CHROM. 5631

NON-DESTRUCTIVE CHROMATOGRAPHIC INDICATOR FOR (AND QUANTITATIVE ANALYSIS IN) THE PAPER CHROMATOGRAPHY OF CARBOHYDRATE COMPOUNDS CONVERTIBLE TO BARIUM SALTS

WALLACE M. PASIKA* AND ARTHUR C. WEST III

Department of Chemistry, Texas A&M University, College Station, Texas 77843 (U.S.A.)

(Received June 2nd, 1971; revised manuscript received August 10th, 1971)

SUMMARY

The scope of application of Thorin as a chromatographic indicator has been investigated for various barium salts and has been found useful in column, paper and thin-layer chromatography. A procedure based on the use of Thorin is described for quantitative analysis in paper chromatography.

INTRODUCTION

A number of reports[1-3] document the use of Thorin [1(O-Arsonophenylazo)-2-napthol-3,6-disulfonic acid disodium salt] in qualitative and quantitative analysis of inorganic ions. With one exception[2] where it is used in the direct titration of sulfate, Thorin's general use is in solution colorimetric determinations.

Herein we report on the use of Thorin as a surface adsorption indicator (pink) for the title salts in spot tests, paper and column chromatography. The usefulness of Thorin lies primarily in the fact that it does not chemically interact with the substrate material and that once this material has been located, the indicator can be removed from the substrate material by "suitable washing". This aspect is especially advantageous in small scale preparative column chromatography.

Another aspect that makes the indicator useful is in work with carbohydrate ionic species that do not contain functional groups (*i.e.*, aldehydic, vicinal hydroxides, etc.) necessary for the usual chemical chromatographic indicators. Provided the ionic species can be converted to the barium salt, one can use Thorin as the indicator. The scope of application of Thorin is indicated below.

EXPERIMENTAL

Indicator solution

Thorin was obtained from the Eastman Chemical Co. (stock no. 6748) and used as received. The spray solution A was prepared by dissolving 25 mg of Thorin in 100 ml of aqueous Fisher "gram-Pac" buffer, pH 4, obtained from the Fisher Scientific Co. The spray solution B contained 25 mg of Thorin in 100 ml of distilled water.

* To whom inquiries should be addressed, Chemistry Department, Laurentian University Sudbury, Ontario, Canada.

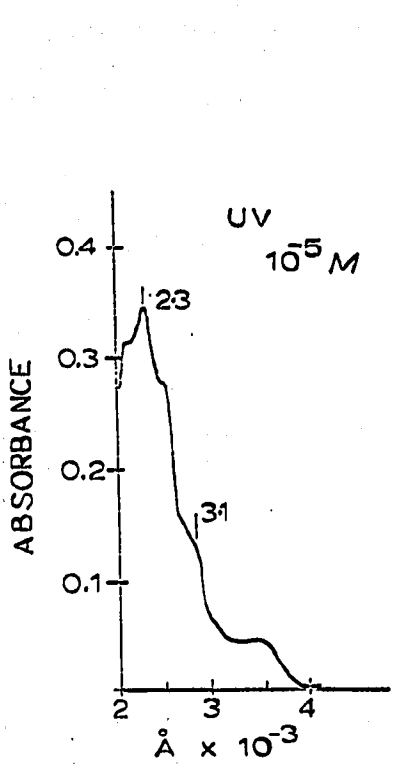
Paper chromatography

(1) Barium methyl α-D-glucopyranoside-2-sulfate (BMGS-2) was spotted on Whatman No. 1 paper to give spots of equal area containing 5, 10, 20, 40, 80, and 100 μg of BMGS-2 per spot. The developing solution in the descending chromatography of these spots was a mixture of ethyl acetate–acetic acid–water (6:3:2). The chromatogram was developed at room temperature for 38 cm after which it was oven-dried at 40° until the odor of acetic acid was no longer evident. The chromatogram was then sprayed with Thorin solution B until the paper was moist. It was dried once more at 40°. (2) Three chromatograms with 5, 10, 20, and 40 μg per spot of BMGS-2 were developed as in section 1. Each was sprayed with a Thorin solution of different concentration. The three solutions contained 25, 50, and 75 mg Thorin per 100 ml of distilled water. (3) Two chromatograms with 10, 20, 30, 40, 50, 60, and 70 μg per spot of BMGS-2 were developed. One was sprayed with Thorin solution A, the other with Thorin solution B. One other chromatogram spotted with barium methyl-α-D-glucopyranoside-6-sulfate (BMGS-6) was developed as in section 1 and sprayed with Thorin solution A.

