# Absorption studies of Creatinine using Kinetic Reaction 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 studing its equivalent absorption with the interaction of wavelength of light using kinetic reaction method with the help of photometer.

**Keywords:** absorption creatinine kinetic reaction photometer



## 1.1 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.

## 1.2.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 |

## 1.3.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

**1.4 METHODOLOGY**

It is purely based on kinetic reaction. The reaction velocity is not related to the substrate concentration and remains constant in the reaction process. As a result, for a given wavelength ,the absorbance of the reacting liquid changes evenly, and the change rate(ΔA/min)is proportional to the activity or concentration of the subject, which is usually the enzyme.

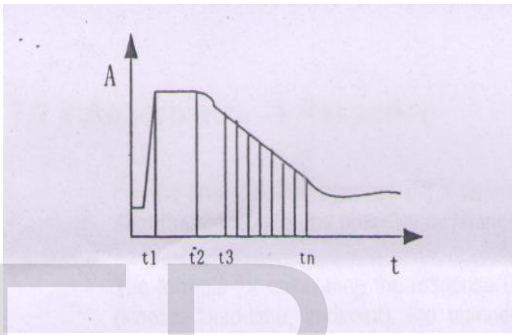



Fig.1.1.kinetic reaction method

In fact, it is impossible for the substrate concentration to be large enough, and the reaction will no longer a zeroth order reaction when the substrate is consumed to ascertain degree.therefore,the theory only stands within certain period. In addition the reaction can become steady only after a certain period. In addition, the reaction can become steady only after certain period because the reaction is complicated at the beginning and there are miscellaneous reactions due to the complex serum compositions. All reagent.

As shown in the above figure1.1,t1 is the time when the reagent is added.t2 is the time when the sample is added. From t3 the reaction becomes steady.tn is the time to stop testing the reaction.t3-t2 is the time delay, and tn-t3 is the monitoring time.

**c) Absorbance and Response**

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

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.

### 1.5 CALCULATING THE RESPONSE OF THE KINETIC REACTION

**single wavelength (for single –reagent )**

As shown in the above figure1.1,

t1- is the time when the regent(volume) is added

t2- is the time when the sample(volume:S)is added.

t3 -is the time from which the reaction becomes steady

tn- is the time when the test result on the reaction is stopped (t3-t2) -is the time delay

(tn-t3)- is the reaction n time

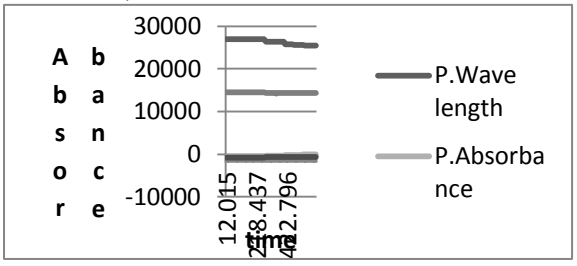
The response is equal to the slope of the linear section between t3 and tn.

### 1.6 EXPERIMENTAL RESULTS[5](TABLE 2)

| S.NO | Time(Sec) X12 | P.Wave length (nm) | P.Absorbance | S.wave length (nm) | S.Absorbance |
|---|---|---|---|---|---|
| 1 | 12.0150 | 26926 | -561 | 14470 | -716 |
| 2 | 24.0310 | 26929 | -562 | 14472 | -717 |
| 3 | 36.0460 | 26933 | -564 | 14469 | -715 |
| 4 | 48.0460 | 26939 | -565 | 14471 | -717 |
| 5 | 60.0780 | 26935 | -564 | 14472 | -717 |
| 6 | 73.3120 | 26936 | -565 | 1447 | -720 |
| | | | | 6 | |
| 7 | 85.3280 | 26938 | -565 | 14476 | -720 |
| 8 | 97.3590 | 26938 | -565 | 14475 | -719 |
| 9 | 110.1710 | 26940 | -566 | 14476 | -720 |
| 10 | 122.2500 | 26940 | -566 | 14478 | -721 |
| 11 | 134.2810 | 26941 | -566 | 14479 | -721 |
| 12 | 146.2960 | 26942 | -566 | 14476 | -720 |
| 13 | 158.2960 | 26942 | -566 | 14478 | -721 |
| 14 | 170.3210 | 26943 | -567 | 14479 | -721 |
| 15 | 182.3280 | 26940 | -566 | 14477 | -720 |
| 16 | 194.3430 | 26944 | -567 | 14480 | -722 |
| 17 | 206.3590 | 26947 | -568 | 14480 | -722 |
| 18 | 218.4370 | 26946 | -568 | 14481 | -723 |
| 19 | 230.3900 | 26947 | -568 | 14481 | -723 |
| 20 | 242.4060 | 26948 | -568 | 14478 | -721 |
| 21 | 254.4210 | 26945 | -567 | 14481 | -723 |
| 22 | 266.4370 | 26948 | -568 | 14477 | -720 |
| 23 | 278.5150 | 26372 | -381 | 14307 | -618 |
| 24 | 290.4530 | 26383 | -384 | 14308 | -618 |
| 25 | 302.6400 | 26386 | -385 | 14308 | -618 |
| 26 | 314.6870 | 26391 | -387 | 14308 | -618 |
| 27 | 326.5310 | 26390 | -387 | 14308 | -618 |
| 28 | 338.6870 | 26404 | -391 | 14310 | -619 |
| 29 | 350.7180 | 26339 | -370 | 14281 | -602 |
| 30 | 362.6090 | 26403 | -391 | 14312 | -621 |

