CHROM. 3874

# THIN-LAYER CHROMATOGRAPHIC METHOD FOR DETERMINING ANTIOXIDANTS IN POLYETHYLENE AND POLYPROPYLENE FILMS

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(First received July 29th, 1968; revised manuscript received October 29th, 1968)

SUMMARY

A thin-layer chromatographic method has been developed for determining phenolic type antioxidants in polyethylene and polypropylene films. This paper describes the extraction procedure used for isolating the antioxidants from the polyolefins, the thin-layer chromatographic separation of various antioxidants used industrially, and a quantitative determination of the antioxidant to detect, in our case, 0.02–0.20 % antioxidant by the use of the double beam scanning densitometer. The six antioxidants studied in this investigation are: 4,4'-butylidene(2-*tert.*-butyl-5-methyl)phenol; 4,4'-thiobis(6-*tert.*-butyl-*m*-cresol); pentaerythritol tetrakis(3,5-di-*tert.*-butyl-4-hydroxyhydrocinnamate); 2,2'-methylenebis(4-methyl-6-*tert.*-butylphenol); octadecyl (3,5-di-*tert.*-butyl-4-hydroxyphenyl)acetate; 2,6-di-*tert.*-butyl-*p*-cresol.

## INTRODUCTION

Antioxidants are used in the food industry to delay or prevent the development of rancidity, and in other industries to enhance the stability of the product. The chemical nature of antioxidants readily permits their analysis, even in low concentrations, by infrared and ultraviolet spectroscopy[1–3]. Distillation[3] and column chromatography[2] have been combined with ultraviolet spectroscopy to determine various combinations of antioxidants; however, the ultraviolet spectra of many additives are often similar, requiring the additional need for a preliminary extraction and infrared analysis to verify the identity.

Antioxidant additives in the plastics industry have given greater durability to polyethylene and polypropylene by decreasing the effect of the polyolefin. The determination of antioxidants in polyethylene and polypropylene[4–6] has been achieved by several authors. However, although the methods are specific in the reported cases, the use of a combination of antioxidants in polyolefins would invalidate the procedures. Although the use of thin-layer chromatography[7–10] is not new for the detection of antioxidants, it was not until SLONAKER AND SIEVERS[11] combined an extraction procedure with thin-layer chromatographic separation that quantitative results were obtained for low concentrations of antioxidants in polyethylene. Since our problem

concerned the separation of various combinations of antioxidants, a thin-layer chromatographic approach was made. This paper is concerned with a different approach toward the isolation of the antioxidants from the polyethylene and polypropylene, which requires only a small sample size (5.0 g) in comparison with the previously reported methods (1 kg); the thin-layer chromatographic separation of various antioxidants, if present in combinations; and a new approach to their quantitative determination whereby, in our case, 0.02–0.20 % antioxidant was detected by use of the double beam scanning densitometer.

## APPARATUS

*Soxhlet extraction apparatus*

The extractor was of medium size, inner diameter 40 mm, top joint 45/50, bottom joint 24/40. The set comes complete with an Allihns condenser, Soxhlet extraction tube and 250-ml flat bottom flask. Extraction thimble: F&S No. 603, size 33 × 94 mm.

*Thin layer plates*

These were Silica Gel G Uniplates (8 in. × 8 in.) and Silica Gel G Uniplates, reverse phase 5 % Dow Silicone (8 in. × 8 in.). The plates were used as received from the supplier after a short equilibration time in the development solvent vapors.

*Capillary pipet*

A 20 $\mu$l capillary pipet was used.

*Chromatographic chamber*

This had an inner diameter 10 3/8 in. × 2 3/4 in. × 10 1/4 in., and was supplied with glass cover. The chamber contains an 8 in. × 8 in. sheet of Whatman No. E-17 filter paper to maintain a saturated solvent atmosphere during development.

*Chromatography sprayer*

*Densitometer*

This was Joyce Loebl Chromoscan, with 0.0–1.0 optical density wedge, a C-cam 1005 aperture, 5-gain, VS lamp, 620 m$\mu$ filter, and a 1:2 gear ratio.

