John Kent
Institute of Pharmaceutical Sciences
Syntex Research
Palo Alto, Calif.

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[x] To whom inquiries should be directed.

Consistency Relations in Solid Solution Melting-Point Diagrams

Keyphrases □ Solid solution systems—melting-point diagrams, consistency relations, thermodynamic analysis □ Melting-point diagrams—solid solution systems, consistency relations

To the Editor:

Solid solution systems have been of pharmaceutical interest in recent years (1–7). The thermodynamic basis for solid solution systems is given in standard texts on solid-state physics or chemistry; *e.g.*, Zhdanov (8) listed the expression for the free energy, F, at temperature T °K of a system containing x mole fraction A and $(1 - x)$ mole fraction B as:

$$F(x, T) = K(T) + 0.5NZ[x^2V_{AA} + (1-x)^2V_{BB} + 2x(1-x)V_{AB}] + RT[x \ln x + (1-x)\ln(1-x)] \quad \text{(Eq. 1)}$$

where:

N = Avogadro's number
Z = coordination number
V_{AA} = interaction energy between two A molecules
V_{BB} = interaction energy between two B molecules
V_{AB} = interaction energy between an A and a B molecule
$K(T) = \int_0^T c\,dT - T\int_0^T \frac{c}{T}\,dT$, where c = heat capacity

The assumptions made here are that: (*a*) only nearest neighbor interactions are considered, (*b*) Stirling's formula is applicable, and (*c*) the heat capacities for the two solids and for the solid solutions are identical. This last assumption is made in all published treatments [*e.g.*, Zhdanov (8) and Ashbee (9)] and is implicit in the use of the terminology $K(T)$ rather than $K(T, x)$. This assumption may not necessarily be a good one (as evidenced by a great deal of thermoanalytical work) but is, nevertheless, made here.

A typical plot of F as a function of composition x at temperatures $T_1 > T_2 > T_3 > T_4$, where T_2 is the eutectic temperature, is shown in Fig. 1. The curves in Fig. 1 are based on $2V_{AB} > V_{AA} + V_{BB}$ (9). The corresponding binary melting-point diagram is shown in Fig. 2.

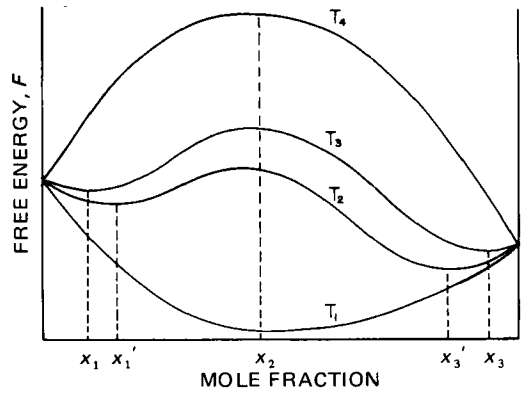

Figure 1—*Free energy* versus *mole fraction of a binary mixture forming a random solid solution. The indicated temperatures are of the rank* $T_1 > T_2 > T_3 > T_4$. *The minima correspond to solid solution compositions at the indicated temperatures (at which the mixture is solid). In this example,* T_2 *could be the eutectic temperature and* T_3 *could be room temperature.*

There are three extrema (at compositions x_1, x_2, and x_3) when solid solutions exist, the two minima (x_1 and x_3) being at the compositions of the solid solutions at the eutectic temperature, T_2. That the maximum coincides with the eutectic composition is, however, not thermodynamically obvious. The values for x_1, x_2, and x_3 satisfy the first derivative equation of Eq. 1 when equated to zero, *i.e.*:

$$\left.\frac{\partial F}{\partial x}\right|_T = NZ\{xV_{AA} - (1-x)V_{BB} + (1-2x)V_{AB}\} + NkT \ln\{x/(1-x)\} = 0 \quad \text{(Eq. 2)}$$

where k is Boltzmann's constant. Inserting $x = x_1$, x_2, and x_3 then yields three equations with three unknowns:

$$x_1V_{AA} + (x_1-1)V_{BB} + (1-2x_1)V_{AB} = -\frac{kT}{Z}\ln[x_1/(1-x_1)] \quad \text{(Eq. 3a)}$$

$$x_2V_{AA} + (x_2-1)V_{BB} + (1-2x_2)V_{AB} = -\frac{kT}{Z}\ln[x_2/(1-x_2)] \quad \text{(Eq. 3b)}$$

$$x_3V_{AA} + (x_3-1)V_{BB} + (1-2x_3)V_{AB} = -\frac{kT}{Z}\ln[x_3/(1-x_3)] \quad \text{(Eq. 3c)}$$

