Substituting now for $\overline{s/D}$ the symbol M_k/C, and for $\overline{s}/\overline{D}$ the symbol M_{kl}/C, where C is a constant, we have

$$\frac{|M_k - M_{kl}|}{C \cdot v_D^2} - K \leqslant \frac{\sigma_s}{\sigma_D},$$

from which we see that on the basis of the inequality between M_k and M_{kl}, conclusions can be drawn as to the ratio between the two standard deviations σ_s and σ_D. This gives the general structure of the relation which Jullander[10] has treated on the assumption of the log normal law governing the distribution of both s and D.

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Determination of Chemical Purity

IN developing the isotope dilution method of amino-acid analysis, we have been concerned with the difficulty of proving the purity of the isolated sample of amino-acid, upon which the accuracy of the determination depends. Elementary analysis and the estimation of reactive groups are not always entirely satisfactory, because the contaminants most likely to be present in such purified material are those which have a very similar composition. Shemin[1] has reported the contamination of glutamic acid hydrochloride by cystine dihydrochloride even after repeated crystallization, and Keston, Udenfriend and Cannan[2] have emphasized the danger of co-precipitation of nearly related amino-acids and their derivatives.

The fact that the solubility of a pure substance is independent of the amount of excess solute has been used as a criterion of the homogeneity of proteins[3] and of amino-acids[4]. In these and other cases conventional methods were used for the determination of solubility. We have found that for substances of relatively low molecular weight a sensitive measure of purity can conveniently be obtained by comparing the vapour pressures of two solutions, one containing a slight excess of solid and the other a large excess. In this way small amounts of impurity, of the order of 1 per cent or less, can be detected in amino-acids.

The apparatus used is a simple differential tensimeter, with xylene as the manometer liquid; water has normally been the solvent. Under the conditions indicated in the table, a recrystallized sample of L-glutamic acid hydrochloride contained sufficient impurity to show a difference in vapour pressure of 6 mm. Synthetic mixtures with known amounts of L-aspartic acid hydrochloride gave proportionately increased differences in pressure, approximately 5 mm. for each 0·1 per cent of added impurity. It is concluded therefore that the original sample of L-glutamic acid hydrochloride contained less than 0·2 per cent L-aspartic acid hydrochloride. Using synthetic mixtures of L-glutamic acid hydrochloride and its enantiomorph, it was found possible to detect the presence of *c.* 0·2 per cent of the D-form, and there may well be occasions on which this method proves convenient for the determination of optical purity.

Purification of 'Analar' glycine yielded a product which (under the conditions indicated in the table) showed a pressure difference of 17 mm. Addition of known weights of DL-alanine to this material again gave proportionately increased differences in pressure (approximately 3 mm. for each 0·1 per cent alanine) and the presence of 0·3 per cent alanine in glycine can readily be detected. If required, it should, of course, be possible to increase the sensitivity considerably.

The simple nature of this method may make it useful as a routine test for the purity of other substances for which more usual tests, such as melting-point determination, are unsatisfactory. Such results as those shown in the table for 'Analar' samples of potassium chloride, sulphanilic acid and phenol suggest that only relatively small amounts of low-molecular weight impurities can be present. This procedure should be especially useful for substances which fail to crystallize.

Substance	Pressure difference (mm. xylene)
1. L-Glutamic acid hydrochloride	6
2. Recrystallized glycine	17
3. 'Analar' potassium chloride	*c.* 1
4. 'Analar' phenol	6
5. 'Analar' sulphanilic acid	16
6. Sulphanilic acid compared with a mixture of sulphanilic and metanilic acids	75

In experiments 1–5, vessel I contained *c.* 0·1 gm. substance and 0·1 c.c. water; vessel II contained *c.* 1·0 gm. substance and 0·1 c.c. water. The temperature of the bath was 54°.

Proof of identity, comparable with that of 'mixed melting points', may be obtained in analogous fashion; solutions saturated on one hand with sulphanilic acid alone, and on the other hand with both sulphanilic and metanilic acids, gave a large difference in pressure.

It must be borne in mind that there are certain limitations (which have been discussed elsewhere[3]) inherent in the use of the 'solubility criterion' of purity. If, however, care is taken to examine solutions with a minimum excess of solid as well as those containing a large excess, and if observations are made with more than one solvent, then strong evidence of homogeneity may be obtained.

We are grateful to the Department of Scientific and Industrial Research for a grant for a special research which has made this work possible, and to the Government Grants Committee of the Royal Society for financial assistance.

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