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Short Communications

Microrefractometry with Abbe-Type Refractometer

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For evaluating the refractive and dispersive constants of a liquid several types of refractometers are available, of which the so-called Abbe-type refractometer [1–3] is the most common.

This type is both rapid and simple by the measurements and has a precision ($\pm$ 0.0001 units in n_D) that is sufficient for most purposes. Because it is provided with compensator [4], the dispersive constants ν (n_D-1/n_F-n_C) may be obtained with close approximation and also n_F and n_C separately [2, 5]. By this the refractometer is also useful for investigations of structure [6] and for quantitative analysis of complicated liquid mixtures [7].

To render a measurement possible it is necessary with a continuous liquid layer between the lower, illuminating prism (Fig. 1 a) and the upper polished prism (Fig. 1 b), where the light is reflected. The required quantities of liquid depend consequently on the dimensions of the free space that exists between the prisms in closed position. The surface of the base is 13 × 28 mm (Abbe-type refractometer model 1 [8]). The height *i.e.* the distance between the prisms, which varies slightly among the instruments, is about 0.15 mm. This makes a volume of about 50 mm^3.

If the test volume is less than the above-mentioned the field of view will be irregularly illuminated and rapidly completely dark according to the fact that air has replaced the liquid film between the prisms. Such factors as the viscosity and volatility of the sample, and the technique used by the supply will also influence the amount that is necessary.

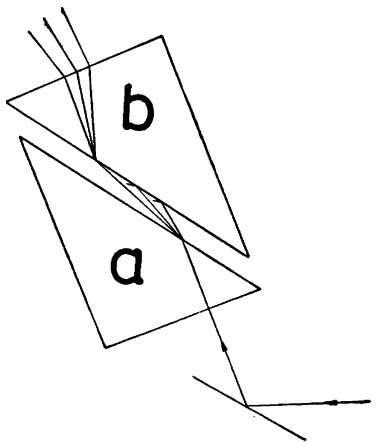

Fig. 1. Schematic diagram of the prism optic of the Abbe-refractometer.

For works with microquantities, *i.e.* a few mm^3, microrefractometers have been designed. Comprehensive reviews of these instruments and works have been made among others by Alber and Bryant [9] and Wilson [10]. Most of these specially designed refractometers are however comparatively tedious and expensive and none unites both the versatility and rapidness of the Abbe-type refractometer. The difference in the last-mentioned factor will be very noticeable by measurements of a large number of test samples *e.g.* by identification of fractions from separation of liquids [11].

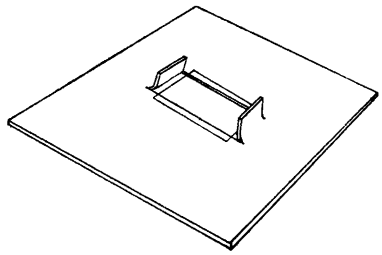

Fig. 2. Inserting of the paper in the measuring plate.

The amount of sample that the Abbe-type refractometer requires can however be reduced if the free space between the prisms is partly filled by a transparent material in which the liquid is adsorbed. Alber and Bryant (*l. c.*) point out that the volume in certain cases may be reduced to about 10 mm^3, if the sample is taken up in a "japanese lens paper" of the size 10 × 15 mm.

This technique has been further developed and here will be described a technique according to which samples of less than 1 mm^3 may be measured. If the free space is limited by a non-adsorbing, inert material (metal) so that only an opening of a few mm^2 is left and a thin paper is fixed in this opening, a very small amount of sample will be sufficient for establishing liquid contact between the prisms. The flow of light will be reduced by this but is still sufficient for fine adjustment of the cross hairs and achromatizing prisms.

The prism surfaces, especially the upper, polished one, are the most sensitive parts of the refractometer and by careless handling the instrument will be damaged [12]. As by this technique a solid body is placed between the prisms great care should be taken. Properly performed no damage of the prisms can happen, partly because the metal only rests between the prisms due to forces of adhesion (not mechanical pressing) and partly because it is made from very soft material.

Making the measuring plate:
Material: 0.08 mm sheet of soft metal i.e. Sn or Al foil, and 0.03 mm paper (resinfree, bleached and hardpressed, of tissue-type).

In the metal foil, 10 × 10 mm, two parallel slits of 5 mm:s length are cut 2 mm apart. A rectangle of 3 mm:s length is cut between the slits and the two laps are unfolded. The paper, 3 × 4 mm, is fitted into the opening and is fixed by folding down the laps (Fig. 2). The plate is then pressed and controlled (micrometer screw) for absolute smoothness and evenness.

Measurement:

The plate is placed with the opening in the middle of the lower prism and the sample is fed from a capillary tube (Fig. 3). By closing the prisms it should be noted that the lower prism is held in as horisontal position as possible until they are locked together, in order to prevent sliding of the plate.

After the measurement the plate is carefully removed and washed in ether. Before measuring an unknown sample, t e technique should be tested at a liquid with

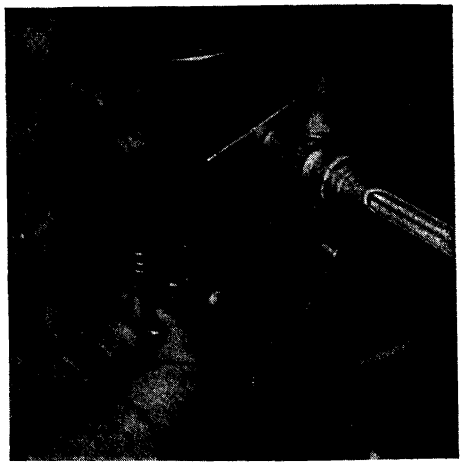

Fig. 3. Photograph of the measuring technique. The sample is fed from a capillary tube to the measuring plate which is resting on the lower prism.

known refractive index in order to control the accuracy and the proper inserting of the paper. The procedure will not take much longer time than a measurement with normal quantities and may be used with all liquids which do not react with the paper. The minimum quantity for a measurement is ¼ – ½ mm³.

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Inability of two Hydantoins to act as Precursors of Pyrimidines in Ribonucleic Acid

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In earlier work we have found that the rat can utilize orotic acid as a precursor for the pyrimidines of polynucleotides [1]. During the course of work on further possible intermediates in the synthesis of polynucleotides in the rat we have investigated the utilization of two N^{15} marked intermediates in the synthesis of orotic from aspartic acid according to Mitchell and Nyc [2] *i. e.* 5-acetylhydantoin and 5(carboxymethylidine)-hydantoin. The latter compound is transformed into orotic acid by alkali under mild conditions. A similar transformation might conceivably be caused by the hydantoinsplitting enzyme recently found in rat tissues by Bernheim and Bernheim [3].

Starting from N^{15} aspartic acid the two hydantoins were synthesized according to the method of Nyc and Mitchell [2]. They contained an over-all excess of 16.2 atom % N^{15}. Each hydantoin was injected subcutanously into two different groups of two rats at a level of 125 mg/kilo of body weight per day. The injections were carried out twice daily with approximately 12 hourly intervals over a period of 3 days. The animals were killed 12 hours after the last injection. In each group the polynucleotides from the pooled livers were prepared and separated into desoxyribo- and ribonucleic acids according to Hammarsten [4]. The pyrimidine nucleosides were prepared from ribonucleic acids according to Reichard [5]. Both the mixed polynucleotides and the pyrimidine ribosides were analyzed for N^{15}.

In no case could a significant incorporation of the isotope be demonstrated thus indicating that none of the two hydantoins had been used for the synthesis of polynucleotide components.

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