where V_{AA}, V_{BB}, and V_{AB} are the unknowns. There is no unique solution to these three equations, because the determinant $D = |x_i, (x_i - 1), (1 - 2x_i)|$ equals zero for all values of x_i; *i.e.*, there is a linear dependence among x_1, x_2, and x_3. If the coefficients of dependence are denoted α_1 and α_2, it follows from Eqs. 3*a*–3*c* that:

$$\alpha_1x_1 + \alpha_2x_2 = x_3 \quad \text{(Eq. 4a)}$$

$$\alpha_1(x_1-1) + \alpha_2(x_2-1) = (x_3-1) \quad \text{(Eq. 4b)}$$

$$\alpha_1(1-2x_1) + \alpha_2(1-2x_2) = 1-2x_3 \quad \text{(Eq. 4c)}$$

The solutions to Eqs. 4*a*–4*c* are:

$$\alpha_1 = (x_2 - x_3)/(x_2 - x_1) \quad \text{(Eq. 5)}$$

$$\alpha_2 = (x_3 - x_1)/(x_2 - x_1) \quad \text{(Eq. 6)}$$

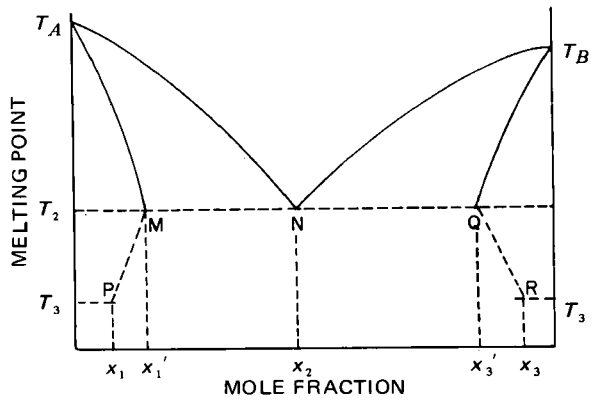

Figure 2—*Binary melting-point diagram corresponding to the free energy diagram in Fig. 1.* T_A-N *and* T_B-N *are liquidus lines and* T_A-M *and* T_B-Q *are solidus lines.*

For Eqs. 3*a*–3*c* to have solutions, the right-hand sides must also be subject to the linear dependence coefficients; *i.e.*:

$$\alpha_1 \ln [x_1/(1-x_1)] + \alpha_2 \ln [x_2/(1-x_2)] = \ln [x_3/(1-x_3)] \quad \text{(Eq. 7)}$$

Inserting Eqs. 5 and 6 into Eq. 7 and multiplying through by $(x_2 - x_1)$ then give:

$$(x_2 - x_3) \ln [x_1/(1-x_1)] + (x_3 - x_1) \ln [x_2/(1-x_2)] = (x_2 - x_1) \ln [x_3/(1-x_3)] \quad \text{(Eq. 8)}$$

Equation 8 is a consistency relation and should apply to the two minima and the maximum. Since the minima at the eutectic temperature are known and are at the solid solution compositions, x_2 can be found; in this fashion, it can be checked whether x_2 occurs on or about the eutectic composition.

Two examples will illustrate the utility of Eq. 8. If $x_2 = 0.5$, then Eq. 8 implies that:

$$(0.5 - x_3) \ln [x_1/(1-x_1)] + (x_3 - x_1) \ln (1.0) = (0.5 - x_1) \ln [x_3/(1-x_3)] \quad \text{(Eq. 9)}$$

The second term is zero, and the solution is $x_1 = (1 - x_3)$; *i.e.*, even when V_{AA} is different from V_{BB}, a maximum at $x_2 = 0.5$ implies symmetry.

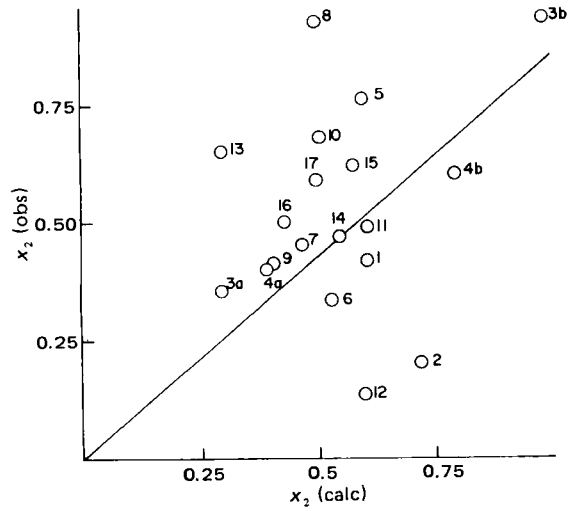

Figure 3—*Plot of the observed* versus *calculated eutectic composition in binary mixtures. The data are from Table I. Numbers correspond with systems shown in Table I.*

