

Measurement of Total Alkaline Phosphatase Activity in Human Serum using Charcoal Activated Para-Nitro Phenyl Phosphate

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Abstract: A one step Charcoal treated Alkaline phosphatase (ALP) reagent has been developed without affecting its basic ingredients. Conventionally, ALP measurement is based on the detection of phenol produced by an enzymatic reaction in 37 °C at pH10 using substrate para-nitrophenyl phosphate (pNPP). In present study, measurement range of ALP activity was noted from 6.41 to 628 U/L in serum samples. Experimental results are compared to these obtained by spectrophotometric measurement in bovine serum. Excellent Correlation exists and close agreements are noted between the both charcoal treated and without charcoal treated reagents having similar substrate (pNPP) properties. Use of charcoal has great advantages in delaying photo degradation of pNPP improving its shelf life & stability.

Keywords: ALP, Charcoal, pNPP, Spectrophotometry, Bovine Serum.

1. Introduction

Although introduced more than 50 years ago, the measurement of alkaline phosphatase (ALP, EC 3.1.3.1) activity in serum as an aid in the diagnosis of hepato-biliary and bone disease enjoys continued popularity¹. Also, alkaline phosphatase comprises a group of enzymes that catalyse the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate². In healthy adults, this enzyme is mainly derived from the liver, bones and in lesser amounts from intestine, placenta, kidneys and leucocytes³. Several approaches in the determination of alkaline phosphatase activity are commercially employed⁴. The level of alkaline phosphatase activity can be measured and upon enzymatic induced hydrolysis of the substrate para nitro phenyl phosphate (pNPP) in the presence of magnesium ions. These phosphatases hydrolyse pNPP & ultimately produce end product yellow chromogenic substance para nitrophenoxide, measured at 405nm⁴. Considering sensitivity, linearity & reaction kinetics, pNPP is the ideal choice & Expert panel on Enzymes of the IFCC have preferred to recommend AMP buffer.

The main objectives of this study is to evaluate the use of Charcoal as a monocomposite package as a new innovative approach for enhanced shelf life of Alkaline phosphatase reagents without affecting enzyme kinetics & sensitivity..

2. Methods & Result

The principle of method and reagent preparations follows as per guide line of IFCC. The basic properties of the reagent constituents remain same as conventional method excepting addition of Charcoal represented as follows.

2 -Amino-2 methyl-1-propanol 1 mol/L,
4-Nitrophenyl phosphate 1 mmol/L
Magnesium acetate 2.0 mmol/L

Zinc sulfate 1.0 mmol/L
N-(2-Hydroxyethyl) ethylenediaminetri acetic acid (HEDTA)
2 mmol/L
Phenol 1.5 mol/L
Charcoal 20 mg/ml
pH : 10.2±0.05
Temperature : 37±0.1°C

In the present study, reagent grade charcoal, preferably activated charcoal or active charcoal was used during preparation of conventional reagents for specifically recycling pNPP and improving stability. The activator charcoal has been treated with oxygen to open up millions of thing pores between the carbon atoms. Background absorbance of Charcoal treated reagent measured photometrically at 405 nm, over a period of 45 days, represented in the following Table 1.

Table 1:

Days	Batch-1	Batch-2	Batch-3	Batch-4
0	0.065	0.056	0.067	0.075
10	0.097	0.089	0.102	0.110
30	0.115	0.121	0.118	0.135
45	0.131	0.132	0.136	0.143

Comparative studies for serum ALP measurement were undertaken in autoanalyser by using two separate alkaline based (pH10) pNPP reagents having charcoal in one segment and without charcoal in others. In both cases, sample/reagent ratio is 1:50, measurement wavelength 405 nm and it follows Rate principles and type of reaction is positive.

Result shows excellent correlation & harmonization studies reveals close agreement represented in the following table (Table 2) and (Fig.1 & 2).

Regression analysis shows positive correlation (R^2 0.982).

