Spectrographic Determination of Calcium in Microbiological Culture Media

E. JOHN EASTMOND
Western Regional Research Laboratory, Albany, California[1]
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INTRODUCTION

ONE of a series of investigations undertaken at this Laboratory regarding the utility of asparagus-butt juice as a microbiological culture medium[2] has involved the effect of various metallic constituents on mold growth and bactericide yield. In order to follow changes in metallic composition of the medium as growth progresses, fairly rapid methods of analysis were considered desirable.

Media prepared from various lots of juice naturally varied somewhat in chemical composition. Typical samples contained approximately 6 to 7 percent total solids, about two-thirds of which was reducing sugar. Total ash varied from 0.5 to 0.7 percent. Potassium salts formed the major part of the ash, with magnesium, sodium, calcium, silicon, and various other minor and trace elements also present. X-ray powder photographs[3] of the ash indicated potassium was present chiefly as the chloride with smaller amounts as the sulfate.

Much of the advantage of speed of direct spectrographic methods is sacrificed in the usual procedure for organic materials by long ashing or wet oxidation. It is difficult to prevent losses during manipulations and to eliminate contaminations from reagents and vessels involved in such treatments. With the present problem, preliminary tests indicated that spectrographic methods would be advantageous over other available methods only if determinations could be made directly or at least without application of long ashing treatments.

Direct analyses of organic solutions have been carried out in only a few cases. The Lundegårdh flame method has been used for the direct analysis of milk samples[4] and diluted urine samples[5] and has been mentioned as probably applicable to laked blood, blood serum, and blood plasma.[5] It was not possible to try this method on culture media since the necessary equipment was not available. Furthermore, except for the materials mentioned above, it appears to have been necessary to ash or otherwise eliminate organic material before introduction into the flame. It is probable that any method which requires atomizing or spraying of the sample into the source would be rather unsuccessful with this culture medium because of its high sugar content and tendency to foam.

In the present method, while the sample as received is not introduced directly into the source, only a minimum of preparation is necessary, and reduction of the organic content is carried out rapidly and directly on the electrode, thus decreasing the possibility of loss and contamination.

TREATMENT OF SAMPLES AND PREPARATION OF STANDARDS

Attempts were made to evaporate the sample directly on the electrode for introduction into a suitable spectral source. The tendency of the sample to foam on heating made it impossible to prepare an electrode with a uniform deposit suitable for use in a spark or high voltage a.c. arc. However, satisfactory burning characteristics and spectral intensities could be obtained with samples charred directly on graphite electrodes and then excited in a low voltage d.c. arc.

The chief problem in order to use such a method was to prepare standards that could be treated similarly and would give similar burning characteristics and a satisfactory working curve. No samples of culture media were available which were sufficiently free of calcium. The complexity of composition of the media made it questionable that correct results would be obtained from a necessarily simpler synthetic

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
[2] H. Humfeld and I. C. Feustel, Proc. Soc. Exper. Biol. and Med. **54**, 232 (1943).
[3] The author is indebted to K. J. Palmer of this Laboratory for carrying out the x-ray test.
[4] H. Lundegårdh, *Die quantitative Spectralanalyse der Elements* (Gustav Fischer, Jena, I. 1929; II. 1934).
[5] J. Cholak and D. M. Hubbard, Ind. Eng. Chem. Anal. Ed. **16**, 728 (1944).

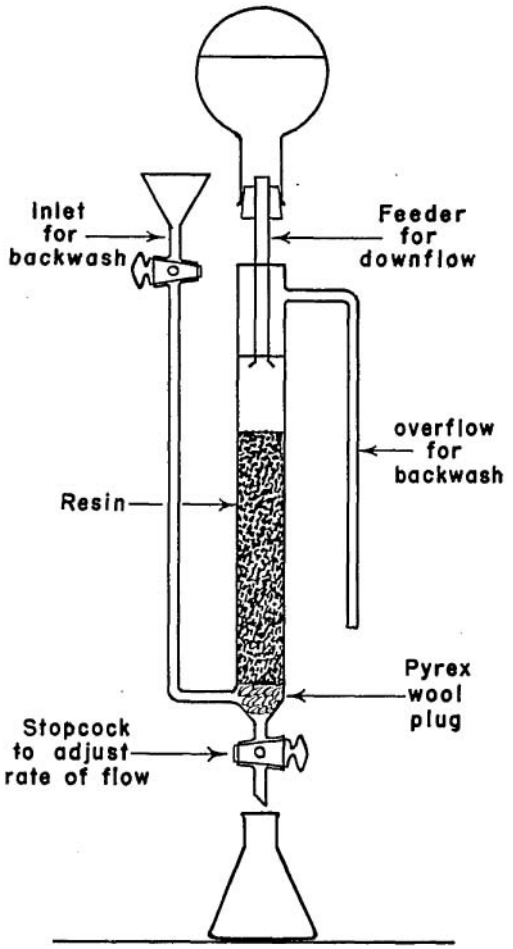

FIG. 1. Ion-exchange resin column for removal of calcium from culture media.

mixture approximating the inorganic composition in an organic base solution such as sugar. It remained to attempt removal of the calcium from the actual medium.

