STATISTICAL ANALYSIS OF SPECTROPHOTOMETRIC DETERMINATIONS OF BORON

ESTUDO ESTATÍSTICO DE DETERMINAÇÕES ESPECTRO-FOTOMÉTRICAS DE BORO

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INTRODUCTION

The red compound formed when curcumin reacts with boric acid in the presence of oxalic acid (rubrocurcumin) has been used in the determination of boron [1,2,3]. Trichloroacetic acid has been used instead of oxalic acid as the colour intensifier[4]. Poor precision generally reported in this method of analysis has made the application of curcumin for boron analysis of a not too widespread use, as observed by Spicer and Strickland[4].

During analytical determinations of boron content in graphites, in this laboratory, it was observed that the results were inconsistent and that a general lack of reproducibility existed among the various analysis made on the same sample, unless unusual controls of the various operations, not generally adopted in ordinary analytical procedures, were made. Small variations in the drying time of rubrocurcumin, in the atmospheric moisture condition, in the rate of evaporation, in the percentage of water in the alcohol used for analysis, in the temperature of drying seemed to give results completely scattered.

Chirsnside, Claley, and Proffitt[5] had also called attention to the fact that the colour produced by this reaction is influenced by such variables as temperature, time of heating, proportion and manner of addition of reagents. Spicer and Strickland[2] observed that the amount of water in the ethyl alcohol used should not be more than 25 nor less than 5% by volume and that a high reproducibility of the atmospheric moisture conditions should be kept.

Albinatti and Pasqués[3] observed that circumstances such as lack of simultaneity in drying sample and standard is very critical, being absolutely necessary that the evaporation be carried on simultaneously, as non compliance with these conditions will give inconsistent results.

The variations observed by the present authors, however, were not of an order of magnitude such that a qualitative examination of the data would permit to ascribe to one or to some of the mentioned factors the cause for the lack of reproducibility. A more rigorous examination would be necessary in order to find which of these factors were responsible for the lack of reproducibility in the analysis of boron. It was decided to make a statistical analysis of the data to check the level of significance of the differences found. A factorial design[6] was made in

which the factors to be studied were the ones which had not already been exhaustively examined in the mentioned papers[2], meaning volume of crucibles and temperature of drying. Consequently it was necessary, at least, two levels of temperature in order to study the influence of this factor. One level was set a 100ºC as it is usual[4]. The other level was set at 55ºC, as in the case where the method applied is the one in which oxalic acid is used as colour intensifier[7]. This choice of temperature values might seem unrealistic since it takes about one hour for drying at 100ºC and eight hours at 55ºC, what would not be practical; however it was chosen in this way so that the effect would be really detected and allow a definite interpretation of statistical analysis.

This paper describes the statistical aspects of the problem of ascertaining whether the results were actually different or whether they were due to experimental errors. The importance of design and statistical interpretation in the detection and correction of sources of errors in analytical procedures, by these methods, have been largely emphasized by Youden[8], Box[9], and Martin[10], as well as by Shewell[11].

Experimental

Preliminary experiments were made with boric acid and curcumin using trichloroacetic acid or oxalic acid as colour intensifier. It was observed that the method with oxalic acid was much more influenced by small variations on the various operations than with trichloroacetic acid. The chemical aspects of the various operations with oxalic acid had been exhaustively examined by Spicer and Strickland[2]. Trichloroacetic acid method seems to be less subjected to small variations of the operations procedures[4] but even so some factors seemed to affect the results with this last reagent. The present authors noticed that of special importance was the volume of quartz crucibles used for evaporation of the boric acid-curcumin mixtures; the results showed to be highly influenced by the volume of the crucibles, the readings in the spectrophotometer being lower for the experiments carried out with larger crucibles. For this reason the volume of crucibles was one of the factors included in the mentioned factorial design.

An interaction of the factors temperature, volume of crucibles and range of concentration of boron, might occur and for this reason concentration of boron

was also included as one of the factors to be studied and check if in the range of amounts of boron used (2.5 to 7.5 micrograms of boron) Beer's law was followed.

The chief virtue of the trichloroacetic acid curcumin method is its very high sensitivity and it is likely to be of most value in determining amounts of boron appreciably less than 1.0 microgram. Although the choise of the range 2.5 to 7.5 micrograms had been dictated by situation originated in actual analysis of samples of graphites, it was decided to examine the case where the amount of boron was less than 1.0 microgram .

In the course of preliminary experiments it was observed that some loss of boron would occur if the solutions were not made alkaline before evaporation. In order to verify the importance of the errors introduced by no alkalinization of the solutions the whole set of experiments was repeated with amounts of boron corresponding to 0.33μg, 0.66μg per crucible and alkalinizing the solutions, before drying with sodium hydroxide. The other factors, meaning temperature and volume of crucibles, were kept the same as in the experiments without alkalinization.