Quantitation of sugar sulfate in paper chromatography

The UV and visible spectra of Thorin in water were obtained (Figs. 1 and 2).

Plots of absorbance *vs.* concentration for these regions are shown in Figs. 3 and 4.



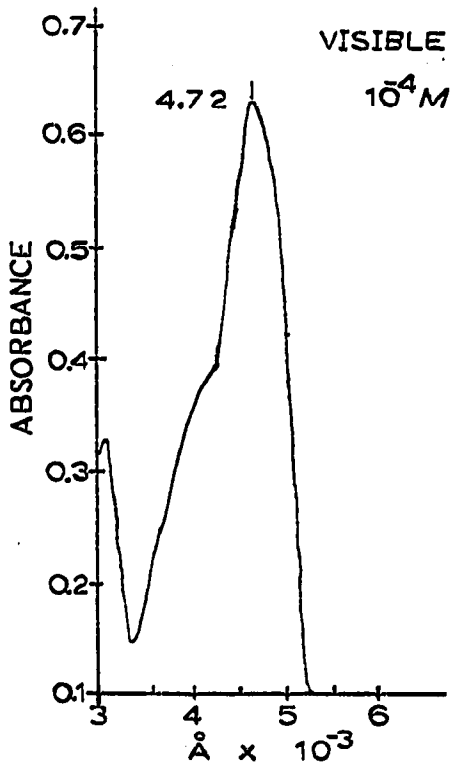
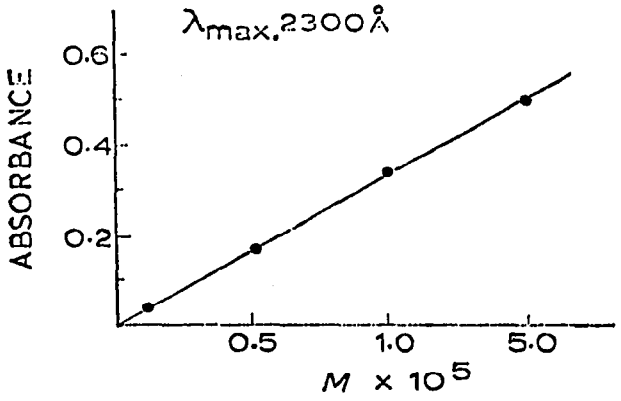


Fig. 1. UV spectrum of Thorin in water at a concentration of 1×10^{-5} *M*; $\lambda_{max.} = 2300$ Å. Instrument Cary 14.

Fig. 2. Visible spectrum of Thorin in water at a concentration of 1×10^{-4} *M*; $\lambda_{max.} = 4720$ Å. Instrument Cary 14.



