Effect of Initial Surface Pressure—Figure 4 shows the π–A curves of cholesterol films at initial surface pressures of 0 and 7 dynes/cm. prior to and following a 35-min. exposure to 175 ± 25 p.p.m. nitrogen dioxide.

Exposure to NO_2, in the case of the film adjusted to an initial surface pressure of 7 dynes/cm., resulted in a steady decrease in pressure, reaching a value of zero in about 20 min. Furthermore, following a 35-min. exposure to NO_2, the film exposed at this higher surface pressure exhibited a greater PAL of cholesterol.

The orientation of cholesterol molecules in a monomolecular film is markedly influenced by the surface pressure of the film. At zero pressure the molecules would be expected to be essentially flat on the surface, occupying relatively large areas. At 7 dynes/cm., the cholesterol molecules are closely packed and occupy an area that indicates they are in a vertical position with the 3-hydroxy groups anchored in the subphase. Thus, at zero pressure, both polar sites of the cholesterol molecule (*i.e.*, the 3-hydroxy group and the 5–6 double bond) would likely be associated with the aqueous subphase. At 7 dynes/cm., only the 3-hydroxy group would be associated with the subphase, while the double bond would be in the gas phase.

Since the PAL of cholesterol upon exposure to NO_2 is greater when the molecules are in the vertical position, it appears that association of both polar sites with the subphase inhibits the oxidation of cholesterol by NO_2. Such a postulation is supported by Altshuller and Cohen (7), who reported that the oxidation of olefins by NO_2 occurred in the gas phase along with some nitration, while little or no oxidation was observed (though nitration did occur) in the aqueous phase reaction.

CONCLUSIONS

While it appears that the rate of loss of cholesterol from a monomolecular film is dependent on the concentration of NO_2, the rate of loss at levels of NO_2 normally found in polluted air ($\approx$0.5 p.p.m.) would be very slow. Thus, only at relatively high levels of NO_2 would the loss of cholesterol be significant, even over several hours. This can be seen from the extrapolated portion of the plot in Fig. 5 of the rate of loss of cholesterol *versus* the concentration of NO_2.

Whether these results can be related to the effect of NO_2 on cell membranes or to the *in vivo* exposure of humans or animals to NO_2 has not been established. However, the work of Steadman *et al.* (8), in which animals were exposed to wide ranges of concentrations of NO_2 for varying periods of time, does show some correlation. These workers noted that animals exposed to NO_2 concentrations of 70 p.p.m. for 8 hr. suffered pulmonary edema and vascular congestion with high mortality rates. On the other hand, exposure to a concentration of 0.5 p.p.m. even for as long as 90 consecutive days produced no apparent untoward effects. Thus, it may be that the loss of cholesterol from surface films, which would be very slow at a NO_2 concentration of 0.5 p.p.m. and relatively fast at a concentration of 70 p.p.m., gives some indication of the loss of this essential lipid from cell membranes and of the observed clinical effects of NO_2.

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Crystal Pseudopolymorphism of Cephaloglycin and Cephalexin

RALPH R. PFEIFFER, K. S. YANG, and MARY ANN TUCKER

Abstract □ The systematic investigation of the crystallization of cephaloglycin and cephalexin leads to a better understanding of the part that pseudopolymorphic crystal transitions play in the analysis, processing, and formulation of these and many other pharmaceutical compounds. The employment of solubility *versus* solvent composition diagrams to detect various crystal forms of compounds is discussed. This appears to be a convenient and sensitive method for detecting new crystalline phases. It should find application whenever crystallizations are performed with more than one solvent, and particularly when instability of the compound at elevated temperatures prevents the use of conventional thermal methods or when poor crystal development limits the use of microscopic methods. Interpretation of vapor pressure–composition relationships for various crystal forms of these compounds points to the advisability of obtaining such data for all pharmaceutical solids.

Keyphrases □ Crystal pseudopolymorphism—cephaloglycin, cephalexin □ Cephaloglycin, cephalexin—pseudomorphic crystallization □ UV spectrophotometry—analysis □ X-ray powder diffraction—analysis □ NMR spectroscopy—analysis

It is the responsibility of the pharmaceutical chemist to become familiar with the crystallizing properties of drugs in order to control the crystal form, habit, size, size distribution, degree of crystallinity, and state of aggregation of the drug particles. These parameters often determine the acceptability of bulk properties (mixing, tableting, filling, dusting, *etc.*) and pharmaceutical performance (dissolution, biological availability, chemical and physical stability, suspendibility, rheology, *etc.*). Haleblian and McCrone (1) adequately documented the broad aspects of this argument in a recent review article on polymorphism, wherein they