METHOD

The boric acid solutions were placed in quartz crucibles of 25,30 and 50 ml, 0.1 ml of sodium hydroxide were added for the set of experiments where the effect of alkalinizations would be investigated, and slowly evaporated on a hot place. After evaporation one milliliter of trichloroacetic acid solution and one milliliter of curcumin solution were added to the dried precipitate and the crucibles were placed in an oven at the temperatures of 55°C or 100°C until constant weight.

In order to determine the time for constant weight, testimony samples were run, in parallel with the actual experiments, for the drying operations. From time to time the testimony crucibles were removed and weighted until no change of weight was detected. Fifteen minutes after this time the crucibles with the samples for analysis were then removed from the oven. This procedure was better than to withdraw the crucibles, with samples for analysis, from the oven, periodically, cool and weigh; this would offer much greater chance of variable results than the practice of heating for a definite time.

After the crucibles had cooled the dry residues were dissolved with ethyl alcohol and the volume made to 25 ml. For the amounts of boron used in the experiments in the range of 2.5 to 7.5 μg, the 25 ml solutions were too concentrate for readings in a spectrophotometer and for this reason they were diluted ten times with alcohol. The solutions were placed in quartz cells one by two centimeters and taken to a Uvispek spectrophotometer and readings were made at 5,500 angstroms with a slit of 0.10 mm. Blanks with the same amounts of trichloroacetic acid and curcumin were run each experiment.

REAGENTS

Ethyl alcohol - 95 percent v/v.
Boric acid solution - 10 μg per ml in boron
Trichloroacetic acid solution - 163 g per l
Curcumin solution - 0.125 percent w/v in ethyl alcohol
Sodium hydroxide - 0.1 normal

All reagents were of analytical grade.

DESIGN OF EXPERIMENTS

A 2^3 factorial design was chosen, with the following factors: volume of crucibles (V), amount of boric acid (C), and temperature (T). In order not to make the size of the blocks too large a confounding of the interaction of the three factors (VCT) was made with a comparison between blocks (days of experiments) and in this way the size of the blocks was reduced by half. The loss in efficiency in the estimate of the interaction VCT is not important, since the interactions of high order are seldom significant[9]. The drawback of this design is that it would not be possible to check Beer's law in the interval of concentration to be used; the concentration factors should figure in the experiments in three levels, at least. In order to obtain the information concerning Beer's law in the interval of concentration of interest a preliminary complete factorial design 2 x 3 = 6 with seven repetitions (42 determinations) was made.

In this preliminary design the temperature was kept at 55°C, the capacity of crucibles at 25 and 30 ml and the amount of boron at 2.5, 5.0 and 7.6 micrograms covering the range of the actual analytical work mentioned in the Introduction. The distribution of the six combinations VC in each block was casualized using Fisher and

Yates' Tables[12]. This preliminary design showed that the main effect of V was highly significant amounting practically to certainty (level of significance less than 0.1%). This shows that the analytical results would depend on the crucibles size; the larger crucibles furnished consistently lower readings. The main effect of C was highly significant (level of significance much less than 0.1%), as it would be expected, and it being so this effect was subdivided in a "linear term" and a "quadratic term" using the coefficients of orthogonal polinomies for n' = 3, Fisher and Yates[12]. The analysis showed that only the first term was significant and that consequently, in the range of concentration of interest in this work, Beer's law was followed. This conclusion is important for the present work since it allows the use of the factors C at only two levels, provided the maximum limit of boron, in each crucibles, is 7.5 micrograms.

The magnitude of the sum of squares for the parcel "between blocks" - strongly suggested that there were significant differences in the readings with nominally identical samples if the chemical analysis were not made in the same day.

The interaction VC was not significant even at the 10% level and volumes of crucibles and amounts of boron can be considered practically independent.

The information the preliminary experiments gave, suggested a factorial design of four pairs of blocks (days) with four observations in each block (a total of 32 determinations). The efficiency of estimate of the main effects and of the interactions of the factors taken two by two is not diminished, rather improved, in relation to a design without confounding. The analysis of variance corresponding to the chosen design is given in Table I.

TABLE I

Analysis of Variance for the Designed Experiments

Source of Variation	Degrees of Freedom	
Between pairs of blocks (days)	3	
Interaction VCT	1	
Error (residual variance between blocks, by difference)	3	7
Between blocks		
Main effect of V	1	
Main effect of C	1	
Main Effect of T	1	

Source of Variation	Degrees of Freedom
Interaction VC	1
Interaction VT	1
Interaction CT	1
Error (within blocks, by difference)	18
Within blocks	24
Total	31

The blocks were randomized within each pair and by mere chance their sequence resulted the same on each occasion. The contents of each block (the order of analysis within each day), imposed by the adopted technique of confounding, was also distributed at random. Table II shows the contents of the blocks as actually obtained for the analysis without previous alkalinization for evaporation.

