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Effect of Temperature on Determination of Boron by Azomethine-H Method

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Abstract: This study, aimed to evaluate the effect of sample solution temperature during and after colour development on precision and accuracy of B determination by azomethine-H method, revealed that value of absorbance of a given standard was highly dependent on temperature of solution during colour development. Absorbance values were unstable with respect to temperature even after the colour development period of one hour. Concentrations of B determined in the extracts obtained by hot 0.02*M* $CaCl_2$ and hot water for ten medium Typic Haplusterts were higher when colour was developed at 30 and 40°C as compared to concentrations of the same solutions determined at 15°C. The magnitude of increase depended upon the non-clarity of the extracts. This study indicates that temperature of the sample solutions should be maintained constant between 15 and 20°C throughout the period of colour development and absorbance measurement for higher sensitivity and accuracy of B determination. (***Key words:*** *Boron determination, azomethine-H, temperature, hot water soluble, hot calcium chloride soluble*)

Azomethine-H is being used extensively throughout the world in the colorimetric determination of boron due to its simplicity in the procedure of colour development. Buffer masking reagent has been

introduced in the procedure to eliminate the ionic interferences and to improve the sensitivity of determination. Katyal (unpublished) indicated that azomethine-H method was highly precise and observed minor day to day variation in the absorbances of same standard during 3 day analysis using the method recommended by Wolf (1974). However, analysis of B in standard samples using azomethine-H in our laboratory showed a large variation in slope (absorbance/ concentration ratio) of the standard curve during different seasons of the year. This might be due to the variation in atmospheric temperature which, in turn, might have influenced the process of colour development with B by azomethine-H. Ferran *et al.* (1988) also observed that colour developed at 17°C showed higher absorbance than at 22°C. But temperature did not influence the kinetics of colour development. However, none of the widely used methods of B determination by azomethine-H has given emphasis on temperature for colour development as a factor controlling determination of B (Wolf 1974; Parker & Gardner 1981; Gupta 1979; Offiah & Axley 1993).

A series of laboratory experiments were conducted to evaluate (i) the effect of temperature of the sample solution on sensitivity and accuracy of colorimetric B determination in the azomethine-H procedure and (ii) the effect of temperature on the determination of available B content in soils.

Materials and Methods

In the first experiment, the effect of temperature on the absorbance of 0.5 and 1.0 mg B L^{-1} (H_3BO_3 in aqueous solution) was studied using azomethine-H method recommended by Gupta (1979). For this purpose, 5 mL of aliquot containing 0.5 and 1.0 mg B L^{-1} was taken in triplicate in 30 mL corning glass tubes along with distilled water as blank. Two mL buffer masking reagent was added and mixed. After that 2 mL of 0.45% azomethine-H (2% in 1% ascorbic acid) was added to each tube and mixed. These solutions were kept for one hour in temperature controlled water bath for colour development maintaining the temperatures in the range between 10 to 40° (± 0.5°C). Absorbances of the solutions were measured at 420 nm using Gilford UV-VIS spectrophotometer (model 250) attached with a rapid sampler. Before aspirating sample solutions, distilled water at temperature as that of sample solution was aspirated several times to minimize temperature change within flow cell.

In the second experiment to study the effect of changes in temperature after colour development period on the absorbance values, standard solutions containing 0 and 1.0 mg B L^{-1} were taken in triplicate and colour was developed in 100 mL conical flask at 15°C and 40°C as per above procedure (but ratio of sample : buffer masking reagent : azomethine-H was 20:8:8). After 1 h, the absorbances of the solutions were measured at different temperatures while increasing the temperature from 15 to 40°C and decreasing the temperature from 40 to 15°C (as the case may be).