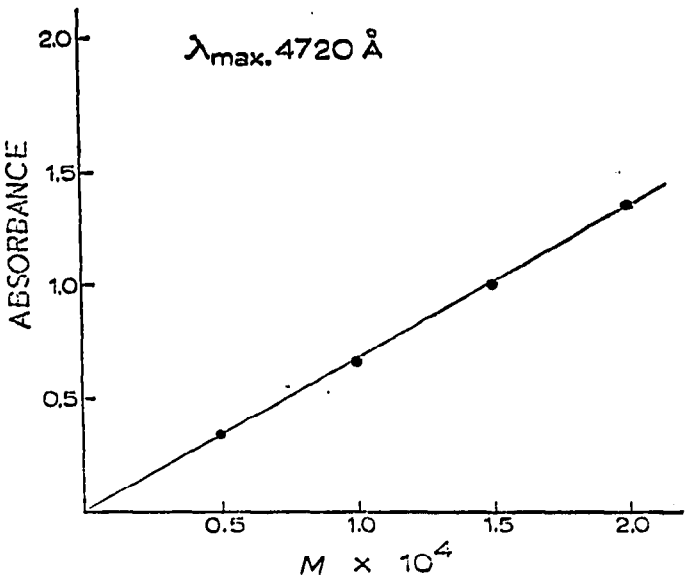


Fig. 3. Beer's law plot for Thorin in water-UV region. The lower detection limit is $\sim 10^{-6}$ *M*.

Fig. 4. Beer's law plot for Thorin in water-visible region. The lower detection limit is $\sim 10^{-5}$ *M*.

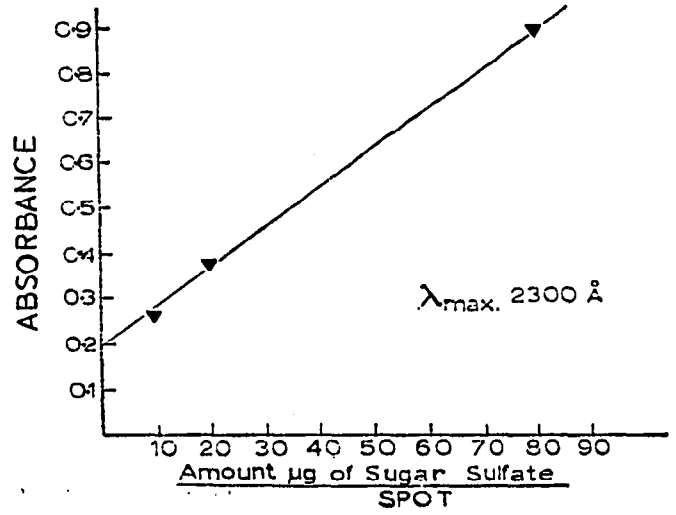


Fig. 5. Plot of absorbance of Thorin dissolved from BMGS-2 spot *vs.* the amount of BMGS-2 known to be present in the spot.

The 10, 20, and 80 µg spots mentioned in section 1 were cut out and placed in 5-ml volumetric flasks which were brought to volume with distilled water. The Thorin on the paper dissolved readily. The UV spectrum of each solution was obtained on a Cary 14. From each spectrum the absorbance at 2300 Å was obtained and plotted *vs.* the known amount of BMGS-2 spotted (Fig. 5).

All of the chromatographed spots of section 3 had their areas measured by tracing them onto millimeter graph paper and counting the squares. The spots on the Whatman paper were then cut out and placed into a 5-ml volumetric flask into which distilled water was added to volume. The UV absorbance at 2300 Å was determined for each solution and plotted against sugar (µg) per spot (Fig. 6) as well as *vs.* spot area (Fig. 7).