The use of synthetic ion exchange resins in water softeners for removal of calcium and magnesium is well known. The more recent use of resins for de-ashing sugar solutions suggests the possible use for the present purpose. It was found that calcium could be almost completely removed from the medium by use of a cation exchange column of Amberlite IR-100 operated in a "potassium cycle." A potassium cycle was chosen since it would leave the major inorganic constituent of the medium unchanged. No lengthy tests have been made to establish optimum conditions for column operation. A satisfactory medium could be obtained by the following treatment in the simple column shown in Fig. 1:

Amberlite IR-100 (potassium cycle).

(1) Backwash (preliminary).
Backwash column bed (in place) with redistilled water. Wash off color. Allow to settle and drain to one inch of top of bed (volume of bed used approximately 100 ml).

(2) Regeneration (preliminary).
Connect 4 percent HCl downflow. Run 500 ml at 3 volumes per hour. Down-rinse with 500 ml redistilled water.

(3) Load with potassium. Connect 3–4 percent KCl downflow. Run 1 liter at 3 volumes per hour. Down-rinse with 300 ml redistilled water.

(4) Operation.
Connect medium solution downflow. Run 1 liter at 3 volumes per hour. Discard first volume of solution through column.

A new column should be "broken in" by repeating steps (1)–(3) several times before any medium is treated. The cycle may be repeated if additional medium is needed.

The medium obtained by this procedure was found to be sufficiently free of calcium to be suitable as a base for preparation of standards. Approximate determinations by the method of addition indicated that the calcium content was less than 0.00005 percent. The use of this medium assured similar qualitative composition and arc burning characteristics for standards and samples.

Standards were prepared from this treated medium by adding known amounts of calcium. Calcium nitrate ($Ca(NO_3)_24H_2O$) was added to five samples to prepare standards varying from 25.6 to 1000 micrograms of calcium per ml.

SELECTION OF CONTROL ELEMENT AND SPECTROSCOPIC BUFFER

Strontium was selected from a number of possibilities as a control for calcium. The lines Ca II 3179.33A ($^2P_{3/2}-^2D_{5/2}$) and Sr II 3464.46A ($^2P_{3/2}-^2D_{5/2}$) were chosen from several theoretically possible analysis pairs. These two lines, in a region conveniently photographed on ordinary blue sensitive spectrographic plates and relatively free from interfering bands and lines, gave reproducible intensity ratios in practical tests. Although Sr 3464 did not appear in qualitative spectrograms of the culture media, traces

of strontium were detected by the presence of more sensitive lines. Estimates by the method of addition indicated that this contamination was less than 0.0001 percent. Over 100 times this amount was added as a control. Moreover, representative samples of the culture media showed very little variation in strontium content. The error introduced by strontium contamination was therefore only a fraction of one percent.

In order to compensate for possible variations in the major inorganic constituents of the media, a buffer of potassium salt was added to the sample. Either KCl or KH_2PO_4 was found to be suitable for this purpose. The presence of the relatively large amount of this salt was also found to reduce foaming when the sample was placed on the electrode, and the sample dried more uniformly than when no additional salt was present.

ANALYTICAL PROCEDURE

Ten ml of standard or unknown medium is pipetted into a Pyrex dropping bottle. An equal volume of buffer-control solution of 0.012 percent strontium and 2 percent KCl in redistilled water is added. About 0.3–0.4 ml of the mixture is placed on the electrode and charred, a drop at a time, by heating the base of the electrode in a microburner flame.

The electrode is cut from $\frac{1}{4}$-inch special purity graphite (regular spectroscopic graphite contains considerable calcium) with a special drill[6] to form an annular ring cavity with a center post of $\frac{3}{32}$-inch diameter. The cavity is $\frac{3}{16}$ inch in outside diameter and $\frac{1}{16}$ inch in depth. The same type of electrode has been used previously[7] but the dimensions given above have been found satisfactory for the present samples.

The loaded electrode is used as the lower (+) electrode and burned at 15 amperes in a 250-volt d.c. arc. The sample is burned to completion as indicated by the marked change in the sound of the arc and the simultaneous drop in current. A large Littrow quartz spectrograph is used, the 10-inch plate covering the range from 2650 to 3850A. A long focus lens is used at the slit to form an image of the arc on the collimating lens. A diaphragm 6 mm high and full lens width immediately in front of the collimator masks out the image of the incandescent graphite poles.

The transmissions of the Ca 3179 and Sr 3464 lines are measured on a direct-reading densitometer and the relative intensities calculated from the photographic calibration curve by means of a calculating board. The working curve obtained from measurements of a series of exposures on the five standard samples is shown in Fig. 2. The points plotted are averages of a number of exposures on different plates.