In the third experiment, the effect of temperature of sample solution on the determination value of available B in soils was studied. The available B content in soils was extracted by two methods, *viz.* hot water soluble (HWS) method of Gupta (1979) and hot 0.02*M* $CaCl_2$ soluble (HCS) method of Parker and Gardner (1981). Ten Typic Haplusterts of Bhopal district (Madhya Pradesh) were used in the experiment. The pH (1:2 in 0.01*M* $CaCl_2$), organic carbon and $CaCO_3$ content in the soils ranged between 6.3 and 7.4, 4.9 and 12.7 g kg^{-1} and 23 to 31 g kg^{-1}, respectively. Each soil was extracted five times and after extraction, replicated extracts were combined together to get pooled extracts for each soil. This was done to eliminate the probable source of variation during extraction as it is not within the purview of our study. Colour in the extracts was developed at 15, 25, 30 and 40°C and absorbances were measured at 420 nm after allowing 1 h for colour development as described in experiment I. Absorbances of the extracts (without developing colour) were also, measured at 420 nm with respect to distilled water after mixing 5 mL extract, 2 mL buffer masking reagent and 2 mL distilled water (in place of azomethine-H) as an indication of nonclarity of the extracts.

Results and Discussion

Results (Fig. 1) showed that absorbance of 0.5 and 1.0 mg B L^{-1} solutions varied considerably among the treated temperature range of 10 to 40°C. The absorbance values were maximum at 15°C and decreased below or above this temperature. The values at 25°C and 40°C were about 77 and 41 per cent of the maximum value at 15°C. This shows

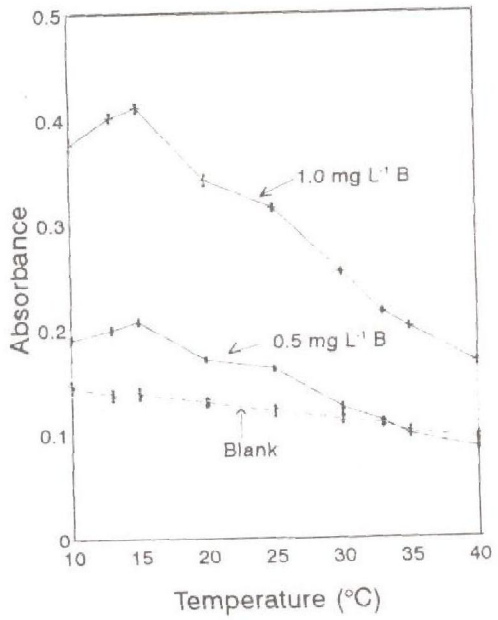

Fig. 1. Changes in the absorbance values (at 420 nm) of B standards with solution temperature during colour development period. Bars represent standard errors of values averaged across replications

that magnitude of colour development by azomethine-H is highly sensitive to temperature during colour development and therefore, slope of the standard curve is changed with changes in temperature (Fig. 2). A gradual decrease in the absorbance values of blank solution (with respect to distilled water) with increase in temperature was also recorded.

Limit of detection (LOD): The LOD was calculated from the results of 20 replicate analyses of blank by following the method of Greenberg *et al.* (1992). The values were 0.02 mg L^{-1} at 15 and 20°C, 0.04 at 25°C and 0.08 at 40°C temperature.

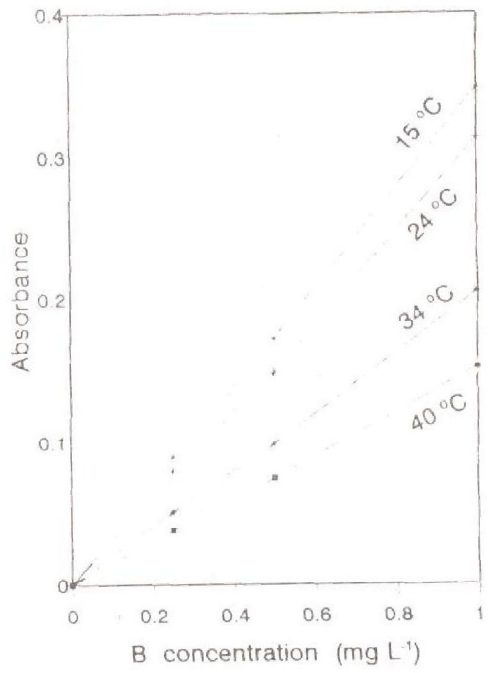

Fig. 2. Changes in slope of B standard curve with temperature of colour development

Precision is measured as inverse of relative standard deviation (RSD) calculated from 20 replicate analysis of 1.0 mg L^{-1} B standard solution. The RSD values were 0.39, 0.48, 0.85 and 1.29% at 15, 20, 25 and 40°C temperature, respectively.

