J. Pharm. Pharmac., 1976, **28**, 766–769 *Received June* 14, 1976

Stability of salicylamide-caffeine complex at different temperatures and its thermodynamic parameters

M. DONBROW*, E. TOUITOU AND H. BEN SHALOM

Pharmacy Department, School of Pharmacy, Hebrew University of Jerusalem, P.O.B. 12065, Jerusalem, Israel

The stability constants for formation of complexes of salicylamide with caffeine have been measured between 15 and 45°, by means of the solubility method. There was a linear solubility increase at all temperatures but phase diagrams indicated that at 15 and 25° an additional phase was formed which was found to be an insoluble 1 : 1 complex. The enthalpies and entropies of interactions were evaluated. They indicate that the interaction is exothermic and enthalpy controlled.

Studies on dissolution of salicylamide in complexing media (Donbrow & Touitou, to be published) required determination of the stability constants of these complexes at different temperatures. The interaction of caffeine with salicylamide has only been investigated at 37° (Reuning & Levy, 1968). In the present work, the temperature range has been extended from 15° to 45° and thermodynamic data have been evaluated from the stability constants.

MATERIALS AND METHODS

Materials. Salicylamide (Sigma Chemicals) and Caffeine anhydrous (Merck) had the correct literature m.p. and were of N.F. grade.

Determination of stability constants. The method used was that of Higuchi & Zuck (1953) based upon solubility increase. The system was shaken for 72 h, this period having been found sufficient for equilibration at 15 and 25°. Aliquot portions, after filtration at the temperature of reaction, were treated with Trinder's reagent (Trinder, 1954) modified by omission of mercuric chloride, this having been found to give the same extinction coefficient at the 525 nm maximum as Trinder's reagent.

It was shown that caffeine if present in the solution does not interfere with the salicylamide assay (Reuning & Levy, 1968).

RESULTS AND DISCUSSION

The phase diagrams at 15 and 25° are shown in Fig. 1. The solubility of salicylamide increases linearly with caffeine concentration indicating that the complex formed is substantially monomolecular with respect to caffeine. At both temperatures, the formation of a plateau indicates that the solution is saturated with complex. This is evident from the fact that (i) the caffeine concentration is considerably below saturation and (ii) the plateau is finite, terminating in a fall in total salicylamide concentration due to exhaustion of the excess solid salicylamide and consequent precipitation of salicylamide from solution as complex on further addition of caffeine. A portion of the solid precipitated at the end of the plateau region was analysed spectrophotometrically and found to have a 1 : 1 molecular ratio of the components.

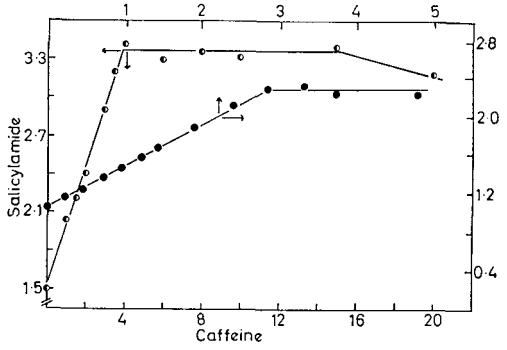

FIG. 1. Phase diagrams of salicylamide-caffeine-water systems at 15° and 25°. Both expressed as concentration $\times 10^2$ (M).

Stability constants were calculated from the slope of the initial linear portion of the diagrams or at the higher temperature, from the calculated slope (Table 1) using equation (1) (Donbrow & Jan, 1965):

$$K = \frac{S}{(1-S)A_0} \qquad .. \quad (1)$$

where K = stability constant; S = slope; A_0 = solubility of salicylamide in water.

Apparent $K_{1:1}$ values are listed in Table 1. They indicate the extent to which binding is strengthened with decrease of temperature. In the present

* Correspondence

Table 1. *Stability constants and solubilities for salicylamide-caffeine systems at different temperatures.*

Temp °C	Salicylamide Sol.[a] A_o M × 10^2	Slope	Stability constant $K_{1:1}$ M^{-1}	% Sol. increase[b]
15	1·06	0·434	72·7	41
16	1·10	0·438	70·8	40
25	1·50	0·465	57·9	31
37	2·90	0·552	44·1[c]	19
45	4·28	0·616	37·6	14

[a] Saturation values. [b] % Increase in salicylamide solubility per mM caffeine added. [c] $K_{1:1}$ from Levy & Reuning (1968). Other values calculated by present authors.

treatment, possible formation of a 2 : 1 salicylamide-caffeine complex is neglected. Reuning & Levy (1968) examined evidence bearing upon the formation of such a complex obtained using the solubility and the membrane permeation methods and, by mathematical analysis, ruled out the presence of the higher complex in significant amounts. At the lower temperatures used in the present study, although stabilization of both the 2 : 1 and 1 : 1 complexes might be expected, the proportion of 2 : 1 to 1 : 1 complex formed should be even further reduced as compared with 37°.