TABLE II

Design Followed for the Spectrophotometric Determinations

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
vct	vc	t	vc	v	vc	vct	vt
t	vt	vct	vt	t	(1)	v	(1)
v	(1)	v	(1)	vct	ct	t	ct
c	ct	c	ct	c	vt	c	vc

In Table II (vct) is the combination ($V_1C_1T_1$), t the combination ($V_0C_0T_1$), v is ($V_1C_0T_0$), vc ($V_1C_1T_0$), (1) is ($V_0C_0T_0$), and so on. The data obtained in this experiment are given in the same order in Table III (optical density).

TABLE III

Experimental Results (optical density)

First Pair		Second pair		Third pair		Fourth pair	
1st block	2nd block	3rd block	4th block	5th block	6th block	7th block	8th block
0.337	0.369	0.190	0.345	0.128	0.330	0.355	0.176
0.197	0.173	0.331	0.171	0.189	0.174	0.160	0.172
0.150	0.189	0.151	0.182	0.345	0.350	0.182	0.360
0.367	0.374	0.365	0.374	0.349	0.132	0.362	0.362

For calculation purposes the data on Table III were codified by multiphying the values by 1,000 and subtracting 200. The analysis of variance is presented in Table IV.

TABLE IV

Analysis of variance of the experimental results

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F+
Between pairs of blocks	3	1,830.8	610.3	4.21
Interaction VCT	1	175.8	175.8	1.21
Remainder (by difference)	3	435.1	145.0	-
Between blocks (days)	7	2,441.7		
Main effect of V	1	4,072.5	4,072.5	42.34xxx
Main effect of C	1	273,615.0	273,615.0	2,844.53xxx
Main effect of T	1	205.0	205.0	2.13
Interaction VC	1	357.8	357.8	3.72(?)
Interaction VT	1	30.0	30.0	(.3.21)
Interaction CT	1	504.0	504.0	5.24+
Remainder (error) by difference	18	1,731.5	96.19	-
Within blocks	24	280,515.8		
Total	31	282,957.5		

+ Following the usual simbology[9] three asteriscs indicate a level of significance less than 0.1% and one asterisc a level between 1 and 5%. "Question mark" corresponds to a level between 5 and 10% ("possibly significant"[13]). The value of F in parenthesis corresponds to a 18 against 1 degree of freedom comparison.

The calculation of the various items in Table IV was made in accordance with the set up given by Davies[6]. For the experiments made with alkalinization of the solution the design was the same one, i.e., 2^3, with confounding of the interaction VCT in eight blocks or days, giving 32 readings.

DISCUSSION

The analysis of variance confirms the high significance of the main effects of V established in the preliminary experiences. On the other hand the data in Table IV for temperature show that its main effect is not significant. Since the number of degrees of freedom of the residual variance is sufficiently high, 18, it is concluded that the temperature of drying does not affect the results. This means that the time of drying can be shortened by using high temperature (up to 100°C); besides, a rigorous control of temperature is useless. This conclusion, however, is subjected to a certain caution, since the interaction CT has shown to be of some significance.

The interaction VC appears as only "possibly significant". If we bear in mind that the main effects of the two factors V and C are highly significant, it is plausible to consider V and C as independent as shown by the preliminary experiments, at least within the accuracy of the present work. Practically, this conclusion means that it is possible to use any volume of crucibles, provided that it is the same for all samples in the chemical analysis as well as for the blancks and calibration of the spectrophotometer.

The statistical analysis made does not show any interaction VCT; this interaction would only have been detected if the effect were highly significant.

The comparison between pairs of blocks was also of no significance. By making the null-hypothesis of no significance of the differences between blocks and comparing the means squares of the parcell between blocks (2,441.7/7 = 348.8) with the residual variance within the blocks (96.19) one gets F = 3.63 which is of significance at the level of 5%; the preliminary experiments showed a still greater significance.

The significance of variation between blocks shows that the choice of the "day" to define the blocks was judicious; in this way it was possible to increase the precision of comparisons appreciably. Experiments where this precaution of choosing the "day" to define the blocks was not taken, had shown that the results were erratic and a statistical analysis practically impossible.