## REAGENTS

*Extraction solvent*

Four volumes of reagent grade *n*-heptane were added to one volume of practical synthetic grade *n*-octane.

*Detection system*[7,8]

Phosphomolybdic acid (3.0 g) was dissolved in a 100-ml volumetric flask with ethanol and diluted to volume with water. The solution was filtered, if necessary, to remove any solids prior to use. The solution must be prepared fresh every two days.

*Development solvent*

*System A (for identification of antioxidants)*. 240 volumes of ethanol were added to 80 volumes of distilled water.

*System B (for quantitative analysis)*. 300 volumes of practical grade cyclohexane were added to 6 volumes of reagent grade methanol.

*Standard solutions*

0.10–0.11 g of the antioxidant to be determined were weighed into a 200-ml volumetric flask and diluted to volume with the extraction solvent. If standards other than 0.10 % (this is based upon a concentration of 5 mg/5 g) are needed, the following equation is used to calculate the percentage antioxidant in the standard.

$$P = \frac{(5)(W_a)}{(W_b)}$$

Where $W_a$ = the sample weight of the antioxidant used to prepare the standard
$W_b$ = sample weight of the polyolefin.

## EXTRACTION PROCEDURE

The polyethylene or polypropylene film, 5.0 g ($W_b$) (precut into strips approximately 1 in. × 1 in.) is weighed into the extraction thimble, 130 ml of the extraction solvent are added to the 250-ml flat bottom flask and a few glass beads; the extraction apparatus is connected and heated to boiling. After 3 h, the system is allowed to cool to room temperature, and the solvent drained into the 250-ml flat bottom flask. The extraction thimble and extraction apparatus are rinsed down with 25–50 ml of the extraction solvent and drained into the 250-ml flask (a turbid solution due to the precipitation of extracted polyolefin may be seen upon cooling; however, this is not uncommon nor should any special attention be taken).

The contents of the 250-ml flask are quantitatively transferred to a 250-ml Pyrex glass beaker (a few glas beads are added to prevent bumping), and the contents concentrated to a 25–30 ml volume by evaporation on a hot plate in a well-ventilated hood. Once the volume is concentrated to 25–30 ml, it is quantitatively transferred to a 50-ml beaker, concentrated to 6–7 ml, quantitatively transferred to a 10-ml volumetric flask, allowed to cool, and diluted to volume with the extraction solvent. If the final solution is turbid or contains precipitate, it is filtered through a Whatman No. 12 filter paper into a 4-dram vial, discarding the first few milliliters; the vial is stoppered.

## CHROMATOGRAPHIC PROCEDURE

Two 20-$\mu$l aliquots of both the sample and the synthetic standard are applied to the thin-layer plate, the spots are dried with a heat gun, and the chromatograms eluted for 30–40 min in the development solvent. The resulting chromatogram is dried with a heat gun, sprayed with the detection reagent, redried, exposed to ammonium hydroxide vapors, and scanned using the Joyce Loebl Chromoscan. The areas of each zone were calculated by triangulation (area calculated by multiplying

the height times the width at half height). The quantitative results are calculated from the following ratio:

$$\% \text{ antioxidant in the polyolefin} = \frac{A_b \times P}{A_a}$$

Where: $A_a$ = the average numerical area recorded for the synthetic standard;
$A_b$ = the average numerical area recorded for the sample;
$P$ = the actual percentage of the antioxidant present in the synthetic standard based upon the weight of the sample.

## DISCUSSION

The direct application of the polyolefin to the thin-layer plate was believed impossible (the polyolefin would hinder migration of the zones making positive identification and interpretation of the resulting chromatogram difficult) and thus a preliminary extraction procedure was incorporated into the method. The extraction procedure described is both simple and straightforward, the only source of error being the extraction of small amounts of the polyolefin. If any of the polyolefin is extracted it must be filtered prior to application to a thin-layer plate, otherwise blockage of the micropipet and a noticeable change in $R_F$ values will be found.