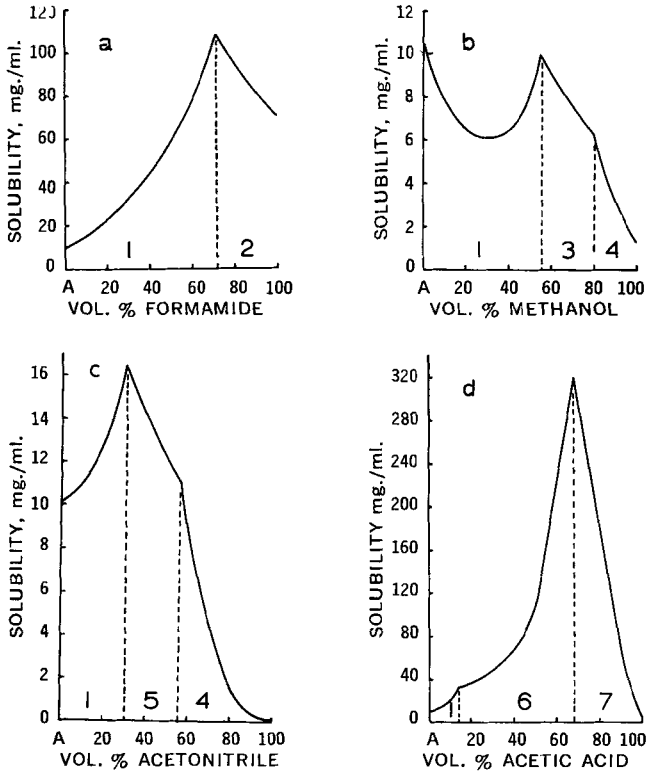


Figure 1—*Solubility of cephaloglycin in binary solvent mixtures at 25°. A = water in all cases. Arabic numerals refer to solid phases in Table I.*

also discussed thoroughly the preparation and characterization of true polymorphic[1] crystal forms by thermal methods. The related and equally pertinent phenomenon of pseudopolymorphism, *i.e.*, modification caused by inclusion of solvent in the crystal structure, apparently has received no such general treatment in the pharmaceutical literature, although many specific cases of pseudopolymorphism in drugs have been reported (2–4).

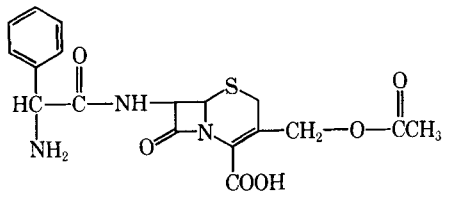
This article suggests some new approaches to the study of pseudopolymorphic crystal transformations as they apply to pharmaceutical manufacturing practice. As a case in point, it reports the crystallizing properties of the antibiotics cephaloglycin (I) (5) and cephalexin (II) (6), both of which occur in a wide variety of solvated crystal forms.

EXPERIMENTAL

Reagents—The cephaloglycin and cephalexin each had a microbiological potency (5, 6) of >970 mcg./mg.; phase-solubility analysis in water indicated a purity of 97 ± 1%. (Both analyses were corrected for volatile components.)

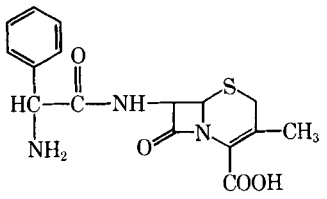
Solvents and other chemicals were reagent grade and were taken from freshly opened packages without further purification.

Equilibration with Solvents—Finely ground cephaloglycin (dihydrate) or cephalexin (monohydrate) was suspended and equilibrated at 25° in a variety of solvents and solvent mixtures. In a typical experiment, 11 tubes, containing a total of 5 ml. of two miscible solvents in ratios ranging from 1:0 to 0:1, were prepared. A quantity of the compound, sufficient to ensure the presence of a



cephaloglycin

I



cephalexin

II

substantial amount of solid phase throughout the equilibration period, was added to each tube. Three hours of shaking on a vibrating mixer,[2] with occasional manual stirring to disperse any lumps, proved satisfactory for the present purpose. The tubes were then centrifuged, and the clear supernates were analyzed spectrophotometrically at 260 mμ for dissolved cephaloglycin or cephalexin. A preliminary solubility *versus* solvent composition diagram was constructed from the data. The crystals, wet with mother liquor, were examined under a polarizing microscope and by X-ray powder diffraction to detect changes in the solid phase.

