Matching Factors of Beckman Spectrophotometer Cells

BY H. SHEFFER AND H. J. HADOW

This note describes some sources of error which are of importance if the highest accuracy is demanded of the Beckman Quartz Spectrophotometer. One of these instruments (Model DU) is being used here to measure the turbidities of solutions of polystyrene in methyl ethyl ketone as standards for calibrating a light-scattering apparatus.[1] At a wave length of 546 mμ, the transmissions of such solutions in 5-cm. silica cells are in the range 93–97% of that of the solvent. These correspond to turbidities of 0.014 to 0.006 cm.$^{-1}$.

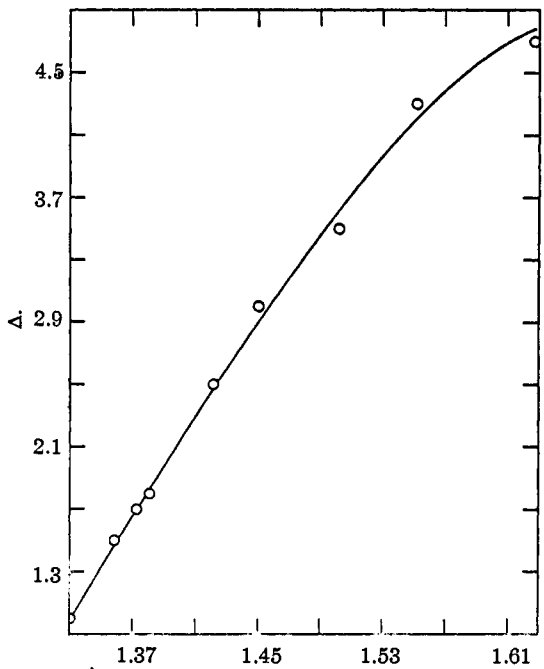

Fig. 1.—Δ plotted against refractive index of solvent.

Since there is a considerable range of wave lengths over which both the red-sensitive (RS) and the ultraviolet sensitive (UVS) phototubes may be used, it was decided to use one to check the other. With solvent in each, the 5-cm. cells were matched at 546 mμ using the RS-phototube, the correction being about 0.1%. The turbidity of a solution was then determined at this wave length using both the RS and UVS-tubes. Applying the matching correction of 0.1%, the transmissions measured by the two phototubes differed from one another by about 1%. This represents the very large discrepancy of 10–30% in turbidity. In addition there was no indication at this stage as to which tube gave the more nearly correct result. It was finally found that a separate matching factor must be determined using the UVS-tube and that, provided the appropriate factor was applied, results with the two phototubes agreed to within 0.1% in terms of transmission.

Since the matching factor is a measure of the *relative* intensity of light transmitted through the two cells filled with the same solvent, it was very surprising to find that its magnitude depended on the photo-tube used. The effects of slit width and of stray light were first eliminated as possible explanations for this behavior. The transmission of colored glass matched against air was found to be independent of the phototube used. However, the difference in the two matching factors rose to 3–4% using 10-cm. cells, indicating that the effect was more important with the longer cells.

When the wave length was increased over the range 400–600 mμ, it was found that the matching factors (10-cm. cells), using the RS-tube, varied in a random way between 0 and 0.5%, while the corresponding factors, using the UVS-tube rose gradually from about 1 to 3%. This led to the thought that differences in refractive index might be responsible for the large variations found with the UVS-phototube. In order to investigate this point the following solvents (in ascending order of refractive index) were used: water, acetone, ethyl acetate, ethyl methyl ketone, dioxane, cyclohexanone, benzene, *o*-dichlorobenzene and quinoline. With a transmission setting of 100% for benzene in one of the 10-cm. cells, readings were taken for each of the solvents in the other cell of the pair. This procedure was carried out using both phototubes at a wave length of 600 mμ and the results, expressed as RS-phototube reading minus UVS-phototube reading for each solvent (Δ), are shown plotted against refractive index in Fig. 1. From this it is apparent that the difference in matching factors increases markedly with increasing refractive index under these conditions. Values of Δ which varied similarly with refractive index were obtained at other wave lengths and also on a second Beckman Spectrophotometer.

In these instruments the light beam is not parallel but diverges slightly. Thus, even if the cells are lined up perfectly parallel to one another and perpendicular to the beam front, the spot of light striking the phototube will vary in area depending on the length of the cell and on the refractive index of the medium in it. If the cells are off by small angles, which is the case in practice, the position of the light spot will change slightly as well as its area. It appears, therefore, that a change in position of the light beam falling on the face of the phototube makes quite a difference in the response of the UVS-tube while that of the RS-tube is relatively unaffected. This point was tested by changing the angle of one of the cells by small amounts relative to the horizontal axis. With benzene in both 10-cm. cells, one cell was used as the standard while the position of the other was altered. Two successive changes of a little over one degree altered the difference in the matching factors for the two phototubes from +2.4 to 0 and from 0 to

(1) Doty, Affens and Zimm, *Trans. Faraday Soc.*, **42B**, 66 (1946).

−2.2. The effect of refractive index on the position and size of the light spot was measured directly by photographing the spot near the face of the phototube. A 30% decrease in area of the spot was observed when a 10-cm. cell filled with water was introduced into the beam. Further successive decreases in area of the order of 5% were observed when the water (R.I. = 1.33) was replaced first by dioxane (1.42) and then by benzene (1.50) and small changes in the positions of the spots were also noticed.

