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SPECTROPHOTOMETRIC DETERMINATION OF CALCIUM WITH GLYOXAL BIS (2–HYDROXY–ANIL)

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Abstract

A selective method is described for the spectrophotometric determination of calcium using glyoxal bis (2-hydroxy-anil) as chromogenic agent. A comprehensive study of interferences and reagent variables is given.

CONTENTS

1. INTRODUCTION

Very few methods are available for the spectrophotometric determination of traces of calcium. Most of the published procedures are unselective and the reagents unstable (Sandell, 1959). An extensive purification of the sample solution is usually necessary.

Recently Goldstein and Stark–Mayer (1958) proposed the Schiff base glyoxal bis (2–hydroxy–anil) as the basis of a specific spot test for calcium. The interference of strontium and barium was removed with sodium carbonate, and heavy metals were complexed with cyanide. In this paper glyoxal bis (2–hydroxy–anil) is used as a chromogenic agent for the quantitative spectrophotometric determination of calcium,by extraction of the red calcium complex with sec–octyl alcohol and direct measurement of the colour in the organic phase. Of 42 elements tested, only Bi, Fe, F, Mn, Pb, rare earths, Sr, Ta, Te, Th, Ti and Zr interfere when present at a concentration of less than one hundred times the calcium concentration.

2. EXPERIMENTAL

2.1 Apparatus and Reagents

Spectrophotometer: A "Unicam" S.P. 600 spectrophotometer with 1 cm glass cuvettes was used for all the experimental work.

Glyoxal bis (2–hydroxy–anil): Dissolve 2.2g of o–amino phenol (purified by recrystallization from toluene) in 500 ml of water by heating to 80° C. Add 2.0g of 30 % glyoxal, stir, and keep at 80°C for 30 min. Cool, and allow to stand in a refrigerator for 12 hrs. Filter off the crude compound and recrystallize from the minimum quantity of methanol. The glyoxal bis (2–hydroxy–anil) is used as a saturated solution in ethyl alcohol, which is stable for six weeks if not exposed to strong light.

Octyl alcohol: B.D.H. reagent grade.

All chemicals used were of reagent grade quality.

2.2 Recommended procedure

Pipette a 1 ml aliquot of the sample solution, previously neutralized with NaOH, into a 50 ml separating funnel. Add the following reagents in the order indicated, shaking well between each addition; 0.50 ml glyoxal bis (2–hydroxy–anil), 0.20ml 10% KCN, 0.20ml 10% NaOH, 0.20ml 10% Na_2CO_3, 5.00 ml sec–octyl alcohol. Shake well for 1 min. Transfer to a 10 ml centrifuge tube and centrifuge at a medium speed for 2 mins. Carefully pour a portion of the red upper octyl alcohol phase into a dry 1 cm cuvette, and measure the absorbance of the sample versus a blank at 545 mμ. The calcium content of the solution may be deduced from a calibration curve.

2.3 Wavelength–absorbance curve, and adherence to Beers' law

The calcium–glyoxal bis (2 hydroxy–anil) complex, when measured against a reagent blank exhibits a well–defined absorbance peak at 545 mμ. At this wavelength the molar absorptivity of the complex in sec–octyl alcohol is 1.56×10^4 (corresponding to 0.0026 μg Ca/cm^2 on the Sandell scale). Beers' law is obeyed up to at least 2 μg Ca/ml in the organic phase.

2.4 Stability of colour and effect of temperature

The calcium–glyoxal bis (2–hydroxy–anil) complex appears to exist in the sec–octyl alcohol as an organo–sol, since some 70 min after formation, the "colour" begins to settle out. Absorbance readings should thus be taken within one hour.

Reproducible results may be obtained only within a relatively narrow range of temperatures. At temperatures below 21° C the octyl alcohol phase becomes cloudy, but may be cleared by warming slightly. Above 32°C the complex precipitates irreversibly. In the optimum temperature range of 23 to 30°C, absorbance readings are constant.

2.5 Effect of varying reagent concentrations

1. Sodium hydroxide: The strength of the NaOH solution used in the recommended procedure (10%) was varied, while all other parameters were kept constant. At NaOH concentrations below 5% formation of the complex was incomplete, but above this value constant results were obtained up to at least 25% NaOH.