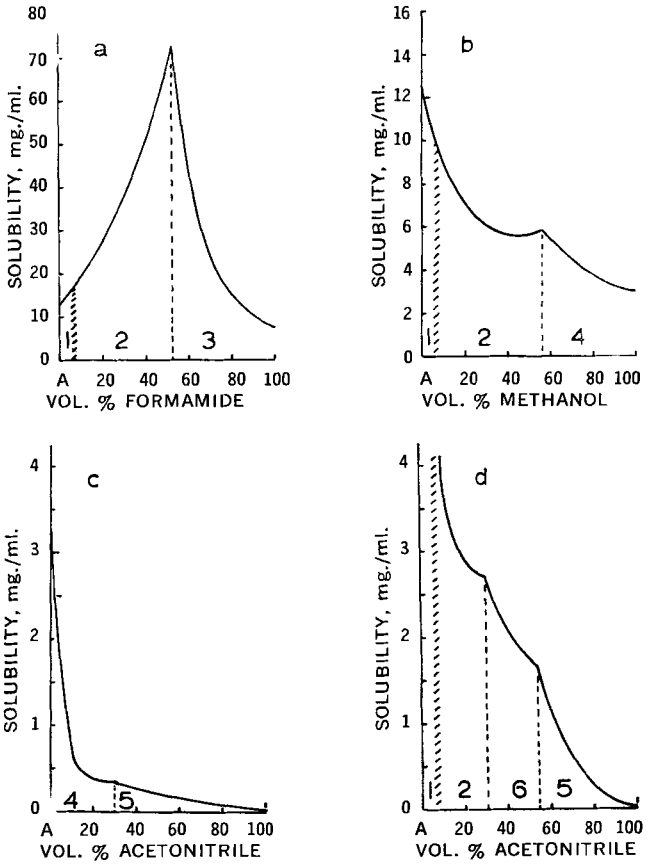


Figure 2—*Solubility of cephalexin in binary solvent mixtures at 25°. A = water in Sections a, b, and d. A = methanol in Section c. Arabic numerals refer to solid phases in Table II.*

[1] True crystal polymorphism involves forms of identical chemical composition. Thus, a compound may exhibit pseudopolymorphism by forming variously solvated crystals; each of these solvates (including the unsolvated form) may exhibit polymorphism.

[2] Vibromixer, model El, AG. für Chemie-Apparatebau, Zurich, Switzerland.

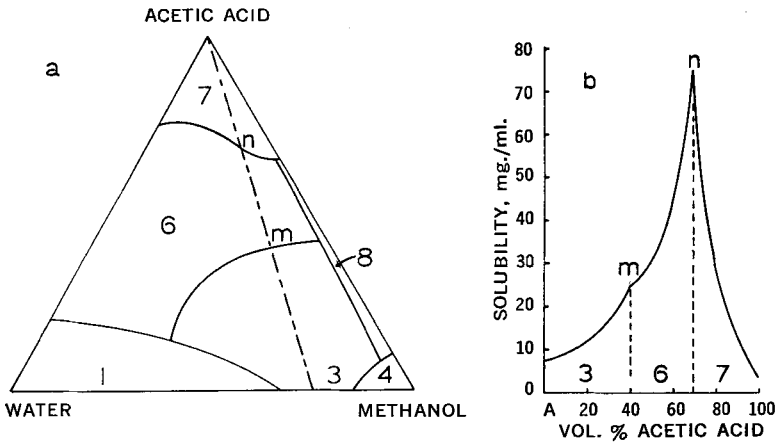


Figure 3—*(a) Stability zones of cephaloglycin crystal forms in mixtures of water–methanol–glacial acetic acid. The broken line indicates the compositions used in Section b. (b) Typical solubility diagram (also Fig. 1, Section d) used to locate zone boundaries in Section a. A = 75% methanol–water. Points m and n correspond in the two sections. Arabic numerals refer to solid phases in Table I.*

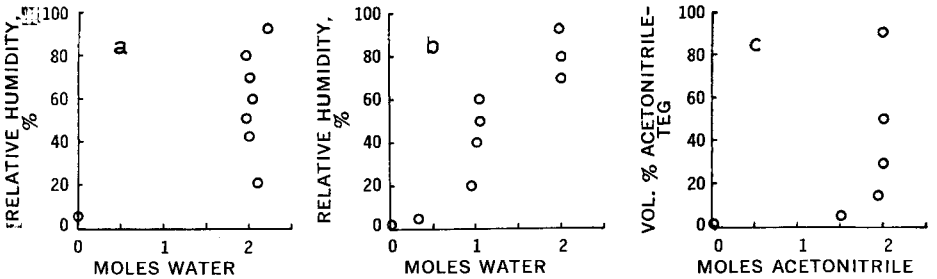


Figure 4—*Crystal–vapor equilibria: a, cephaloglycin–water; b, cephalexin–water; and c, cephalexin–acetonitrile. The ordinate in Section c describes the volume composition of acetonitrile–triethylene glycol (TEG) used to provide a range of acetonitrile vapor pressures whose exact magnitude was not determined.*

Preparation of Pure Reference Phases—To obtain the various crystalline phases in a homogeneous form, each phase was crystallized from the totally dissolved state. A solution (about 10% w/v) of the antibiotic in the appropriate solvent mixture, *i.e.*, a composition that had yielded a new solid phase in the equilibration, was prepared by acidification with an amount of concentrated hydrochloric acid[3] equivalent to the amount of solute. Precipitation of the zwitterion in the desired crystal form was then effected by slow addition of an equivalent amount of concentrated ammonium hydroxide.[3] The resulting crystals were removed by filtration and dried under conditions that would not disrupt the crystal stoichiometry (see *Discussion*). Analysis by selected methods followed. These methods always included X-ray powder diffraction and some of the following methods: elemental analysis, thermogravimetry, titration, spectrophotometry (NMR and UV), and specific analysis for water (Karl Fischer).

