Spectrophotometric determination using anthrone for sugars separated by cellulose thin-layer chromatography

Although thin-layer chromatography (TLC) has found wide use for the separation of many compounds, its applicability with simple sugars has been limited. The principal difficulty has been the very small quantities of sugars which could be separated satisfactorily on silica gel plates. This difficulty has been overcome using cellulose as the sorbent layer[1]. The usefulness of TLC in sugar analysis could be increased if the separated sugars could be quantitatively estimated by a suitable procedure.

The anthrone reaction of DREYWOOD[2] has been shown to be suitable for the analysis of small quantities of simple sugars separated by paper chromatography[3–5]. This communication discusses the suitability of the anthrone reaction for the estimation of the quantities of some sugars separated on cellulose thin-layers.

Procedures

Chromatography. Plates are washed with a detergent, rinsed well with tap water, followed by distilled water and finally methanol (A.R. grade).

The cellulose slurry is prepared as follows: 15 g cellulose 300 MN (Macherey, Nagel & Company, no binder) are mixed with 90 ml of a deionized water–methanol solution (5:1, v/v) by adding small portions of the solution to the powder and mixing well. A homogeneous, bubble-free slurry results which is sufficient to cover five 20 × 20 cm plates and two 5 × 20 cm plates. A 0.37 mm thick layer is applied with an adjustable applicator (Desaga-Brinkmann).

The plates are dried in a hood for 2 h and then stored in a desiccator cabinet overnight.

The origin is marked 2 cm from the bottom of the plate. The layer is broken horizontally 15 cm above the origin and vertically 0.5 cm in from each side of the plate. Standards and samples are spotted at 1.5 cm intervals using a micropipette.

The plates are developed twice in an ascending manner with a 15 min drying time between developments. The solvent used is formic acid–*tert.* butanol–methyl ethyl ketone–deionized water (15:40:30:15, v/v, A.R. grade)[6].

Location and collection of samples. After development the plate is masked and the locator strips are sprayed with a suitable detection reagent. Heat is applied with an industrial air drier.

The plate then is sectioned as illustrated in Fig. 1 so that each square contains one sugar. Blanks are taken at the same height as the sample.

The unwanted layer is scraped off the plate and discarded. Each square then is scraped into a clean 16 × 100 mm Pyrex culture tube. The samples must be removed by scraping with a single-edge razor blade because the layer adheres to the plate much more tenaciously then does silica gel.

Removal of sample from layer. Approximately 6 ml of 90–95 % acetone (v/v) is added and the sample is agitated gently to break up the cellulose and elute the sugar. Each sample then is filtered with suction through a medium porosity Hirsch filter into a clean tube. The samples are taken to dryness in a vacuum oven at 60° and 90 mm Hg.

Analysis. The reagents used are: 2 % anthrone (Distillation Products) dissolved

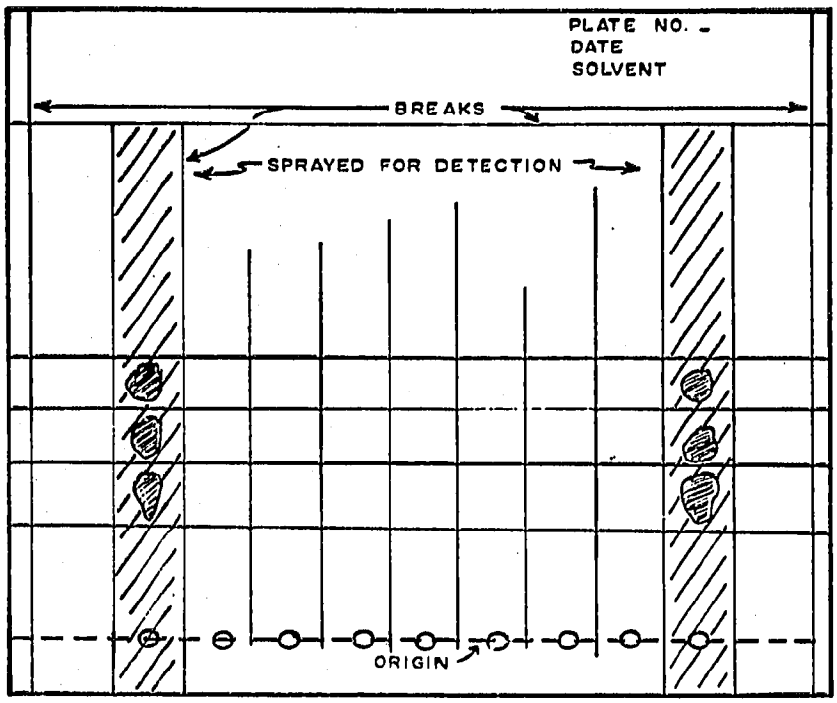

Fig. 1. TLC plate developed and ready for scraping.

in reagent grade ethyl acetate according to the method of LOEWUS[7]. Concentrated sulfuric acid, reagent grade (96 ± 0.7 %).

The dried samples are placed in an ice-water bath and 2 ml deionized water, 0.5 ml anthrone reagent and 4 ml concentrated sulfuric acid are added in that order. The tubes are covered with Parafilm, shaken gently while cold to achieve partial mixing, and then the tube is inverted ten times to mix the reagents completely. Caution!

Tubes containing ketohexoses are heated in a water bath at 60° for 5 min while tubes containing aldohexoses such as glucose, or disaccharides such as sucrose are heated at 80° for 30 min to develop maximum color. After appropriate heating the tubes are cooled by placing them in a room temperature water bath for a few minutes. Absorbance is determined on a Beckman DU spectrophotometer at 625 mμ and a slit-width of 0.1 mm. Deionized water is used as a reference.

Discussion

The anthrone reaction provides a satisfactory means for the quantitative analysis of sugars separated on cellulose thin-layer plates. Beer's Law is followed within the range 5 γ to 100 γ. The upper limit of suitable separation of glucose from fructose is 100 γ.

In this laboratory the procedure is used routinely for the determination of free sugars in extracts of plant tissue. After the tissue has been extracted and the extract made to a known volume aliquots are spotted and the determinations carried out. No further purification of the extract is required. The variation between plant parts and between sample dates is much larger than the variation in the amount of sugar found in samples from different plates. A deviation between plates of 3 γ within the range 5 γ to 50 γ is normal.

Although the anthrone reaction is relatively non-specific for carbohydrates, fructose can be distinguished by the conditions of time and temperature required to obtain maximum color formation. Glucose, sucrose, oligosaccharides and polysaccharides such as cellulose cannot be differentiated in this manner. Thus, in the analysis of simple sugars contamination can be a serious problem if the working area is not kept free of cellulose particles.

Standards must be run on each plate because a single calibration curve which would be applicable for several plates could not be obtained. The blank values for cellulose-TLC are higher than those obtained using paper chromatography. This results partially from the small particle size which increases the probability of hydrolysis of the cellulose and production of oligosaccharides. Interfering compounds can be kept to a minimum by using the aqueous acetone as the eluant since their solubility is limited in this solvent.

Other colorimetric procedures for the determination of sugars should also prove applicable to the system described above.

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