714. *Determination of the Molar Composition of Complexes of Xanthine Derivatives by Interferometric Measurement of Refractive Index.*

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Interference refractometry has been employed to obtain the composition of weak complexes formed in aqueous solutions by xanthines with various organic acids. Caffeine has been shown to form complexes with benzoic, phenylacetic, phenylpropionic, phenylbutyric, and *m*-hydroxybenzoic acids, and the results have been correlated with those from other methods. Some advantages over previous methods of detecting and determining the stoicheiometry of the complexes are discussed.

Direct methods have not hitherto been found applicable to the determination of the molecular ratios of weak complexes formed in dilute aqueous solutions by many organic donor-acceptor pairs, such as caffeine–benzoic acid. The complexes are usually attributed to polarisation or dipole-dipole interaction and their formation does not appear to be accompanied by changes in pH, polarographic half-wave potential, or spectra sufficient for the use of the usual direct specific techniques. Such complexes are readily formed by many molecules including xanthines, tetracyclines, dioxobenzodiazines, aromatic amines, acids, and amino-esters,[1] and may be of importance biochemically in transport and enzyme mechanisms as well as in pharmaceutical formulation.

The molecular formulæ used in calculating the stability constants of such complexes are usually inferred by mathematical analysis of experimental data. When a distribution method can be employed [2] the dependence of the complexing reaction on each concentration term may be determined and some estimate made of the molecular ratios and stability constants of the major reaction occurring.

A solubility method, based upon the increase in solubility of a sparingly soluble compound in solutions of the more soluble one, has been applied by Weil-Malherbe,[3] Andrews and Keefer,[4] and Higuchi and his co-workers to a variety of organic systems. The solubility curves have been classified by Higuchi [5] into three general groups, some of which are amenable to mathematical analysis. In many cases there is uncertainty that the stoicheiometric ratio so obtained is constant over the whole concentration range and indefinite or non-integral ratios have been observed which indicate that more than one complex is present. When insoluble complexes have separated, molecular ratios determined by chemical analysis of the solids have been assumed to hold also over the lower concentration range used in the calculation of the stability constants. A further limitation of this method is the invariance of the activity of the less-soluble compound throughout the study, which prevents the investigation of polynuclear complexes of this compound, so that the reaction may be treated only as a function of one concentration term.

[1] Gans and Higuchi, *J. Amer. Pharm. Assoc.*, 1957, **45**, 458; Guttman and Higuchi, *idem*, 1957, **46**, 4; Haddad, Sciarrone, and Higuchi, *idem*, 1959, **48**, 588.
[2] Higuchi and Zuck, *J. Amer. Pharm. Assoc.*, 1952, **41**, 10; 1953, **42**, 132.
[3] Weil-Malherbe, *Biochem. J.*, 1946, **40**, 351.
[4] Andrews and Keefer, *J. Amer. Chem. Soc.*, 1949, **71**, 1723, 3644.
[5] Higuchi, Sciarrone, and Haddad, *J. Med. and Pharm. Chem.*, 1961, **3**, 195.

Giles and his collaborators [6] determined the molecular ratios of a number of hydrogen-bonded complexes by measuring the refractive-index changes of two component mixtures, in a number of solvents. A linear relation between the square of the refractive index and concentration was demonstrated for the pure components and additivity proved for compounds incapable of interaction. When, however, the two compounds reacted to form one or more complexes in solution, maxima or minima appeared at the points corresponding to the molecular ratios of the complexes. The method is thus similar in some respects to Job's spectrophotometric method of molar ratio determination (see Vosburgh and Cooper, ref. 7), and the mathematical basis has been summarised by Eaton.[8]

Giles was unable to detect complex-formation arising from dipole-dipole interaction in a series of selected donor–acceptor pairs, which did not, however, include xanthines. The method has here been examined for these systems and since many of the compounds are of very low solubility in water and are known to give weak complexes, a more sensitive interferometric method has been developed.

Experimental

Instrument.—A Hilger Rayleigh interference refractometer (Model M154) for liquids, fitted with a constant-temperature jacket, was used in conjunction with a tungsten lamp as light source. A number of readings were checked by using sodium and mercury vapour-line sources; no differences were observed. The maximum sensitivity of refractive-index measurements obtainable with this instrument is of the order of 10^{-7}, as compared with 10^{-4} and 10^{-5} in Abbé and Pulfrich refractometers of the type used by Giles. The instrument is a differential type and is particularly suitable for the determination of molecular ratios, where differences in refractive index between two solutions are more significant than absolute values. The cell block was maintained at constant temperature.