Standard solutions (100 mg/200 ml) of the previously mentioned antioxidants were prepared and 20 $\mu$l of each solution were applied to thin-layer plates and eluted with the development solvent. The $R_F$ values of the antioxidants investigated are shown in Table I. System A was used for identification purposes while System B was used for quantitative analysis.

TABLE I

$R_F$ VALUES OF ANTIOXIDANTS

System A: Silica Gel G, reverse phase 5% Dow Silicone; developing solvent, ethanol–water.
System B: Silica Gel G Uniplates; developing solvent, cyclohexane–methanol.

| *Antioxidant* | *$R_F$ value* | |
|---|---|---|
| | *System A* | *System B* |
| 4,4′-Butylidenebis(2-*tert.*-butyl-5-methyl)phenol | 0.80 | 0.0 |
| 4,4′-Thiobis(6-*tert.*-butyl-*m*-cresol) | 0.84 | 0.0 |
| Pentaerythritol tetrakis(3,5-di-*tert.*-butyl-4-hydroxyhydrocinnamate) | 0.60 | 0.29 |
| 2,2′-Methylenebis(4-methyl-6-*tert.*-butylphenol) | 0.76 | 0.34 |
| Octadecyl (3,5-di-*tert.*-butyl-4-hydroxyphenyl)acetate | 0.33 | 0.70 |
| 2,6-Di-*tert.*-butyl-*p*-cresol | 0.71 | 0.72 |

The final aspect of this investigation was concerned with the collection of quantitative data to check both the stability and the precision of the method. The method was made quantitative by spraying the eluted chromatogram with an ethanolic solution of phosphomolybdic acid; the latter yields blue zones for visual detection and visual reflectance measurements on the densitometer. The densitometer measures the blue zone giving a numerical count (integration is internal to the instrument, but not exact for measurement in our case) and a pictorial representation

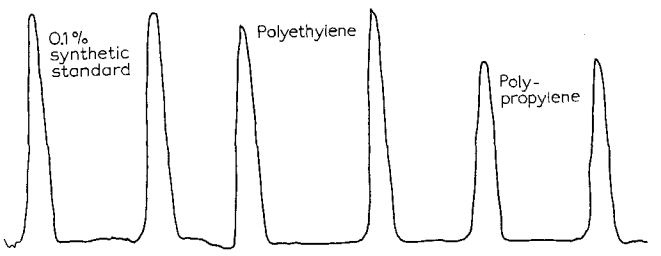


Fig. 1. Chromatographic peak representation using the Joyce Loebl densitometer. Instrument conditions: Joyce Loebl Chromoscan, 0.0–1.0 optical density wedge, C-cam, 5-gain, VS lamp, 620 mμ filter, 1:2 gear ratio. Standard contains 0.1 % 2,2′-methylenebis(4-methyl-6-*tert.*-butylphenol).

(Fig. 1) from which the area under the peak can be calculated by triangulation (area calculated by multiplying the height times the width at half height).

In order to check the variables in the thin-layer procedure, solutions of 2,2′-methylenebis(4-methyl-6-*tert.*-butylphenol) were prepared in the 0.02–0.20 % range and run according to the chromatographic procedure. A plot (Fig. 2) of the data produces a straight line which passes through the origin. Although this clearly indicates that the percentage antioxidant is directly proportional to the areas recorded, the individual trial runs vary in some cases more than desired. This variation in results can be readily explained from the standpoint of the inability to spray the phosphomolybdic acid solution evenly and thus produce the same colored zones. As will be shown later, the method of spraying the plate will determine to a large degree the depth of the blue color, thus preventing determination of the percentage directly from the calibration curve or from a calibration constant; however, it is important to remember the linear relationship of the concentration to the area. After attempts at eliminating the variation due to the uneven spraying were fruitless, a synthetic standard was introduced into the system. The synthetic standard, which can be prepared in any desired concentration, was spotted alongside the sample, thus exposing both the sample and the standard to the same conditions. The antioxidant content in the polyolefins, prepared to contain approximately 0.1 %, was determined by use of the calibration curve (Fig. 2) and by comparison of the area of the zone from

