=3secThen ,Response of reaction mixture R=-63-1(-564)=501

Reaction time is=t3-t2=49-24=25 sec

VI. RESULTS OF EXPERIMENT (TABLE 2)[10]

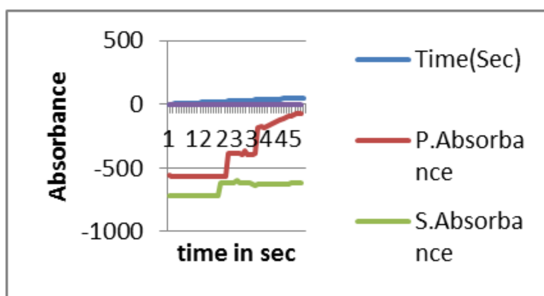
HUMAN SERUM(Female,age:27years):

S.NO	Time(Sec)	P.Wave length(nm)	P.Absorbance	S.wave length(nm)	S.Absorbance
1	1	26926	-561	14470	-716
2	2	26929	-562	14472	-717
3	3(*t1)	26933	-564	14469	-715
4	4	26939	-565	14471	-717
5	5	26935	-564	14476	-717
6	6	26936	-565	14476	-720
7	7	26938	-565	14475	-721
8	8	26938	-565	14476	-721
9	9	26940	-566	14478	-720
10	10	26940	-566	14479	-721
11	11	26941	-566	14476	-721
12	12	26942	-566	14478	-720
13	13	26942	-566	14479	-722
14	14	26943	-567	14476	-722

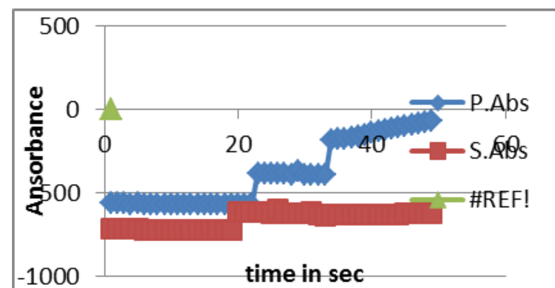
15	15	26940	-566	14478	-723
16	16	26944	-567	14479	-723
17	17	26947	-568	14477	-721
18	18	26946	-568	14480	-723
19	19	26948	-568	14480	-720
20	20	26945	-568	14481	-618
21	21	26948	-567	14481	-618
22	22	26945	-568	14478	-618
23	23	26948	-568	14481	-618
24	24(*t2)	26372	-384	14477	-618
25	25	26372	-385	14308	-619
26	26	26383	-387	14308	-602
27	27	26386	-387	14308	-621
28	28	26391	-391	14310	-621
29	29	26390	-370	14281	-622
30	30	26404	-391	14313	-620
31	31	26339	-393	14314	-618
32	32	26403	-392	14311	-626
33	33	26410	-390	14308	-634
34	34	26405	-185	14320	-630
35	35	26400	-178	14310	-630
36	36	25785	-179	14381	-629
37	37	25765	-170	14313	-629
38	38	25768	-159	14314	-627
39	39	25739	-149	14311	-629
40	40	25708	-140	14308	-629
41	41	25679	-129	14320	-627
42	42	25651	-122	14334	-629
43	43	25620	-113	14328	-627
44	44	25546	-104	14327	-626
45	45	25523	-97	14326	-624
46	46	25500	-89	14326	-622
47	47	25491	-86	14322	-621
48	48	25460	-75	14325	-621
49	49(*t3)	25441	-69	14322	-620

VII. GRAPHS

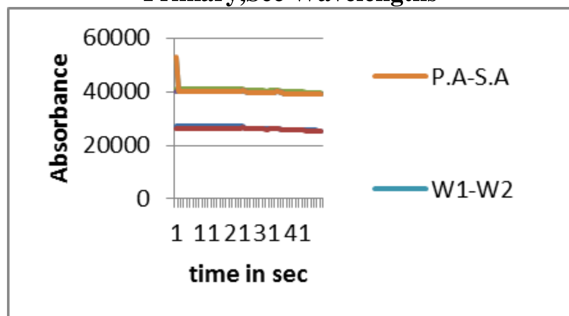
Graph-1: Response of Creatinine Absorbance with two different interference filters



Graph-2 :X-Y :Scattered chart



Grapgh:3 Chart Showing the variation of Absorpton Vs Primary,Sec Wavelengths



VIII. CONCLUSIONS

From the above observations it is concluded that by using optical spectrometry using tungsten halogen lamp as source and interference filters the creatinine absorption characteristics by UV light were observed. Moreover the reaction time for measuring creatinine was found to be 25 seconds and response of reaction mixture is at absorption of 501nm.

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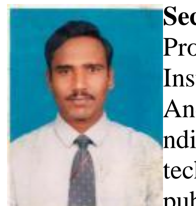

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