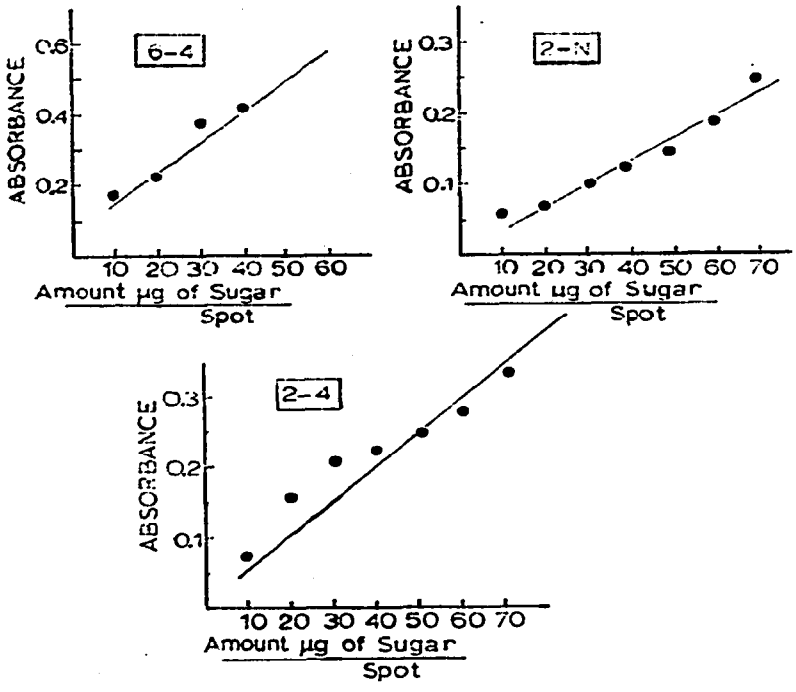


Fig. 6. Plots of absorbance of Thorin dissolved from sugar sulfate spots *vs.* the µg amount of sugar sulfate per spot: 6–4, BMGS-6, Thorin solution A; 2-N, BMGS-2, Thorin solution B; 2–4, BMGS-2, Thorin solution A.

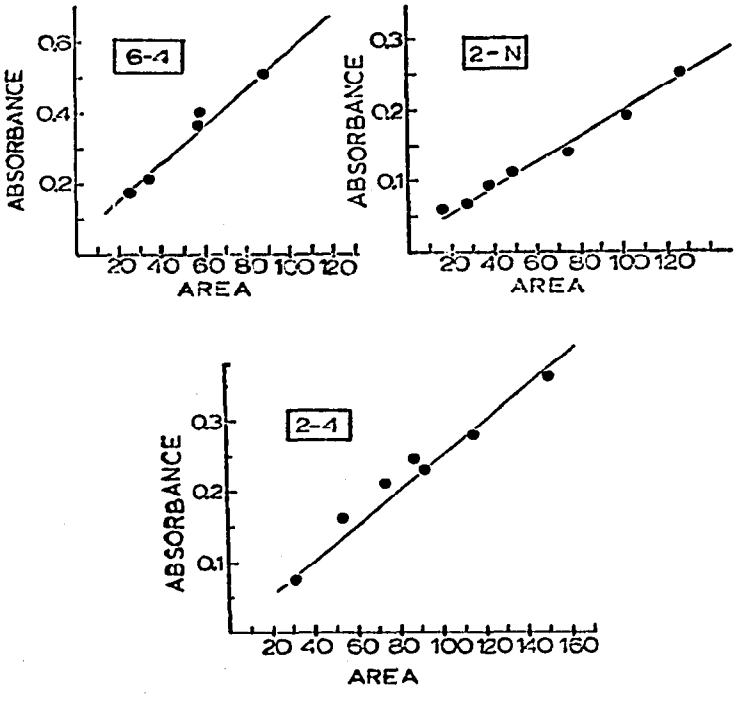


Fig. 7. Plots of absorbance of Thorin dissolved from sugar sulfate spots *vs.* the areas of the sugar sulfate spots: 6–4, BMGS-6, Thorin solution A; 2-N, BMGS-2, Thorin solution B; 2-4, BMGS-2, Thorin solution A.

From the Thorin (solution A) sprayed paper from which the 10, 20, 30, 40, and 50 μg BMGS-6 areas were cut, areas identical in size were obtained to serve as blanks. The UV absorbance of these blanks was compared to the UV absorbance of the Thorin from the sugar spots. The data are presented in Table I.

TABLE I

UV ABSORPTION OF THORIN DISSOLVED FROM BMGS-6 (pH = 4) SPOTS AND FROM BLANKS OF EQUAL AREA SIZE

BMGS-6 per spot	*10 µg*	*20 µg*	*30 µg*	*40 µg*	*50 µg*
BMGS-6 absorption	0.17	0.22	0.38	0.42	0.51
Blank absorption	0.22	0.31	0.30	0.41	0.43
Difference	−0.05	−0.09	+0.08	+0.01	+0.08
Average of Difference	+0.03				
Standard Deviation $\sigma = 8.1 \times 10^{-2}$					

Column chromatography

A small column (~ 10 cc) was packed with a cellulose slurry (ethyl acetate–acetic acid–water (6:3:2)) and washed with the same solvent system. 500 µl of 5×10^{-4} *M* Thorin solution were applied to the top of the column with a Hamilton microliter syringe and eluted with the above solvent system. The effluent was collected in a 25-ml volumetric flask. After 25 ml were collected a comparison solution containing 500 µl of 5×10^{-4} *M* Thorin in 25 ml of the above solvent was prepared. The absorbance at 2300 Å for both solutions was obtained.