All the experimental work was made by the same analyst and in nominally identical situation; consequently one should expect that the experimental error would be the same in the preliminary experiments and in the experiments given in Table III. In fact, by comparing the residual variances of both statistical analysis one gets a value for F = 1.46 (18 against 30 degrees of freedom; double-tailed test[13]); this value for F is not significant. As a consequence, instead of calculating the standard deviation independently for the preliminary experiments and for the experiments reported in Table III, it is possible to get a more precise estimate of the error by combining the two parcels for the "remainder" in both sets of experiments; the variation coefficient calculated in this way turned out to be 0.034 meaning a relative error of 3.4%; this value gives a measurement for the reproducibility of results and it represents the error of analysis made on pure solutions. Evidently, this error may be larger when the analysis is made on ores or other materials.

The choice of the relative error to characterize the experimental accuracy instead of the standard deviation was made because a larger deviation in absolute value might be expected with higher concentrations. A check for homogeneity of variance was not made since a certain lack of homogeneity in the experimental field affects very little the accuracy of analysis of variance even when the data are far from being homogeneous.[13]

The experiments carried out with alkalinization of the samples before evaporation showed the importance of making the various operations for actual samples and blanks in parallel, since it was observed significant differences between blocks or days of analysis, Table VII. However, no significance was indicated for the factor "volume" and this fact shows that the larger evaporation effects for larger crucibles-volumes had been suppressed by the alkalinization of the solutions.

For the experiments in which the solutions were alkalinized the operation sequence for calculation is presented in Table V (Casualization), Table VI (Numerical Data, Optical Density) and Table VII (Analysis of Variance).

TABLE V

Design followed for the experiment with alkalinized solutions

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
c	(1)	vct	vc	ct	t	(1)	vct
vct	vt	c	ct	vc	v	vc	v
t	vc	v	vt	vt	c	ct	t
v	ct	t	(1)	(1)	vct	vt	c

TABLE VI

Experimental results for the experiment with alkalinized solutions

First pair of blocks		Second pair of blocks		Third pair of blocks		Fourth pair of blocks	
1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
0.415	0.223	0.408	0.415	0.423	0.231	0.225	0.408
0.407	0.245	0.415	0.425	0.414	0.209	0.413	0.211
0.199	0.414	0.210	0.235	0.223	0.418	0.417	0.229
0.210	0.419	0.212	0.221	0.225	0.408	0.219	0.418

TABLE VII

Analysis of variance for experiments with alkalinized solutions

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	F	
Between pairs of blocks	3	22.8	7.6	(13.43)	x
Interaction VCT	1	684.5	684.5	6.70(?)	
Remainder(by difference)	3	306.2	102.1		
Within blocks	7	1,013.5	144.8	3.10	x
Main effect of V	1	136.1	136.1	2.91	
Main effect of C	1	302,253.1	302,253.1	6,465.31	xxx
Main effect of T	1	84.5	84.5	1.81	
Interaction VC	1	112.5	112.5	2.41	
Interaction VT	1	120.1	120.1	2.57	
Interaction CT	1	136.1	136.1	2.91	
	6	302,842.5			
Remainder (error) by difference	18	841.5	46.7		
Within blocks	24	303,684.0			
Total	31	304,697.5			

CONCLUSIONS

The readings made on samples nominally identical, on different days, differ significantly. This difference, shown by the statistical analysis, might be caused by varions in the atmospheric moisture condition in the laboratory.

The size of the crucibles does not affect the results significantly, unless the solutions are made alkaline prior to evaporation. The effect "size" is inde - pendent of amount of boron as well as of temperature.

As a consequence of the first and second conclusion above one should - choose only one size of crucibles for the analysis, which should be carried out at the same time for samples and standards.

The temperature of drying does not affect the results, meaning that higher temperature can be used allowing an economy of time. Besides, a rigorous control of temperature is not necessary.

The relative error for each reading is of the order of 3.4%, for the range of 2.5 to 7.5 g of boron (without alkalinization) and 2.2% for the range of 0.33 to 1 µg of boron (with alkalinization).

The statistical analysis of the preliminary experiments showed that Beer's law is followed for the amounts of boron used, with a maximum of 7.5 µg per crucible, with spectrophotometric quartz cells of 1 x 2 cm and the solution of the red compound formed made in 95% ethyl alcohol.

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SUMMARY

One of the methods for analysis of boron is by absorption spectrophotometry of solutions of the red compound that boric acid forms with curcumin. The course of this reaction is very much affected by various factors giving erratic results. Some of these factors, which are cause for errors, can be detected by not-too elaborate methods for interpretation of data; other factors, however, are not

of easy interpretation as the probable cause of inconsistency among various experi - ments. This paper presents the application of modern statistical methods to the - study of the influence of some of the mentioned factors on quantitative determinations of boron. The application of these methods provided objective ways of establishing - significant effects for the various factors involved.