Harmonization studies reveal that there is no appreciable

change in ALP activity between two sets (p NPP with charcoal & without charcoal) of reagents. Moreover, distribution of majority values are symmetrical on both sides along the regression line (difference is ± 5 U/L) against irrespective of difference concentration of ALP activity, excepting in one occasion.

Precision Check: Harmonization studies of ALP activity were under taken in 2 different throughput auto analyzers (100 tests / hour & 400 tests / hour) for precision checking of Charcoal based pNPP reagents represented in the following Table 3 & Fig. 3 & 4.

Table 2

pNPP Without Charcoal	pNPP with Charcoal	Average	Difference
104	103	103.5	-1
148	151	149.5	-3
48	40	44	-8
75	78	76.5	3
48	40	44	-8
46	41	43.5	-5
76	80	78	4
55	59	57	4
77	78	77.5	1
52	57	54.5	5
72	69	70.5	-3
100	94	97	-6
152	147	149.5	-5
102	105	103.5	3
47	39	43	-8
46	40	43	-6
61	60	60.5	-1
59	58	58.5	-1
60	60	60	0
102	100	101	-2
78	80	79	2
61	60	60.5	-1
75	78	76.5	3
65	61	63	-4
45	39	42	-6

Table 3

AUTOQUANT-100	AUTOQUANT-400	Average	Difference
57.91	56.8	57.355	1.11
71.27	72.51	71.89	-1.24
72.99	75.61	74.3	-2.62
51.59	50.2	50.895	1.39
58.28	61.3	59.79	-3.02
81.29	76.42	78.855	4.87
65.33	63.24	64.285	2.09
80.55	79.2	79.875	1.35
77.21	76.41	76.81	0.8
53.45	54.02	53.735	-0.57
113.95	114.7	114.325	-0.75
104.3	95.67	99.985	8.63
61.25	58.93	60.09	2.32
96.68	97.13	96.905	-0.45
68.3	68.25	68.275	0.05
77.58	79.3	78.44	-1.72
66.44	65.12	65.78	1.32
90.94	88.2	89.57	2.74
79.06	81.2	80.13	-2.14
44.54	48.6	46.57	-4.06