Spot tests

Varying amounts (0.2 to 40 µg per spot) of BMGS-6 were spotted on Whatman No. 1 paper and sprayed with Thorin solution B. Barium dextran sulfate, barium carboxymethyl dextran, and barium nucleic acid were also spotted. Dextran sulfate (degree of substitution (DS) = 0.56) and carboxymethyl dextran (DS = 0.88), were converted to the barium salt with the aid of an ion-exchange resin. Calf Thymus DNA was precipitated from solutions by the addition of $BaCl_2$ and washed several times to remove free Ba^{2+}. No limits were determined.

Cation–anion independent migration

$Ba(OAc)_2$, $BaCl_2$, and BMGS-6 were spotted on Whatman No. 1 paper and developed for 30 cm with the ethyl acetate–acetic acid–water system (6:3:2). After drying the paper was sprayed with Thorin solution A. R_F values were obtained for the three compounds.

A synthetic mixture of the barium salts of methyl-α-D-glucopyranoside-6-sulfate and 2,6-disulfate with methyl-α-D-glucopyranoside was spotted on silica gel G plates with and without calcium sulfate binder. One set (with and without $CaSO_4$) of plates was developed with ethyl alcohol–water (90:10) and another set with ethyl acetate–acetic acid–water (60:30:20).

Barium benzoic acid was thin-layer chromatographed with ethyl acetate–acetic

acid–water (60:30:20). After drying the plate was scanned with UV radiation and the benzoic acid spot located; the plate was sprayed with Thorin solution A.

DISCUSSION

The lower limit of detection of BMGS-2 in paper chromatography with the ethyl acetate–acetic acid–water system was 10 μg per spot.

The higher the concentration of Thorin in the spray solution, the more difficult it is to rigidly define the boundaries of the pink spot that indicates the presence of the barium compound. We decided on 25 mg of Thorin per 100 ml of H_2O. One could get by using less, thereby increasing the contrast between spot and background.

It appeared to us that better contrast between spot and background was obtained when the Thorin spray solution was made up in distilled water and had a pH near to 7.

For spot tests, the limit of detection was 0.2 mg BMGS-6 per spot. Spots of the macromolecular compounds gave the characteristic pink color when sprayed with the Thorin solution.

R_F values for the $Ba(OAc)_2$ $BaCl_2$ and BMGS-2 were 0.66, 0.40, and 0.55, respectively. In the case of the thin-layer chromatography of the synthetic mixture sugar sulfate and glucose, the Thorin detected spots were coincidental with acid-charred spots of the sugar sulfates. The UV detected spot of barium benzoic acid was coincidental with the Thorin detected spot. All of the above observations indicate that the barium cations do not migrate independently of the anion and that the pink spot on the chromatogram indicates compound and not Ba^{2+} exclusively.

It was reasoned that the amount of Thorin defining a spot is directly proportional to the amount of substrate making up the spot. This should follow from the fact that the area of a developed chromatographic spot is proportional to the amount of material spotted. Initially the Thorin-substrate proportionality was substantiated by the data of Fig. 5, where the UV absorbance of Thorin dissolved from 10, 20, and 80 mg spots is plotted *vs.* the μg amount of BMGS-2. This relationship was further substantiated with the chromatograms of section 3 (Fig. 6). Fig. 7 shows plots of the absorbance of Thorin *vs.* the area of the developed spots. Both Fig. 6 and Fig. 7 indicate the validity of the above reasoning by virtue of the direct linearity they exhibit.

