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RESEARCH ARTICLE

ABSORPTION STUDIES OF CREATININE USING SINGLE-REAGENT AND SINGLE WAVE LENGTH 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 endpoint method with the help of photometer.

Introduction:-

Creatinine reacts with Picric Acid in an alkaline medium to form an Orange colored complex. 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 ketoacids. These measurements are based on the reaction between Creatinine and Picric Acid which yields orange colored complex consequently measured by a photometer. However these measurements plays major role in renal system function which deals with the correct identification of (GFR) Glomerular filtration rate [7].Glomerular filtration rate (GFR) can be used to asses renal function. There are two types, actual and estimated GFR. In actual GFR, the equation UV/P is used where U and P are the concentrations of creatinine in the urine and plasma respectively, with V being the amount of urine produced in 1 minute. Since creatinine levels vary during the day, for V and U to be accurate, urine needs to be collected over 24 hours. This makes the test time consuming for the patient. To avoid the 24 hour collection of urine, an estimated GFR (eGFR) can be used in which only the plasma creatinine is used. Estimated GFR can be successfully used to identify patients which would need an actual GFR [7].

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

Working reagent preparation:-
Prepare Working Reagent by mixing equal volume of Reagent 1 (Picrate Reagent) with Reagent2(Sodium Hydroxide) to make up the desired volume. Mix gently for 2 minutes. Reagent 3 is ready to use.

Reagent storage and stability:-
Prior to reconstitution:-
Unopened Reagents 1, 2 are stable at Room Temperature (15 – 30 ºC) and reagent 3 is stable at 2-8 ºC until the expiry date mentioned on the container label.

After reconstitution, The "Working Reagent" is stable for 7 days at 2-8°C.

Equipment and specifications:-

a. **Light source:-**
b. **Inner Reaction bath window:-**
c. **Outer Reaction bath window:-**
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

Experiment lay out:-

The optical setup is shown in the following fig 1.1consists of the above discussed components such light source (LS-1), optical lens system, cuvette for serum sample, primary and secondary interference filters and the photo detector [10].

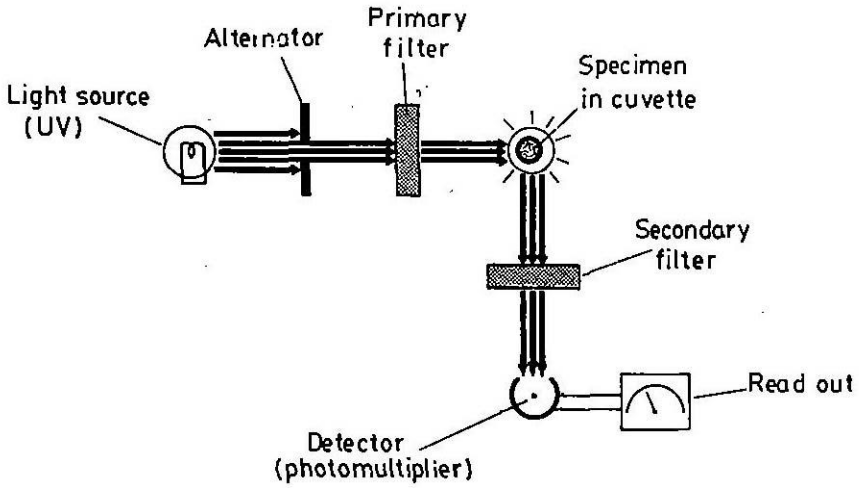


Fig 1.1:-Optical setup.

Methodology:-

It is purely based 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 reaction 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, the time when the sample is added is t2, are measured. The reaction starts when they are mixed. At time t3 the reaction reaches the equilibrium and the absorbance reading is taken. The reaction time is t3-t2.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 [10].

As shown in the above figure, when a 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

Calculating the response of the end-point reaction:-
Single –reagent and single wavelength:-