Using the Law of Mass Action, it can be shown that the ratio of the two complexes present, R, is given by:

$$R = [S_2C]/[SC] = K_{2:1}[S] \quad .. \quad (2)$$

where $[S_2C]$, $[SC]$, $[S]$ are the concentrations of the 2 : 1 complex, 1 : 1 complex and salicylamide, respectively, the last being the solubility under the saturation conditions used, and $K_{2:1}$ is the stepwise formation constant of the higher complex. The ratios at any two temperatures are given by equation (3):

$$R_1/R_2 = {}^1K_{2:1}[S]_1/{}^2K_{2:1}[S]_2 \quad .. \quad (3)$$

where the added 1 and 2 define the terms at the respective lower and higher temperatures. The solubility of salicylamide falls very steeply with temperature drop, *ca* 300 % over 21°, which is of a higher order than the rise in K value observed in this and similar systems* (Higuchi & Zuck, 1953), hence $[S]_1/[S]_2$ is much lower than ${}^2K_{2:1}/{}^1K_{2:1}$, and there should consequently be a considerable reduction in the ratio R of the two complexes at low temperatures.

The K values in this system fall into the general pattern observed for complexes of caffeine with benzoic acid derivatives. They lie between the values of benzoic acid, and its halogen and alkyl substitued derivatives (16–25 at 15°, 13–18 at 30°) and those of *p*-hydroxybenzoic acid and its esters (90–100 at 15°, 50–100 at 30°) (Higuchi & Zuck, 1953). Higuchi & Drubulis (1961) noted that the presence of free OH groups in the Ar ring enhances complexation. It seems probable that the mechanism in salicylamide complexation is similar to that in the other caffeine-aromatic substrate systems.

Thermodynamic constants for the interaction have been calculated and are in Table 2. The enthalpy, ΔH, was obtained from the slope of a plot of log K against 1/T (slope 860) which gives the expected straight line relation, showing that the enthalpy is

Table 2. *Thermodynamic constants for the salicylamide-caffeine interaction.*

Temp °C	$-\Delta H$ kJ mol^{-1}	$-\Delta G$ kJ mol^{-1}	$-T\Delta S$ kJ mol^{-1}	$-\Delta S$ jK^{-1} mol^{-1}
15	16·4	10·3	6·1	21
16	16·4	10·3	6·1	21
25	16·4	10·1	6·3	21
37	16·4	9·9	6·5	21
45	16·4	9·6	6·7	21

* Obtained from plot of log $K_{1:1}$ (M^{-1}) against 1/T (deg K^{-1}) which gave a slope of 860.

independent of temperature. This has been proposed as a test for the presence of a single 1 : 1 complex or 1 : 1 isomeric complexes (Orgel & Mulliken, 1957; Kuntz & Johnston, 1967). The linearity observed would seem to justify the simplifications made earlier in data treatment. The free energy of binding, ΔG, and the entropy of the reaction, ΔS, were calculated from the relationships:

$$\Delta G = -RT \ln K \quad .. \quad .. \quad (3)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad .. \quad .. \quad (4)$$

From the thermodynamic data, it is seen that the reaction is exothermic, the enthalpy, ΔH, (− 16·4 kJ mol^{-1}) being within the range encompassed by benzoic acid (− 12·6 kJ mol^{-1}), benzoate (− 13·9)

* The only 2 : 1 caffeine complex for which the temperature dependence of K values has been studied by an unequivocal method is caffeine-(benzoic acid)$_2$ in which the overall formation constant doubles between 30° and 0° (Higuchi & Zuck, 1953). The $K_{2:1}$ stepwise constant, calculated from the data, shows a much lower temperature dependence, rising by 30 % over the same range.

and butyl paraben (− 29·4) (Higuchi & Zuck, 1953). Since these are overall values, representing net changes resulting from formation of complex, alterations in solvation of substrate and complex molecules and changes in water structure, they cannot be subjected to detailed analysis but are of the order expected for complexation involving weak intermolecular forces.

The entropy change, ΔS, is $-21\ J K^{-1} mol^{-1}$ which is below that reported for butyl paraben (− 63), and above that of benzoic acid (− 16·8). Its negative value suggests a higher degree of ordering in the system in the complexed state, and a general correlation with and similarity to the other compounds in the series, K, ΔH and ΔS values running parallel to each other.