Table I—Referenced Binary Systems with Observed and Calculated Values of Eutectic Composition

Composition Number	System	Reference	x_2 (obs)	x_2 (calc)
1	Chloramphenicol–urea	5	0.37	0.62
2	Sulfathiazole–urea	7	0.20	0.73
3*a*	Deoxycholic acid–menadione	6	0.36	0.30
3*b*	Deoxycholic acid–menadione	6	0.94	0.98
4*a*	Quinine–phenobarbital	6	0.40	0.39
4*b*	Quinine–phenobarbital	6	0.60	0.79
5	Theophylline–phenobarbital	6	0.76	0.60
6	Phenacetin–phenobarbital	6	0.33	0.53
7	Acetaminophen–phenobarbital	6	0.45	0.47
8	Phenytoin–phenobarbital	6	0.93	0.50
9	Aspirin–phenobarbital	6	0.35	0.40
10	*p*-Nitroaniline–7-(2-hydroxyethyl)theophylline	2	0.68	0.51
11	*m*-Methoxybenzoic acid–7-(2-hydroxyethyl)theophylline	2	0.49	0.61
12	Aminopyrine–7-(2-hydroxyethyl)theophylline	2	0.13	0.65
13	Anthranilic acid–caffeine	2	0.65	0.30
14	Benzoic acid–7-(2-hydroxyethyl)theophylline	2	0.47	0.55
15	Benzidine–7-(2-hydroxyethyl)theophylline	2	0.62	0.58
16	Aminopyrine–allobarbital	2	0.50	0.38
17	*p*-Nitrophenol–7-(2-hydroxyethyl)theophylline	2	0.59	0.50

In the second example, it is assumed that x_2 does not occur at 0.5. The following figures are used: $x_1 = 0.2$ and $x_3 = 0.85$. These values inserted in Eq. 8 give the following equation in one unknown (x_2):

$$(x_2 - 0.85) \ln \{0.2/0.8\} + (0.85\text{-}0.2) \ln \{x_2/(1-x_2)\} = (x_2 - 0.2) \ln \{0.85/0.15\} \quad \text{(Eq. 10)}$$

The solution is found by trial and error and is $x_2 = 0.43$.

This method was used to determine x_2 from known values of x_1 and x_3 (solid solution compositions at the eutectic temperature); the values were taken from reported binary melting-point diagrams (2, 5–7). The calculated values of x_2, denoted x_2(calc), are tabulated *versus* the eutectic composition, denoted x_2(obs), in Table I and plotted in Fig. 3.

If x_2(obs) = x_2(calc), then a least-squares fit regression line of the data should be such that the 95% confidence limits of the intercept should include zero and the 95% confidence limits of the slope should include unity. This is the case since the least-squares fit equation is x_2(obs) = (0.37 ± 0.75)x_2(calc) + (0.27 ± 0.45). The data, as shown in Fig. 3, are scattered and the fit obviously is not excellent ($r = 0.22$, which is significant at the 75% level).

If x_2(obs) = x_2(calc) is plotted as a confined least-squares fit, then the 95% confidence limits on the slope should include unity, as indeed they do; the least-squares fit of the confined line is x_2(obs) = (0.85 ± 0.40)x_2(calc) (Fig. 3). Other tests fail to reject the hypothesis that x_2(obs) = x_2(calc). For instance, the differences x_2(obs) − x_2(calc) in Table I should be normally distributed about a mean of zero. A χ^2-test fails to show them to be nonnormal ($\chi^2 = 0.2 \ll \chi^2_{2,0.05} = 6$). Therefore, the data apparently are in-

dicative of the proposed theory and data treatment method.

(1) K. Sekiguchi and N. Obi, *Chem. Pharm. Bull.*, **9**, 866(1961).
(2) K. Sekiguchi, Y. Ueda, and Y. Nakamori, *ibid.*, **11**, 1108(1963).
(3) *Ibid.*, **11**, 1123(1963).
(4) K. Sekiguchi, N. Obi, and Y. Ueda, *Chem. Pharm. Bull.*, **12**, 134(1964).
(5) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *J. Pharm. Sci.*, **54**, 1145(1965).
(6) J. K. Guillory, S. Huang, and J. Lach, *ibid.*, **58**, 301(1969).
(7) W. L. Chiou and S. Niazi, *ibid.*, **60**, 1333(1971).
(8) G. S. Zhdanov, "Crystal Physics," Academic, New York, N.Y., 1965, pp. 335–338.
(9) K. H. G. Ashbee, in "Problems in Solid State Physics," H. J. Goldsmid, Ed., Academic, New York, N.Y., 1968, p. 191.

J. T. Carstensen [x]
Shabbir T. Anik
School of Pharmacy
University of Wisconsin
Madison, WI 53706

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[x] To whom inquiries should be directed.