Thus colour development for B analysis at temperature between 15 and 20°C gave higher sensitivity (*i.e.* low LOD) as well as precision of determination.

Changes in Temperature of Sample Solution

In several laboratories, a variation in air temperatures between the sample processing (laboratory) room and the instrument room is observed. The air temperature in the instrument room is generally kept low to maintain the sensitivity and longivity of various electronic components. Depending upon the temperature differences, there may occur sharp changes in sample temperature during spectrophotometric determination when samples are brought into the instrument room.

Results (Fig. 3) show that absorbance values were unstable with respect to temperature even af-

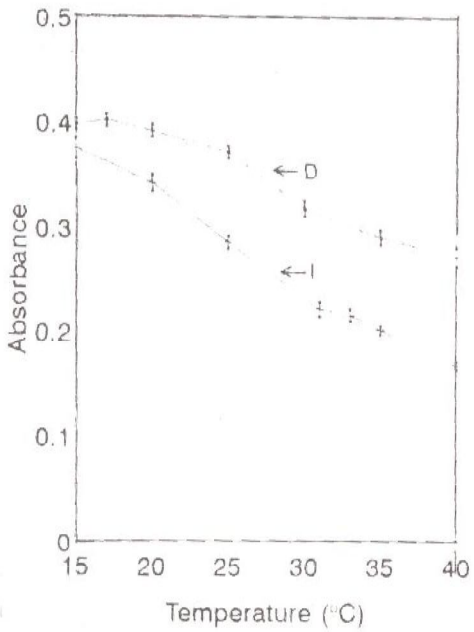

Fig. 3. Changes in the absorbance values (at 420 nm) of 1.0 mg B L^{-1} with changes in solution temperature after colour development period. Bars represent standard errors of values averaged across replications.

ter the colour development period of 1 h. Absorbance value of same B standard decreased at high temperature and increased at low temperature of sample solutions. As slope of the standard curve changes with temperature, there could be a tremendous error in determination if temperature of the sample solution changes during B determination. Sixteen to 28 per cent error in determination of B was observed in this laboratory in a batch of 50 samples within 10 minutes after preparation of standard curve when samples after colour development were brought from laboratory (room air temperature 30°C) to instrument room (room air temperature 22°C) for absorbance measurement.

Sample Solution

Non-clarity of the soil extracts is a major problem in the determination of available B by azomethine-H method (Wolf 1974; Parker & Gardner 1981). Although the uses of charcoal (Gupta 1979) or $CaCl_2$ (Parker & Gardner 1981) have been recommended for removing colours in the hot water extracts significant amount of absorbance (a measure of non-clarity) at 420 nm (Table 1) for the extracts alone were obtained by both the procedures in this laboratory. Such a non-clarity in the extracts is supposed to introduce significant amount of error in the boron determination.

As temperature was increased to 30°C, the determination value of B was increased by 11.6 and 3.7 per cent in HCS and HWS methods, respectively (Table 1). Further, increase in temperature to 40°C caused a sharp increase in the determination value of B by 41.9 and 59.2 per cent in HCS and HWS methods, respectively. Since the amount of B present in the extracts should be same, the increase in determination value of B at 30 and 40°C from the value at 15°C may be termed as errors of determination. Significant correlation coefficients were found for error at 30°C (r = +0.711* and +0.722*) and error at 40°C (r = +0.884** and +0.865**) with non-clarity (absorbance values of extracts alone with respect to distilled water) of the extracts of HCS and HWS, respectively. Results of the first experiment in this study has indicated (Fig. 2) that increase in temperature of colour development decreases slope of the standard curve and hence, increases the multiplication factor (MF) (*i.e.* dilution/ slope of standard curve) for calculation of concentration from the absorbance of unknown samples. Such a change may appreciably modify the determined value of HWS or HCS-B. This is because absorbance due to non-clarity of extract (which is error in determination) is not supposed to change with temperature, though it has to be multiplied by higher MF at higher temperature, and thus magnifying error in determination. The amount of B determined did not vary significantly between 15 and 25°C temperature during colour development in both methods of extraction.