There is no direct evidence for classical hydrophobic bonding, originating from the diminution in the amount of ordered 'iceberg' structure in the water layer surrounding the non-polar groups when two such groups associate to form a single cavity instead of occupying separate cavities in the water. This would have led to increase in both the enthalpy and the entropy, on complexation, the latter dominating and rendering the free energy change negative, while the endothermicity would cause bond strengthening with temperature rise (Davis, Higuchi & Rytting, 1974), both contrary to observation. A classical entropy increase term could be hidden within the overall negative entropy change, indeed weakening of complexation in related systems in organic solvents is evidence for hydrophobic bonding (Kristiansen, Nakano & others, 1970), but may equally well be explained by the alternative theory below. However, the driving force for complexation is clearly enthalpic, the entropic energy ($-T\Delta S$) being destabilizing and constituting only some 40 % of the enthalpy value (Table 2).

The thermodynamic results are parallel qualitatively to those calculated by Sínanoğlu & Abdulnur (1964, 1965) in their theory of solvent effects on base stacking in DNA, which attributes the major driving force for the interaction to the large cohesive energy of water, as reflected in its high surface tension. They would be indicative of hydrophobic interaction between the salicylamide and caffeine rings in plane to plane stacking configuration, surface free energy being released by formation of a single cavity in the water in place of two, resulting in a large surface enthalpy decrease.

Negative enthalpies and entropies are characteristic also of many charge-transfer interactions (e.g. Farrell & Westwood, 1970) but there was no spectral evidence for this mechanism in this or similar complexes (Herbstein, 1971; Donbrow & Jan, 1965) and it seems that for spectral changes to appear, donor and/or acceptor properties must be enhanced (Cohen & Connors, 1970). The negative entropy could well arise from the high degree of ordering in a parallel plane alignment of large flat rings, whether caused by π orbital overlap or hydrophobic stacking forces. This factor might well be enhanced in salicylamide by ring enlargement due to intramolecular H bonding between the side chains. The values of the association constants also seem too high for donor-acceptor complexes, which are generally destabilized in polar solvents (Foster, 1969) and intermolecular H bonding would similarly by disfavoured in dilute aqueous solution. Furthermore, for purine interactions vertical stacking is thought to occur preferentially to horizontal H bonding, in water (Ts'o & Chan, 1964).

It is also noteworthy that although addition of caffeine increases the solubility of salicylamide at all temperatures, its relative effect changes with temperature. This is shown in Table 1 by the percentage solubility increase of salicylamide relative to the solubility of the pure substance at the given temperature, which decreases sharply with rise of temperature, as expected from the negative value of the enthalpy. This behaviour may influence the dissolution profile of the drug as well as other pharmaceutical properties.

REFERENCES

Cohen, J. L. & Connors, K. A. (1970). *J. pharm. Sci.*, **59**, 1271–1276.

Davis, S. S., Higuchi, T. & Rytting, J. H. (1974). In: *Advances in Pharmaceutical Sciences*, Vol. 4, London: Academic Press.

Donbrow, M. & Jan, Z. A. (1965). *J. Pharm. Pharmac.*, **17**, 129S–137S.

Farrell, P. G. & Westwood, J. V. (1970). *J. chem. Soc.* (B), 1518–1521.

Foster, R. (1969). In: *Organic Change Transfer Complexes*, Chapter 7. London: Academic Press.

Herbstein, F. H. (1971). In: *Perspectives in Structural Chemistry*, p. 217–8, New York: Wiley.

Higuchi, T. & Drubulis, A. (1961). *J. pharm. Sci.*, **50**, 905–909.

Higuchi, T. & Zuck, D. A. (1953). *J. Am. pharm. Ass. Sci. Ed.*, **42**, 138–145.

KRISTIANSEN, H., NAKANO, M., NAKANO, N. J. & HIGUGHI, T. (1970). *J. pharm. Sci.*, **59**, 1103–1106.
KUNTZ, JR., I. D. & JOHNSTON, JR., M. D. (1967). *J. Am chem. Soc.*, **89**, 6008–6017.
ORGEL, L. E. & MULLIKEN, R. S. (1957). *Ibid.*, **79**, 4839–4846.
REUNING, R. H. & LEVY, G. (1968). *J. pharm. Sci.*, **57**, 1556–1561.
SÍNANOĞLU, O. & ABDULNUR, S. (1964). *Photochem. Photobiol.*, **3**, 333–342.
SÍNANOĞLU, O. & ABDULNUR, S. (1965). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **24**, S-12-S-23.
TRINDER, P. (1954). *Biochem. J.*, **57**, 301–303.
TS'O, P. O. P. & CHAN, S. I. (1964). *J. Am. chem. Soc.*, **86**, 4176–4188.