In the case of an assay, known amounts of the compound along with the unknown amount could be paper chromatographed and from a Fig. 5-type plot for the known and the Thorin absorbance for the spot with the unknown concentration, the concentration of the unknown can be ascertained.

To check whether (a) the sprayed Thorin was caused to interact with the substrate of the spot some time during the processing, (b) the substrate prevents any amount of Thorin from dissolving from the spot in the preparation for UV analysis, (c) there is any significant interaction between the spot dissolved Thorin and the sugar sulfate, and (d) the paper penetration of the Thorin spray is identical through the substrate area as for areas of paper not containing sugar sulfate, blanks of Thorin were subtrated from the Thorin absorbance obtained for a sugar spot. The blanks were cut from the same chromatograms from which the sugar spots were obtained. The results represented in Table I indicate that phenomena (a), (b), and (c) do not

take place, and the one described in (d) does. The residual 0.03 absorbance arises because of the difficulty in cutting exact areas.

A least squares treatment was applied to the data of Figs. 6 and 7. Statistical treatment[4] of the data in Table I and the graphs of Figs. 6 and 7 were carried out. The standard deviation on the differences of Table I is 8.08×10^{-2}. Standard deviations on the slopes and intercepts of the data in Fig. 6 are as follows: run 6-4, $\sigma_m = 1.41 \times 10^{-3}$ and $\sigma_b = 3.87 \times 10^{-2}$; run 2-N, $\sigma_m = 3.98 \times 10^{-4}$ and $\sigma_b = 1.79 \times 10^{-2}$; run 2-4, $\sigma_m = 3.74 \times 10^{-4}$ and $\sigma_b = 1.69 \times 10^{-2}$; while for the data in Fig. 7, we have run 6-4, $\sigma_m = 1.35 \times 10^{-4}$ and $\sigma_b = 7.72 \times 10^{-3}$; run 2-N, $\sigma_m = 1.85 \times 10^{-4}$ and $\sigma_b = 1.34 \times 10^{-2}$; run 2-4, $\sigma_m = 8.52 \times 10^{-6}$ and $\sigma_b = 8.07 \times 10^{-4}$.

More than 95 % of the Thorin is recoverable from the cellulose column with a 25-ml wash. The absorbance at 4720 Å of the comparison solution was 0.100 and that of the effluent > 0.095.

A micro column filled with Whatman chromatographic ashless cellulose powder was prepared. 250 μg of the mixture of mono-((BMGS-6)) and disulfate (Ba^{2+}2,6-disulfate-α-Me-D-glucose)) were added to the top of the column with a microliter syringe and then carefully developed with ethyl alcohol–acetic acid–water (80:1:19) so as not to disturb the bed. A few drops of the Thorin solution were introduced at the top of the column and moved down the column with the same solvent system. As the Thorin moved down the column, its presence gave the column a very faint yellowish color. When the Thorin caught up with the barium sugar sulfate the absorbed band became a bright pink, very easily discernable visually. In the absence of the sugar salt the cellulose had a yellowish color. With continued elution the Thorin passed the barium sulfate band. The Thorin elutes faster than the sulfate and hence can be added periodically during the course of fractionation to track a particular fraction and yet not contaminate the column of the fraction on it.

ACKNOWLEDGEMENT

We appreciate the support given us by the Agricultural Research Service, U.S. Department of Agriculture through Grant No. 12-14-100-9159 (71) administered by the Northern Marketing and Nutrition Research Division, Peoria, Ill.

REFERENCES

1 D. W. Margerum, C. H. Byrd, S. A. Reed and C. V. Banks, *Anal. Chem.*, 25 (1953) 1219.
2 M. Q. Freeland and J. S. Fritz, United States Atomic Energy Commission Report, ISC667, Nov. 28, ((1955)).
3 K. Kodama, *Methods of Quantitative Inorganic Analysis*, Interscience, N. Y., 1963, p. 89.
4 H. W. Salzberg, J. I. Morrow, S. R. Cohen and M. E. Green, *Physical Chemistry*, Academic Press, N.Y., 1969, p. 23.