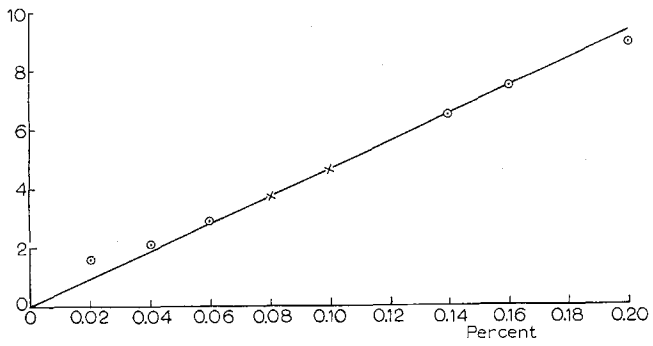


Fig. 2. Calibration curve for 2,2′-methylenebis(4-methyl-6-*tert.*-butylphenol).

the sample to that produced by a synthetic standard. The comparison of the results obtained from the two methods is shown in Table II. As can be seen in the data of Table II, the spraying on these particular zones evidently produced darker zones than those tabulated for the calibration curve, thus yielding high results and showing the need for the use of a synthetic standard.

TABLE II

COMPARISON OF RESULTS

| *Sample* | % *A*[a] *by ratio of a synthetic standard* | % *A*[a] *by calibration curve* |
|---|---|---|
| Polyethylene | 0.095, 0.094, 0.096, 0.092 | 0.172, 0.163, 0.173, 0.156 |
| Polypropylene | 0.084, 0.073, 0.086, 0.080 | 0.146, 0.135, 0.153, 0.136 |

[a] 2,2′-Methylenebis(4-methyl-6-*tert.*-butylphenol).

## RESULTS AND CONCLUSION

In order to show the validity of the extraction in the thin-layer chromatographic procedures, samples of polyethylene and polypropylene were blended with approximately 0.10 % 2,2′-methylenebis(4-methyl-6-*tert.*-butylphenol) and the analysis run according to the recommended method outlined in this paper. The results of these experiments, tested over a 3-day period, along with ultraviolet spectrophotometric data, are shown in Table III. Although there are minor differences in the results of the methods, the thin-layer procedure shows the presence of an additional zone, attributed to the presence of 2,6-di-*tert.*-butyl-*p*-cresol. The presence of this antioxidant, although not determined by the thin-layer chromatographic procedure, could interfere with the ultraviolet procedure thus giving higher results. The extraction procedure, the thin-layer chromatographic procedure, and the quantitative data tabulated by the use of the double beam scanning densitometer offer a new yet simple approach to the determination of various antioxidants in polyethylene and polypropylene. The precision of the method is 10 %, which at low concentration can be tolerated; however, it was not the purpose of this investigation to vary sample size in order to enhance the precision values.

TABLE III

VALIDITY OF THE RECOMMENDED METHOD

| *Sample* | % *Antioxidant*[a] | | | | |
|---|---|---|---|---|---|
| | *1st day* | *2nd day* | *3rd day* | *Average* | *By U.V. analysis* |
| Polyethylene | 0.095, 0.094<br>0.096, 0.092 | 0.103, 0.100 | 0.087, 0.089<br>0.087 | 0.094 | 0.102, 0.105 |
| Polypropylene | 0.080, 0.084<br>0.073, 0.086 | 0.079, 0.078 | 0.078, 0.078<br>0.079 | 0.079 | 0.083, 0.083 |

[a] The antioxidant in this analysis is 2,2′-methylenebis(4-methyl-6-*tert.*-butylphenol).

ACKNOWLEDGEMENT

The author gratefully acknowledges the services rendered by Mr. R. E. WEAVER during the course of this investigation.

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