Chemicals.—Caffeine (B.P.; Evans Medical Ltd.) after recrystallisation from water had m. p. 239°. Benzoic acid (Analar; Hopkin and Williams Ltd.) had m. p. 121° and was 99·8% pure by titration. Phenylacetic acid (British Drug Houses Ltd.) after recrystallisation from light petroleum (b. p. 40—60°) had m. p. 76°. α-Phenylpropionic acid (Eastman Organic Chemicals) was recrystallised from light petroleum (b. p. 40—60°) and then had m. p. 49°. γ-Phenylbutyric acid (Eastman Organic Chemicals) after recrystallisation from light petroleum (b. p. 40—60°) had m. p. 50°. *m*-Hydroxybenzoic acid (British Drug Houses Ltd.) had m. p. 204—207° and was 99% pure by titration. 8-Chlorotheophylline (May and Baker Ltd.) was recrystallised from water; it then had m. p. 311° (decomp.) and was 99·1% pure by B.P.C. method.

Method.—A series of binary mixtures of constant total molarity was prepared from stock solutions of the two components. About 10—12 solutions were required to cover initially the full range of molar ratios and to establish the general pattern of the curve.

In many cases, further solutions were prepared for checking at increments of 5 or 2·5% molar concentration over a range bracketing a change of slope. These increments are essential whenever the position of the break is uncertain. For 3 : 1 or higher complexes, the additional points are also needed to establish the slope of the shorter line unequivocally, since the linear molar percentage scale is subject to large errors in molar ratio at the ends, where the ratio value changes rapidly. In other systems, these intermediate mixtures confirmed the original slopes or served to detect fringe errors but could be dispensed with provided the initial pattern of the curve is clearly defined.

Runs were made in 1-cm. cells in the first instance and repeated in 10-cm. cells if the breaks were small.

After obtaining the instrument zero, one compartment was filled with the next test solution and the refractive-index difference measured in terms of instrument-scale units. Refractive-index differences were kept small to minimise fringe errors by using each solution as a reference

[6] Arshid, Giles, McLure, Oglivie, and Rose, *J.*, 1955, 67.
[7] Vosburgh and Cooper, *J. Amer. Chem. Soc.*, 1941, **63**, 437.
[8] Eaton, Appendix to Arshid, Giles, McLure, Oglivie, and Rose, 1955.

for the next one. Nevertheless fringe errors were observed and eliminated as described above. Temperature uniformity of the glass-block differential cell was established somewhat slowly, and readings fluctuated for some 20 min. for a 1-cm. cell and up to 45 min. for a 10-cm. cell. ΔR values were recorded as the difference between the scale reading (R) and the instrument zero (R_0). The ΔR values were summated in the construction of the graph and the intermediate points are not shown.

Results and Discussion

Fig. 1 shows the results of typical experiments performed as a linearity check. The instrument scale readings were related linearly to concentration over the small ranges used subsequently in complex-formation studies. Strictly, the linear relation should be observed by the square of the refractive index, but Giles and his collaborators [9] have shown

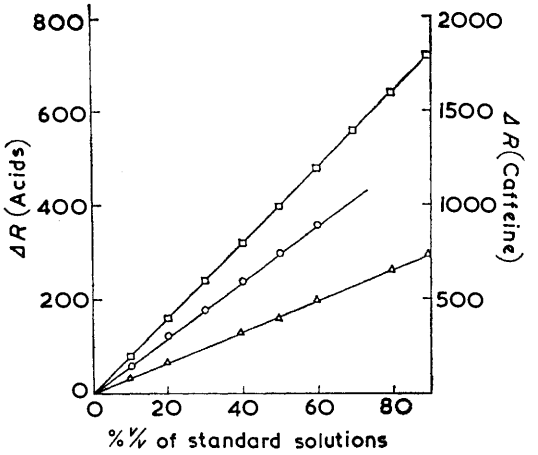

Fig. 1. Relation between instrument scale readings and molar concentration.
—□—□— Caffeine (0·02M, 10-cm. cell)
—○—○— Phenylbutyric acid (0·008M, 10-cm. cell)
—△—△— Phenylacetic acid (0·05M, 1-cm. cell)

that, if the differences are small, direct functions of refractive index may be plotted to avoid tedious computations.

Theoretically, the readings should be additive, provided that complex formation does not occur. An additivity check was furnished by the caffeine–procaine hydrochloride system which gave a linear ΔR-molar percentage curve, with no evidence of any break. The experimental curve was in fact identical with the summed curves of the separate components (Fig. 2).