RESULTS AND DISCUSSION

Reproducibility of the procedure was tested by the following experiments:

(1) Separate preparations were made by adding standard-buffer solution to ten different samples of the same culture medium. Duplicate exposures were made for each of the ten samples and intensity ratios determined according to the procedure described above. Calcium concentrations, read from the working curve, are listed in Table I. The standard deviation from the mean is seen to be 1.8 percent of the mean, maximum deviation 3.3 percent, and the spread

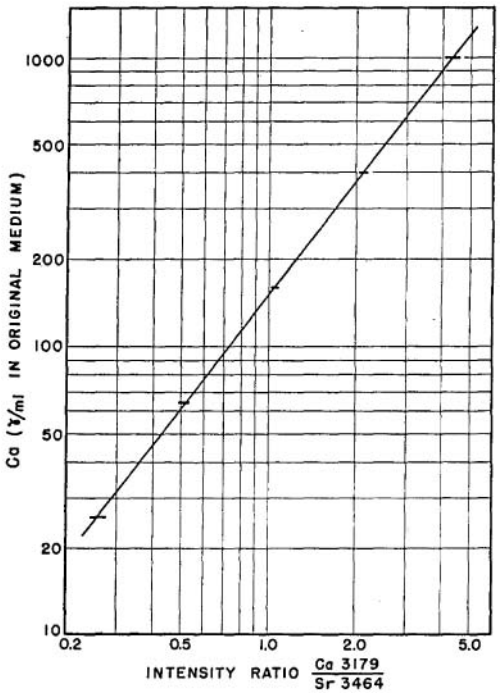

FIG. 2. Analytical working curve for the determination of calcium in culture media.

[6] E. J. Eastmond, J. Opt. Soc. Am. **34**, 621 (1944).
[7] C. R. Jeppesen, E. J. Eastmond, and H. G. Logan, J. Opt. Soc. Am. **34**, 313 (1944).

TABLE I. Calcium determinations on ten separately prepared samples of the same original culture medium.

Sample No.	Micrograms Ca per ml	Sample No.	Micrograms Ca per ml
1	208	6	205
2	212	7	210
3	207	8	205
4	200	9	203
5	211	10	208
Mean		= 206.9 γCa/ml	
Standard deviation from mean		= 3.72 γCa/ml	
Standard deviation (percent)		= 1.8%	
Maximum deviation from mean		= 3.3%	
Spread		= 5.8%	

between the maximum and minimum results, 5.8 percent.

(2) Four samples of the same original medium were prepared with 0, 100, 250, and 500 micrograms of calcium per ml added, respectively. These four samples were analyzed according to the procedure already described. The differences between the amount detected in the unknown with no calcium added and those detected with known amounts added are listed in Table II as the observed calcium added. The results are seen to be within 4 percent of the calculated value.

The precision of results indicated by the tests is much better than was originally expected on the basis of previous experience with the d.c. arc. This is probably due to the elimination of ashing or other pretreatment, and the direct manipulation of the sample on the electrode. Mixing of sample and control as solutions also tends toward better homogeneity.

TABLE II. Results of tests with known amounts of calcium added to a sample of culture medium. Results given in micrograms per ml.

Sample No.	Total calcium observed	Added calcium		Percent difference
		Calculated	Observed	
1	63	0	—	——
2	167	100	104	+4.0
3	321	250	258	+3.2
4	559	500	496	−0.8

The method is not limited to the analysis of asparagus juice media, and has in fact already been used for calcium determinations in other types of media such as those supplemented with corn-steep liquor and whey. Furthermore, indications are that the method will be applicable to other organic solutions—vegetable, citrus and other fruit juices, etc.—with little modification.

The application of this procedure for the determination of other metallic constituents of culture media is being investigated. The preparation of standards is complicated for some elements of interest, since it is difficult to remove them independently from the medium. However, the availability of a Ca-free medium (by the ion exchange method described) has presented an excellent opportunity to compare results from actual and synthetic media. Investigations along this line are now in progress and results indicate that with the use of synthetic standards the method will be applicable for the simultaneous determination of several other elements.

Letters to the Editor

Concerning the Color of the Purkinje Blue Arcs

SIDNEY M. NEWHALL
East Foxboro, Massachusetts
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THE purpose of this note is to correct a false impression regarding our knowledge of the blue arcs of the retina. According to a recent article[1] "the question of color and intensity of the arcs as a function of primary stimulus remains unsettled." This unqualified statement is definitely misleading because an article[2] appearing in this same journal a few years earlier not only furnished extensive data on the "color" of the arcs but also indicated the relationship of their color to the color of the primary stimulus. The earlier paper also supplied quantitative data on the duration of the arcs and contributed to the general theory of the arcs; but these contributions also have been ignored with the result that the article in question is out of date in several respects.

[1] R. L. Dolecek and Jules de Launay, J. Opt. Soc. Am. 35, 676 (1945).
[2] S. M. Newhall, J. Opt. Soc. Am. 27, 165 (1937).