Although for the 5 and 10 cm. cells the differences in matching factors may be large and quite variable with refractive index, good agreement can be obtained using both phototubes as long as the matching factors at a given wave length are obtained separately for each phototube and each liquid used. In this way discontinuities in absorption curves, which are sometimes obtained at the point where the tubes are interchanged, can be eliminated. The source of error discussed is particularly important when solutions varying widely in refractive index are matched against one solvent.

The effect was also determined for 1-cm. cells which are used by the majority of investigators. The difference in the matching factors for several of these cells changed by 0.1–0.3% (in terms of transmission) when benzene in the cells was replaced by water. These amounts become appreciable in terms of optical density only when very low absorptions are being measured.

For work of the highest accuracy, matching factors should be rechecked with any change of operating conditions whatsoever. Due to the zonal variations in spectral sensitivity of phototubes, differences in matching factors may be expected whenever the effective area of the phototube illuminated is altered in any way. In addition to the effect of refractive index, such changes may be brought about by slight localized variations in transmission of the absorption cells due to striae, scratches, etc., and when cells are shifted in orientation with respect to the phototubes.

The authors wish to acknowledge the assistance of Mr. J. C. Hyde.

DEFENCE RESEARCH CHEMICAL LABORATORIES
OTTAWA, ONTARIO RECEIVED SEPTEMBER 11, 1948

Phenoxyacetaldehyde

BY ROBERT J. SPEER AND HENRY R. MAHLER

Hatch and Nesbitt[1] have reported the synthesis of phenoxyacetaldehyde in 45% yield by the lead tetraacetate oxidation of glycerol α-phenyl ether. In connection with the radiochemical program of this Laboratory, the aldehyde in question has been prepared as an intermediate for certain labeled amino acids. It has been found that a modification of the above preparative technique results in an improved yield.

(1) L. F. Hatch and S. S. Nesbitt, THIS JOURNAL, **67**, 39 (1945).

In order to avoid even a temporary excess of oxidant, the order of addition of reagents has been reversed. That is, a slurry of lead tetraacetate (1.0 mole) in benzene was added slowly to a stirred benzene solution of glycerol α-phenyl ether[2] (1.0 mole). This addition was accomplished during *ca.* two hours while the reaction mixture was stirred vigorously at a temperature of 20–25°. After isolation as previously described,[1] the product was fractionated through a 24-in. Widmer column, b. p. 82–83°(4–5 mm.). The aldehyde thus obtained represented a yield of 60% of theory; $n^{20}D$ 1.5360, d^{20}_4 1.1308 (*cf.* Huntress and Mulliken[3] $n^{21}D$ 1.5380, d^{21} 1.1310). Molecular refraction, 37.54 (found), 37.20 (calcd.).

The value of this simple modification has been confirmed by one of us[4] in the case of other sensitive aldehydes.

(2) Supplied through courtesy of the Miner Laboratories, Chicago, Ill.

(3) E. H. Huntress and S. P. Mulliken, "Identification of Pure Organic Compounds—Order I," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 66.

(4) Henry R. Mahler, Thesis, University of California, 1948.

INDUSTRIAL RESEARCH DIVISION
TEXAS RESEARCH FOUNDATION
RENNER, TEXAS RECEIVED NOVEMBER 15, 1948

Ascaridole in Oil of Chenopodium. III.[1] The Characterization of Ascaridole

BY H. HARRY SZMANT AND ALFRED HALPERN

In connection with the investigation of the chemistry of ascaridole in progress in our Laboratories[1,2] it was desirable to reexamine the physical properties of ascaridole in view of the discrepancies noted in the literature.[3] The physical properties of ascaridole are of particular importance since no solid derivative of ascaridole is known.

Oil of chenopodium[4] was fractionated using an all-glass system equipped with a Vigreux column. The physical properties and yields of the various fractions are summarized in Table I.

The fractions obtained from the preliminary distillation above, were analyzed for ascaridole content according to the method of Cocking and Hymas[5] and the results are included in Table I. A portion of fraction "C," which analyzed iodometrically for pure ascaridole, was subjected to a careful fractionation in a two-foot glass-helices-packed column. The physical properties of the fractions obtained during this refractionation are reported in Table II.

(1) For paper II in this series see Halpern, *J. Am. Pharm. Assocn.*, **37**, 465 (1948).

(2) Halpern, *ibid.*, **37**, 161–165 (1948).

(3) Henry and Paget, *J. Chem. Soc.*, **119**, 1714 (1921); Paget, *ibid.*, 829–833 (1938); Paget, *Analyst*, **51**, 180–186 (1930); Khavin, *Ukrain. Gosudarst. Inst. Eksptl. Farm. (Kharkov), Konsul'tatsionnye Materialy* **1939**, No. 6, 165, *cf. C. A.*, **36**, 2997 (1942); Janot and Chaigneau, *Compt. rend.*, **214**, 746–747 (1942); Thoms and Dobke, *Arch. Pharm.*, **268**, 128–137 (1930).

(4) Oil of chenopodium meeting all of the N. F. VIII requirements was obtained from Magnus, Mabee and Reynard, Inc., New York.

(5) Cocking and Hymas, *Analyst*, **55**, 183 (1930).