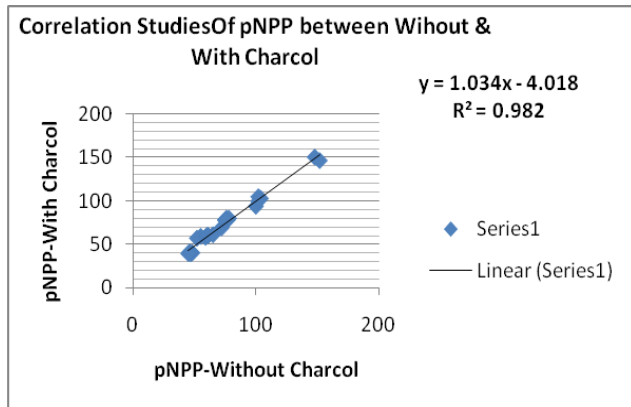


Figure 1

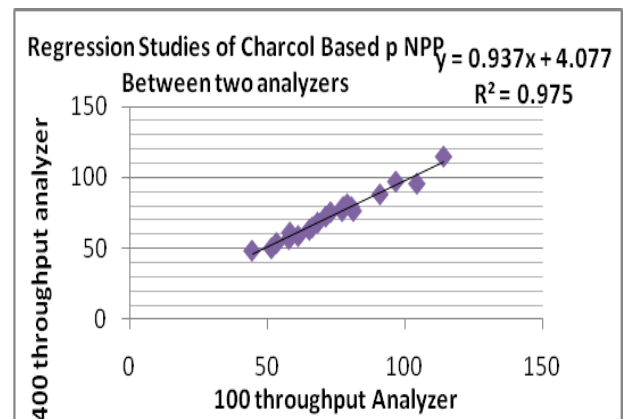


Figure 3

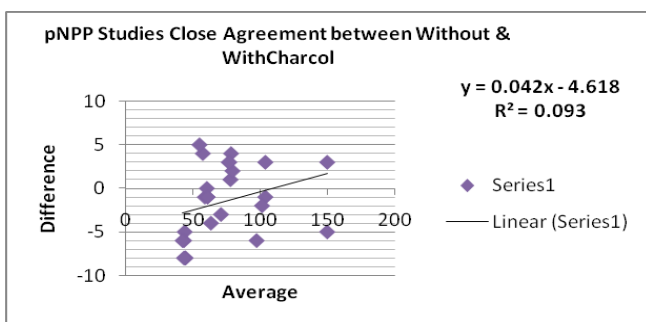


Figure 2

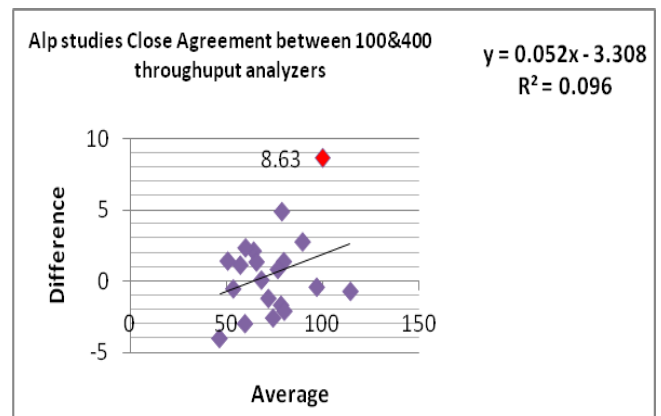


Figure 4

Table 3 & Fig. 3 & 4 demonstrate that ALP activity is strongly precise between the two different throughput analyzers by using Charcoal based pNPP as it is evident by

both regression analysis (R^2 0.975) and close agreement studies. Close agreement studies reveals that in majority cases, the difference of ALP activity (Y axis) is within ± 5 U/L against irrespective of different conc. of ALP activity (X-axis), excepting in one occasion, the difference of ALP activity is 8.63. In majority cases, the data points are symmetrically distributed along the close proximity of the regression line.

3. Sensitivity & Linearity Check

Linearity check was carried out by selecting high reacting serum samples having ALP activity 628 U/L. Sample was serially diluted up to different concentration. Correlation studies were conducted by measuring individual samples having respective concentration and its corresponding to observed response.

Table 5

Serial dilutions concentration of ALP	Observed Response
628	628
314	348.3
157	167.7
78.5	83.64
39.25	40
19.6	17.83
9.81	8.9
4.9	6.41

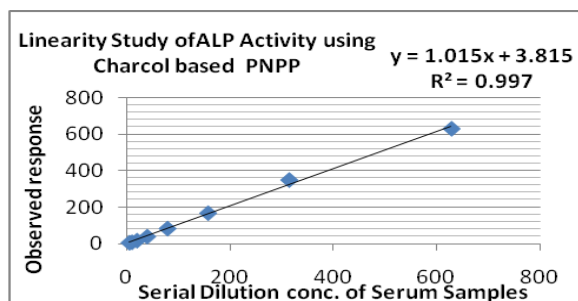


Figure 5

Result shows (Table 5 & Fig. 5) excellent correlation (R^2 0.997) and minimum activity 6.41 U/L was noted.

4. Summary & Conclusion

The present study reveals that there is no appreciable changes in background absorbance of charcoal treated p NPP over a period of 45 days (Table-1). It is plausible to note that, Alkaline Phosphatase activity remains consistent both in Charcoal treated p NPP reagents & p NPP reagents having without charcoal as it is reflected in Table 2, Fig. 1 & Fig. 2 Precision (Table 3, Fig. 3 & 4) and Linearity (Table 5, Fig. 5) studies reveals that Charcoal based pNPP reagents maintains persistent ALP activity without compromise statistical rules.

In conclusion, Charcoal based p NPP reagents can be alternative choice for ALP measurement having slower rate of photo degradation, improving stability, minimizes reagent wastage and savings operating cost.

5. Acknowledgement

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