2. Potassium cyanide: Varying the KCN concentration from 0 to 25% did not affect the results.

3. Sodium carbonate: A slight decrease in absorbance was found if the Na_2CO_3 concentration was reduced below 7%. Above this value results were constant to at least 25% Na_2CO_3.

4. Reagent concentration: Constant results were obtained when the volume of reagent was varied between 0.2 and 0.7 ml.

5. Volume ratio of aqueous and organic phases: The volume of the aqueous phase was altered from 0.5 to 4 ml, but the concentrations of the reagents were kept at the values used in the recommended procedure, and the volume of octyl alcohol was unchanged (5 ml).

It was found that results were constant if the volume of the aqueous phase was greater than 1.5 ml. Below this volume the absorbance readings increased slightly.

2.6 Choice of solvent

Among the solvents investigated for extraction of the calcium–glyoxal bis (2–hydroxy–anil) complex were; sec–octyl alcohol, chloroform, carbon tetrachloride, benzene, hexane, amyl alcohol, toluene, methyl iso–butyl ketone, ethyl acetate, chlorobenzene and chlorotoluene. Only sec–octyl alcohol was found capable of completely extracting the red calcium complex. This solvent had the additional advantage of being lighter than water, which enabled insoluble impurities such as metallic hydroxides, reagent impurities and water to be simply and rapidly removed from the organic phase by centrifuging.

2.7 Study of interferences

Various elements were tested for interference in the determination of calcium using the recommended procedure. A standard amount of calcium, 5 µg, was used throughout the experiment and where necessary, interferences were tested at levels of 1000:1, 100:1, 10:1, 1:1 and 0.1:1, element: calcium. The element was considered to interfere if an error greater than 5% was incurred in the calcium determination. The results are given in Table I.

2.8 Reproducibility of the method

Ten repeat determinations were carried out on 5 µg of calcium using the recommended procedure. At this level the coefficient of variation of the results (n–l) was found to be 2%.

3. REFERENCES

Goldstein, D. and Stark–Mayer, C., (1958). New specific test for calcium – Anal. Chim. Acta, 19: 437–9.

Sandell, E.B., (1959). Colorimetric Metal Analysis. (Interscience Publishers, Inc., New York).

TABLE I

STUDY OF INTERFERENCES IN THE SPECTROPHOTOMETRIC DETERMINATION OF CALCIUM WITH GLYOXAL BIS(2-HYDROXY-ANIL)

Showing the concentration level, 1000:1, 100:1, 10:1, or 1:1 corresponding to foreign ion: calcium, at which interference first occurs. Calcium conc. in all cases 1 μg/ml in the organic phase.

Interference level	Foreign ion	Notes
No interference at 1000:1	acetate, Ag^{+1}, Al^{+3}, As^{+3}, Au^{+3}, Be^{+2}, BO_3^{-3}, Br^{-1}, citrate, Cd^{+2}, Cl^{-1}, Co^{+2}, Cr^{+6}, Cs^{+1}, Cu^{+2}, Hg^{+2}, I^{-1}, Li^{+1}, Mo^{+6}, NH_4^{+1}, Ni^{+2}, NO_3^{-1}, oxalate, PO_4^{-3}, Pt^{+4}, Sb^{+3}, SiO_3^{-2}, Sn^{+2}, SO_4^{-2}, tartrate, V^{+5}, Zn^{+2}	for Ag^{+1}, Au^{+3}, Cd^{+2}, Co^{+2}, Cu^{+2} and Ni^{+2}, KCN must be in excess.
Interference at 1000:1, but not at 100:1	Ba^{+2}, Mg^{+2}, UO_2^{+2}	
Interference at 100:1, but not at 10:1	Bi^{+3}, F^{-}, Fe^{+2}, Fe^{+3}, Mn^{+2}, Nd^{+3}, Sr^{+2}, Th^{+4}	do not use KCN with Bi^{+3}
Interference at 10:1, but not at 1:1	Ce^{+4}, Pb^{+2}, Ta^{+5}, Te^{+6}, Ti^{+4} Zr^{+4}	if Pb present add SO_4^{-2}