Giles's experiments on the systems (*a*) phenol–glucose–triethylamine in water, (*b*) dimethylformamide–phenol in carbon tetrachloride, and (*c*) glucose–phenol in water were repeated in order to confirm the validity of the method; [9] the results were identical with those reported.

Typical ΔR–molar ratio plots for several xanthine–acid mixtures are shown in Figs. 3—5 and the molar ratios at which breaks were observed are summarised in the Table.

The caffeine–phenylacetic acid system gave a single break (Fig. 4) which corresponded to a 1 : 2 complex. This value accords with the stoicheiometric ratio obtained by chemical analysis of the recrystallised solid complex, which separates from aqueous mixtures of caffeine and phenylacetic acid at higher concentrations, and also with the value obtained

[9] Arshid, Giles, Jain, and Hassan, *J.*, 1956, 72; Arshid, Giles, and Jain, *J.*, 1956, 559.

Summary of results.

Solutes a	Solutes b	Total molar concn.	Path length (cm.)	Temp. (°c)	Molar ratio of complex (a : b)
Caffeine	Phenylacetic acid	0·025	1, 10	15	1 : 2
,, ...	,,	0·05	1	20	1 : 2
,, ...	Benzoic acid	0·02	1	12	1 : 2, 1 : 1
,, ...	,,	0·02	10	15	1 : 2, 1 : 1, 2 : 1
,, ...	Phenylpropionic acid	0·02	10	15	1 : 2, 1 : 1, 2 : 1
,, ...	*m*-Hydroxybenzoic acid *	0·04	1	15	1 : 2, 1 : 1
,, ...	Phenylbutyric acid	0·008	10	15	3 : 1
,, ...	Procaine hydrochloride	0·08	1	20	—
,, ...	,,	0·005	10	15	—
8-Chlorotheophylline	Phenylacetic acid	0·0025	10	15	?

* All solutes were in water except that for caffeine–*m*-hydroxybenzoic acid 0·0025N-HCL was used to suppress ionisation.

by mathematical analysis of the solubility phase diagram.[10] Further confirmation is afforded by thermal analysis of anhydrous mixtures, only one eutectic point being observed at the same molar ratio.[11]

Mixtures of caffeine with benzoic acid gave two breaks at 1 : 1 and 1 : 2 when studied in a 1-cm. cell but since the change of slope was very small, the solutions were re-examined in a 10-cm. cell. It is evident from Fig. 3 that another complex having a molar ratio of 2 : 1, caffeine : acid, is also formed. This system has been studied by Higuchi and Zuck [2]

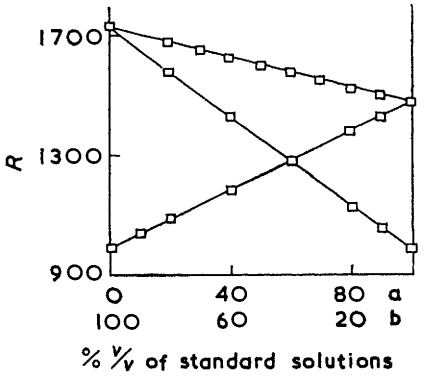

FIG. 2. Additivity of readings.
Cell: 1 cm. a: Caffeine (0·05M in water). b: Procaine hydrochloride (0·05M in water).

by the distribution method; two complexes were postulated, 1 : 2 and 1 : 1. Re-examination of Higuchi's results shows no evidence of a 2 : 1 complex at 30° but there is a distinct though small change in the value of K_1, the stability constant estimated for the 1 : 1 complex, at 15° at the higher caffeine : acid ratios and a somewhat greater change at 0°. These trends indicate a possible dependence of the complex-forming reaction on the caffeine concentration and would accord with the formation of a 2 : 1 complex or higher polynuclear caffeine complex. Guttman and Higuchi [1] observed that dimerisation and tetramerisation of caffeine occur at higher concentrations of free caffeine, particularly at low temperatures. This effect is probably small when the bulk of the caffeine is complexed and should be very small in the interferometric method, when the total molarity is 0·02. There is no evidence of a slope change due to this cause in the results obtained on the simple caffeine solutions (Fig. 1).

Phenylpropionic acid behaves very similarly to benzoic acid in its complexing behaviour, forming 2 : 1, 1 : 1, and 1 : 2 complexes (Fig. 3). Results obtained by Donbrow and Hone[10] by the solubility method show a dependence of K on the caffeine concentration and show

[10] Donbrow and Hone, unpublished.
[11] Sekiguchi, *J. Pharm. Soc. Japan*, 1961, **80**, 669.