| | | | | | |
|---|---|---|---|---|---|
| 31 | 374.7340 | 26410 | -393 | 14313 | -621 |
| 32 | 386.7650 | 26405 | -392 | 14314 | -622 |
| 33 | 398.6870 | 26400 | -390 | 14311 | -620 |
| 34 | 410.7810 | 25785 | -185 | 14308 | -618 |
| 35 | 422.7960 | 25765 | -178 | 14320 | -626 |
| 36 | 435.0780 | 25768 | -179 | 14334 | -634 |
| 37 | 446.8120 | 25739 | -170 | 14328 | -630 |
| 38 | 458.8280 | 25708 | -159 | 14327 | -630 |
| 39 | 470.8750 | 25679 | -149 | 14326 | -629 |
| 40 | 482.8590 | 25651 | -140 | 14326 | -629 |
| 41 | 494.9840 | 25620 | -129 | 14322 | -627 |
| 42 | 507.1560 | 25597 | -122 | 14325 | -629 |
| 43 | 518.9530 | 25571 | -113 | 14322 | -627 |
| 44 | 531.1090 | 25546 | -104 | 14321 | -626 |
| 45 | 543.1250 | 25523 | -97 | 14317 | -624 |
| 46 | 555.1400 | 25500 | -89 | 14314 | -622 |
| 47 | 567.1710 | 25491 | -86 | 14313 | -621 |
| 48 | 579.3120 | 25460 | -75 | 14312 | -621 |
| 49 | 591.3280 | 25441 | -69 | 14311 | -620 |

**1.7 GRAPH(ABSORPTION VS TI ME IN SECONDS)**




**1.8RESULTS AND CONCLUSION (**TABLE:04)

(Primary wave length filter)

| s.no | Time(sec) | Primarywave length(nm) | Primary absorption |
|---|---|---|---|
| 1 | T1=278.515 | 26383 | -384 |
| 2 | T2=410.781 | 25785 | -185 |
| 3 | T3=543.125 | 25523 | -97 |
| 4 | Tn=591.328 | 25441 | -69 |
| 5 | Tn-t3=48.203 | | An-A3=28 |

(secondary wave length filter) **(**TABLE:05)

| S.no | Time(sec) | Secondary wave length(nm) | Secondary absorption |
|---|---|---|---|
| 1 | T1=278.515 | 14307 | -618 |
| 2 | T2=350.718 | 14281 | -602 |
| 3 | T3=422.76 | 14320 | -626 |
| 4 | Tn=398.687 | 14311 | -620 |
| 5 | Tn-t3=24.073 | | An-A3=6 |

Calculations

Primary wave length filter:

Tn-T3=48.203 sec

Absorbance:An-A3=28

Response or the Slope of the linearization portion is=[(391-(-381)]/(362.609-278.515)

=-10/84.094=0.11 Abs per sec

secondary wave length filter:

Tn-T3=24.073sec

Absorbance :An-A3=6

Slope of the linearization portion is=[(-621-(-629)]/(567.171-507.156)=8/60.015=0.133 Abs per sec

(TABLE:06)(comparision of two filters)

| S.no | Response of Primary wave length filter | Response of secondary wave length filter |
|---|---|---|
| 1 | 0.11 Abs per sec | 0.133 Abs per sec |

Hence it is concluded that by using the kinetic eaction method the response of the secondary wavelength filter to the creatinine of the human serum is much better than primary wave length filter.

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