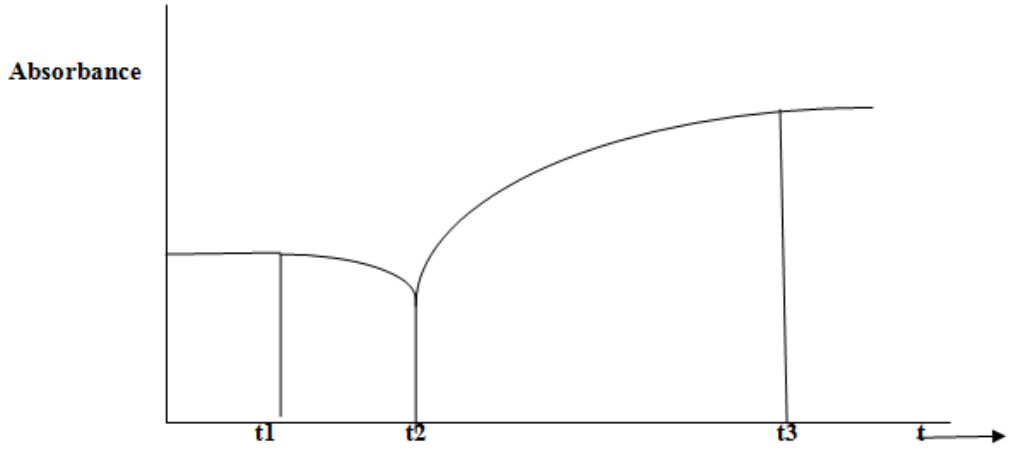


Fig 1.2:- Endpoint reaction with single-regent and single-wavelength.

As shown in the above figure, t1 is the time when the reagent (volume) is added.t2 is the time when the sample (volume: S) is added. 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-K1XA 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
A t2-1=Absorbance at the previous point before t2.
K1=Single reagent test volume coefficient, If the start time of reaction is 0, K1=V/ (V+S); otherwise, K1=1

Results of experiment:-

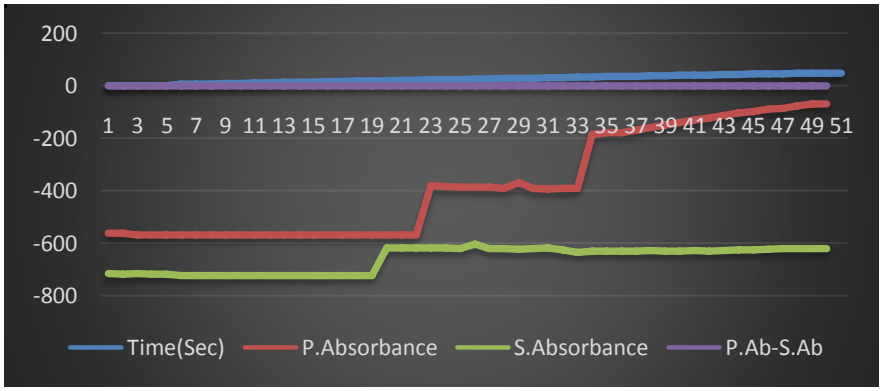
Table-2:-Bs-300 Mindray model auto analyzer.

Sample id: 40803 data type: reaction data sample type: human serum

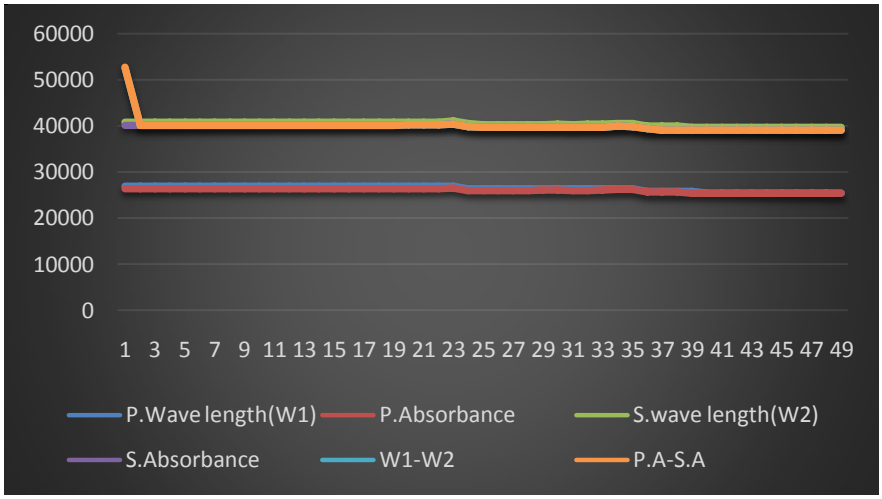
S.NO	Time(Sec)	Primary wave length	Primary Absorbance	Secondary wave length	Secondary absorbance
1	1	26926	-561	14470	-716
2	2	26929	-562	14472	-717
3	3	26933	-564	14469	-715
4	4	26939	-565	14471	-717
5	5	26935	-564	14476	-717
6	6	26936	-565	14476	-720
7	7	26938	-565	14475	-721
8	8	26938	-565	14476	-721
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	-381	14481	-618
24	24	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	25441	-69	14322	-620

In this experiment the time when the reagent is added is t1=20 sec
The time when the sample is added is t2=22 sec
The reaction starts when they are mixed. At t3= 47 sec the reaction reaches the equilibrium and the absorbance reading is taken. The reaction time is t3-t2= 47-22=25 sec

Graphs:-



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



Grapgh:2:- Chart showing the variation of AbsorptionVs. Primary, Sec Wavelengths

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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