Keeping in view the results of the above three experiments, it is inferred that temperature of the sample solutions should be maintained constant between 15 and 20°C throughout the period of colour development and absorbance measurement for higher sensitivity and accuracy as well as lower degree of error in determination of B due to non-clarity, if any, in the extracts.

Table 1. Effect of temperature during colour development on value of HCS and HWS B content

Soil	Absorbance values of extracts alone (without azomethine-H)	Determination values of B (mg kg^{-1}) at temperature			
		15°C	25°C	30°C	40°C
	Hot 0.02M $CaCl_2$ soluble (HCS)				
1. Kachi Barkheda	0.025	0.35^a	0.34^a	0.38^b	0.58^c
2. Pipaliyamira	0.034	0.34^a	0.38^b	0.42^c	0.70^d
3. Bilkisganj	0.012	0.35^a	0.34^a	0.37^a	0.43^b
4. Samry I	0.014	0.60^a	0.62^a	0.64^a	0.64^a
5. Dupadiya	0.017	0.29^a	0.28^a	0.34^b	0.44^c
6. Bhandeli	0.032	0.64^a	0.62^a	0.74^b	0.94^c
7. Samry II	0.021	0.49^a	0.48^a	0.53^b	0.61^c
8. Sarwer	0.025	0.50^a	0.53^a	0.58^b	0.69^c
9. Nabibagh	0.018	0.20^a	0.22^a	0.29^b	0.30^b
10. Ratanpur	0.021	0.43^a	0.46^a	0.54^b	0.73^a
Mean	0.022	0.42^a	0.43^a	0.48^b	0.61^a
	Hot water soluble (HWS)				
1. Kachi Barkheda	0.027	0.36^a	0.39^a	0.39^a	0.57^b
2. Pipaliyamira	0.015	0.15^a	0.14^a	0.16^a	0.24^b
3. Bilkisganj	0.024	0.38^a	0.38^a	0.43^b	0.68^c
4. Samry I	0.033	0.30^a	0.32^a	0.33^a	0.55^b
5. Dupadiya	0.025	0.35^a	0.36^a	0.38^a	0.63^b
6. Bhandeli	0.006	0.37^a	0.34^a	0.35^a	0.42^b
7. Samry II	0.007	0.16^a	0.14^a	0.15^a	0.22^b
8. Sarwer	0.012	0.23^a	0.25^a	0.24^a	0.37^b
9. Nabibagh	0.012	0.10^a	0.10^a	0.12^a	0.29^b
10. Ratanpur	0.004	0.26^a	0.27^a	0.27^a	0.27^a
Mean	0.017	0.27^a	0.27^a	0.28^a	0.43^b

Note: Values (a, b) followed by the same letter in the same row, do not differ significantly ($P < 0.05$)

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References

Ferran, J., Bonvalet, A. & Casassas, E. (1988) *Agrochimica*, **32**, 171.

Greenberg, A.E., Clesceri, L.S. & Eaton, A.D. (1992) In *Standard Methods for the Examination of Water and Waste Water*, 18th edn, APHA-AWWA-WFF, Washington, D.C., U.S.A. p. 1.

Gupta, U.C. (1979) *Can. J. Soil Sci.* **59**, 241.

Offiah, O. & Axley, J.H. (1993) *Boron and its Role in Crop Production*, (Ed. U.C. Gupta), CRC Press Inc., Boca Raton, Florida, p. 105.

Parker, D.R. & Gardner, E.H. (1981) *Commun. Soil Sci. Pl. Anal.* **12**, 1311.

Wolf, B. (1974) *Commun. Soil Sci. Pl. Anal.* **5**, 39.