Toxic Substances Produced by *Fusarium* I: Trichothecene Derivatives from Two Strains of *Fusarium oxysporum* f. sp. *carthami*

Keyphrases □ *Fusarium oxysporum*—cultured, trichothecenes isolated, phytotoxic and dermatitic properties evaluated □ Phytotoxins—trichothecenes isolated from *Fusarium oxysporum*, activity evaluated □ Fungi—*Fusarium oxysporum* cultured, trichothecenes isolated, phytotoxic and dermatitic properties evaluated

To the Editor:

Fusarium oxysporum Schlecht. f. *carthami* Klisiewicz & Houston is involved in the typical wilt disease of safflower (*Carthamus tinctorius* Linn.) (1, 2). However, the nature of the substance or substances responsible for the phytotoxic effects has not been evaluated previously. Since food materials infected with a species of *Fusarium* have often contained substances that produce high mammalian toxicity (3), the presence of *F. oxysporum* in safflower is thus cause for alarm.

Although previous investigations with other forms of *F. oxysporum* furnished biologically active 12,13-epoxytrichothecenes (3, 4), there is no report on this form of the fungus producing any toxic substance in artificial media or the host tissue. The present investigation was designed to isolate and study the substances produced by the fungus in artificial media that are responsible for the pathogenic property.

Two strains (weakly parasitic and virulent) were collected from Varanasi, India (2), and their identity was confirmed by the Commonwealth Mycological Institute, Kew, England [CMI (IMI-166917 and IMI-186539)]. These strains were separately grown in Richard's solution (200 ml) (5) in still culture flasks (1 liter) at 21° for 21 days.

When sprayed on safflower plants, the culture filtrates caused severe scorching of foliage accompanied by marked retardation of stem growth and frequently death of the plants. Even in high dilution (1:100), the culture filtrates inhibited root elongation of 2-day-old seedlings. There was no inhibition of germination of safflower seeds when sown in a medium containing the culture filtrates, but the seedlings showed the usual toxic symptoms.

The effects of the intracellular toxins (from the mycelium) were more severe than those of the extracellular ones (from the culture filtrates). For the extraction of the intracellular toxins, the mycelia were first washed and then macerated with water in a high-speed blender. The extract was passed through a bacteria-proof filter. The effect of the filtrate was tested on the host plant in the usual way.

The toxic substances were isolated from the culture filtrates by solvent extraction. In a typical experiment, the culture filtrate (5 liters) from the weakly parasitic strain at the natural pH (about 3.8) was successively extracted with chloroform (3 liters) and ethyl acetate (3 liters). The aqueous mother liquor was then concentrated to about 500 ml under reduced pressure and again extracted with hot ethyl acetate (3 liters). The three extracts were processed separately.

Evaporation of the solvent from the chloroform extract afforded a brown oil (0.88 g) which, in very low concentration (1–2 ppm), produced phytotoxic effects similar to those shown by the culture filtrates. It showed the presence of about six trichothecene derivatives by TLC (fluorescence under UV light, characteristic Ehrlich-reagent positive spots) (6).

A solution of the oil in ether–hexane (2:1, 50 ml) was filtered and set aside. Colorless crystals (58 mg) resulted, mp 158–160°; $[\alpha]_D^{22}$ +18° (c 0.52, ethyl alcohol); UV: λ_{max} (ethyl alcohol) only an end absorption; mass spectra: *m/e* 366 (M^+). The melting point, optical rotation, and spectral properties of this compound were indistinguishable from those of diacetoxyscirpenol (4β,15-diacetoxy-12,13-epoxytrichothec-9-en-3α-ol) (6).

The oily residue obtained from the ether–hexane mother liquor was dissolved in benzene (10 ml) and chromatographed over a magnesium silicate column (1.2 × 14 cm). Elution with chloroform and chloroform–methanol (98:2) yielded several 20-ml fractions containing a toxic material, as indicated by a rat skin bioassay (7). The residue from the concentrated eluates, when crystallized from hexane–benzene, gave colorless needles (33 mg), mp 148–150°; $[\alpha]_D^{22}$ +17.8° (c 0.48, ethyl alcohol), +16.5° (c 0.52); UV: λ_{max} (ethyl alcohol) only an end absorption; IR: ν_{max} (potassium bromide) 3400 (br), 1722, and 1245 cm^{-1}; mass spectra: *m/e* 466 (M^+, relative intensity 0.5%), 364 ($M^+ - C_5H_{10}O_2$, 22%), 322 (2.5), 305 (2), 304 (7), 291 (14), and 121 (100). The melting point, optical rotation, and spectral properties of this compound were indistinguishable from those of T-2 toxin (4β,15-diacetoxy-8-isovaleroxy-12,13-epoxytrichothec-9-en-3α-ol) (8).