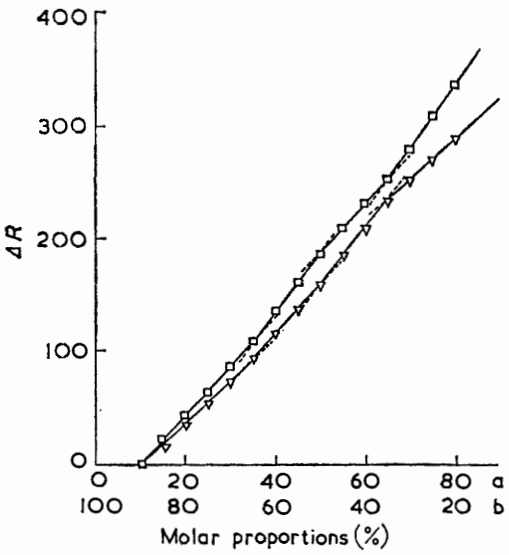

FIG. 3.

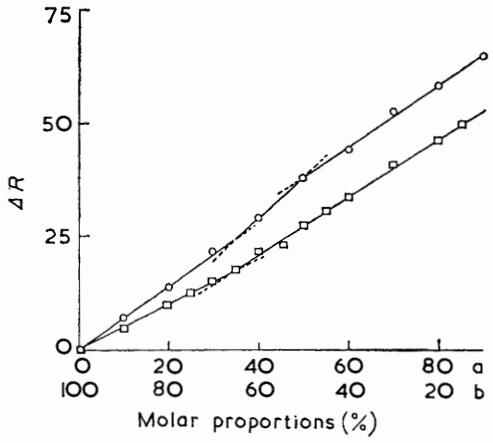

FIG. 4.

FIG. 3. Relation between refractive index (as instrument scale readings) and component ratio.

—□—□— Caffeine–benzoic acid (0·02M in water).
—△—△— Caffeine–phenylpropionic acid (0·02M in water).

Cell: 10 cm. a: Caffeine. b: Acid.

FIG. 4. Relation between refractive index (as instrument scale reading) and component ratio.

—○—○— Caffeine–*m*-hydroxybenzoic acid (0·04M in 0·0025N-HCl).
—□—□— Caffeine–phenylacetic acid (0·025M in water).

Cell: 1 cm. a: Caffeine. b: Acid.

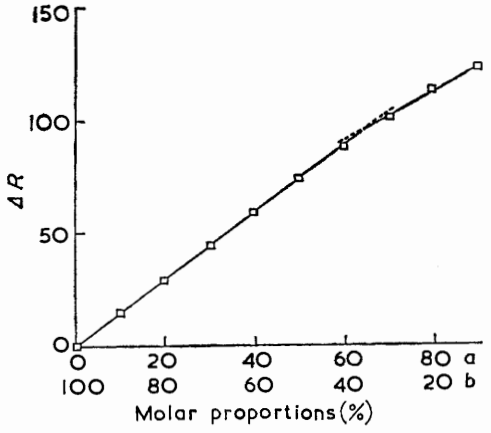

FIG. 5. Relation between refractive index (as instrument scale reading) and component ratio.

Caffeine–phenylbutyric acid (0·008M in water). Cell: 10 cm. a: Caffeine. b: Acid.

the presence of 1 : 1 and 2 : 1 complexes; the 1 : 2 complexes cannot be detected by this method at a constant activity of acid.

A single higher polynuclear caffeine complex is observed in mixtures of caffeine with phenylbutyric acid (Fig. 5). The 3 : 1 molar ratio is the same as that obtained by mathematical analysis of the solubility phase diagram.[10]

The caffeine–*m*-hydroxybenzoic acid system, previously reported by Higuchi and Lach [12] to form 1 : 1 and possibly 1 : 2 complexes, clearly indicated the presence of 1 : 2 and 1 : 1 complexes in the present study (Fig. 4). No complex formation was detected between caffeine and procaine hydrochloride although Lachman, Ravin, and Higuchi [13] have detected a 2 : 1 complex.

Small breaks were obtained in mixtures of 8-chlorotheophylline with phenylacetic acid but the results were inconclusive at the very low concentrations obtainable when this sparingly soluble xanthine derivative is used.

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London, S.W.3. [*Received, January 12th*, 1963.]

[12] Higuchi and Lach, *J. Amer. Pharm. Assoc.*, 1954, **43**, 524.
[13] Lachman, Ravin, and Higuchi, *J. Amer. Pharm. Assoc.*, 1956, **45**, 290.