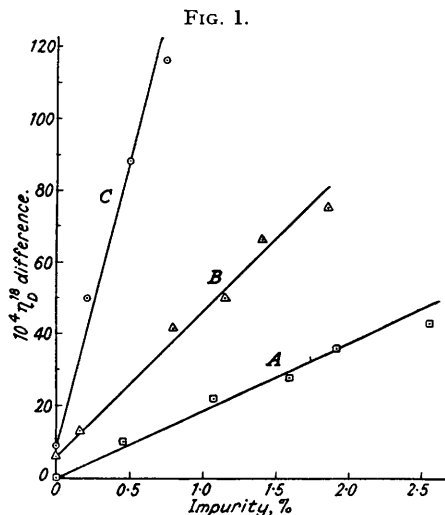


380. Amino-acids and Peptides. Part IV.* The Solubility Criterion of Chemical Purity with Special Reference to Amino-acids: the Use of Refractive-index Measurements.

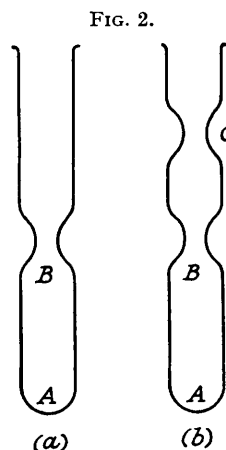
By E. W. WILLIAMS and G. T. YOUNG.

In the application of the solubility criterion of chemical purity, it is necessary to be able to detect differences in the concentration of solutions in equilibrium with varying amounts of excess of solute. This paper describes a method in which refractive-index measurements are used for this purpose.

THE application of the solubility criterion of chemical purity was discussed in Part III of this series,* where a method was described in which differences in solubility are detected by observing the difference in vapour pressure of saturated solutions. It was pointed out that properties other than vapour pressure may provide the basis for such a test, and we describe here a procedure in which refractive-index measurements are used in this way.



- A. Potassium nitrate in "AnalaR" sodium chloride.
 B. DL-Alanine in glycine.
 C. L-Aspartic acid hydrochloride in L-glutamic acid hydrochloride.



In brief, the method involves the preparation of two saturated solutions, one in equilibrium with a slight excess of solute and the other in equilibrium with a large excess. The refractive indices of the two solutions are then measured; if the solute is pure, these values should be identical. When an interferometer is available, this should provide the most sensitive means of determining the difference in refractive index, but for most purposes separate observations with an Abbé refractometer will be found satisfactory, and this procedure has been used in the present work.

The sensitivity of the method under typical conditions has been determined for mixtures containing varying amounts of (a) potassium nitrate in "AnalaR" sodium chloride, (b) DL-alanine in "AnalaR" glycine, and (c) L-aspartic acid hydrochloride in L-glutamic acid hydrochloride. The conditions used are given in the Experimental section. Fig. 1 shows the differences between the refractive indices of the pairs of saturated solutions prepared from each series of mixtures. The differences are seen to be approximately proportional to the amount of added impurity and this method appears to be capable of detecting readily *ca.* 0.3% of the contaminant in the first two cases, and as little as 0.1% in the third case. It should be possible both to increase the sensitivity and to diminish considerably the quantity of material required.

The application of the phase rule to the differential solubility criterion of purity has been discussed in Part III (*loc. cit.*). Refractive-index measurements should normally detect

* Part III, *J.*, 1951, 1279.

differences in the concentration of the saturated solutions, but exceptional cases may be envisaged in which impurities dissolve without affecting the refractive index, for example by a combination of opposing effects or by reason of compensating volume changes on dissolution. Observations in a second solvent will then provide additional evidence. If the nature of the expected impurity is known, empirical experiments with known mixtures should establish a firm basis for the examination of unknown samples. Within this limitation, this method may at times prove more convenient than the vapour-pressure procedure.

As in the latter case, proof of identity analogous to that of "mixed melting points" may also be obtained by this method; a solution saturated with both the known and the unknown material should have the same refractive index as that of a solution saturated only with the known sample, if the two substances are identical. The refractive-index method should find useful applications for many types of compound other than the amino-acids, and it may be especially valuable for the examination of materials which decompose above room temperature.

EXPERIMENTAL.

Method.—The following procedure is typical of that used to establish the purity of a substance, aqueous solutions being used.

A test-tube (*ca.* 8×1 cm.) is drawn out as shown in Fig. 2(a), and the sample (*A*) is weighed into the tube in amount just sufficient to saturate 0.2 ml. of water and to leave a visible excess. The water (0.2 ml.) is then added. A small plug of glass wool is now pushed into the constriction at *B*. A second neck is then formed at *C* [Fig. 2(b)] and the tube is evacuated at the water-pump and sealed by drawing off at *C*. A second tube is prepared similarly, containing approximately five times the weight of solute but the same volume of solvent (0.2 ml.). The two tubes are then immersed in a thermostat, kept about 1° below the temperature at which the refractive indices are to be measured; in this way, the possibility of deposition of solids upon the prism of the refractometer is avoided. In the present work, the thermostat was kept at 17°.

We have found that equilibrium is normally established within 3 days at this temperature. The tubes are then removed and the seals broken to restore atmospheric pressure. They are then resealed and the saturated solutions are centrifuged through the glass wool plugs into the empty ends of the tubes. The tubes are then opened and the refractive indices of the two solutions are determined on the Abbé refractometer.

It is possible to establish equilibrium within a few hours by heating the tubes to 100° after evacuation; there may, however, be some danger of supersaturation on cooling to room temperature, and we have preferred to wait for the longer period without heating.

Examination of Synthetic Mixtures.—The compositions and results are shown in Fig. 1. In each case, one tube contained 0.2 g. of the mixture, and the other 1.0 g.; 0.2 ml. water was added to each.

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[Received, March 3rd, 1951.]