Determination of Solubility *versus* Solvent Composition Diagrams—The solubility determinations were repeated under the earlier conditions but using the proper equilibrium solid phases at the start of the individual equilibrations. In this way the solubility *versus* composition curve for each solid phase could be obtained over its entire stability range without relying on phase transformations. The equilibrium diagrams in Figs. 1 and 2 were constructed using the nonoverlapping parts of these curves. It was found that the same solubility information (agreement within 5%) could be obtained by using admixtures of two appropriately chosen solid phases for equilibrations near transition points. This procedure was used to obtain the information in Fig. 3. It was assumed that the stable (least soluble) phase was essentially controlling the solubility, even in the presence of unconverted metastable crystals.

Equilibration with Vapor—Vapor equilibration chambers were made from 5-lb. ointment jars with tight fitting lids. Each jar contained about 500 ml. of a constant vapor pressure slurry. Water vapor pressures were determined with electric hygrometer[4] sensors. In one study, the jars contained 200 ml. of acetonitrile and triethylene glycol mixed in various ratios, thus providing atmospheres covering a wide range of acetonitrile vapor pressures. Previously analyzed samples of about 1 g., spread thinly in open weighing dishes, were set on trivets within the jars. The dishes were covered and weighed daily for 1 week, although constant weight was achieved after 2 or 3 days. Samples showing significant gain or loss in weight were checked for phase changes by X-ray diffraction. All changes in solvent content or phase identity were found to be reversible upon subsequent exposure of the samples to the appropriate atmospheres.

[3] Possible effects caused by the water and salt thus introduced were ignored.

[4] Hygrodynamics, Inc., Silver Spring, Md.

RESULTS AND DISCUSSION

Because cephaloglycin and cephalexin were each observed to occur in many solvated crystal forms (Tables I–IV), and often in widely varying mixtures of these forms, a systematic study of the crystallizing properties of these compounds was undertaken. The properties of the pure crystalline components under equilibrium conditions were first explored. Mixtures of these components resulting from kinetic factors are subsequently considered.

Crystal–Solution Equilibria—The solubility diagrams resulting from this study (Figs. 1 and 2) provide a clear indication of not only the existence of multiple crystalline forms of cephaloglycin and cephalexin but also the ranges of solvent compositions in which the various forms are stable.

When considering crystal transformations that occur with changes

Table I—Some Solvated Crystal Forms of Cephaloglycin

Moles Solvent/Moles Cephaloglycin[a]	Method[b]
1. $2H_2O$	a, b, c, d, e, f, g
2. Formamide	a, b, e, f
3. Methanol · H_2O	a, b, c, e, f, g
4. Unsolvated (anhydrate)	a, b, c, e, f, g
5. Acetonitrile · H_2O	a, b, d, e, f
6. Acetic acid · H_2O	a, b, c, e, f, g, h
7. 2 Acetic acid	a, b, e, f, g, h
8. Acetic acid · methanol	a, b, e, f
9. Ethanol · H_2O	a, b, e
10. *N*-Methylformamide	a, b, i

[a] Arabic numerals correspond to phases in Figs. 1 and 3. [b] Compositions were determined by: a, uniqueness of X-ray powder diffraction pattern; b, Karl Fischer water; c, thermogravimetric analysis; d, vapor pressure–composition diagram; e, UV spectrophotometry; f, NMR spectrometry; g, elemental analysis; h, titration; and i, qualitative stoichiometry only. The quantitative results confirmed the stated compositions to ±0.1 mole solvent. All forms, when recrystallized from water, had retained the X-ray diffraction properties of the starting material, indicating that no chemical modification of the antibiotic had occurred.

Table II—Some Solvated Crystal Forms of Cephalexin[a]

Moles Solvent/Moles Cephalexin	Method
1. $2H_2O$	a, b, d, e
2. H_2O	a, b, d, e, f, g
3. Formamide	a, i
4. Methanol	a, b, g
5. 2 Acetonitrile	a, d, e
6. Acetonitrile·H_2O	a, b, e
7. *N*-Methylformamide	a, i
8. *N*-Ethylformamide	a, i

[a] Arabic numerals correspond to phases in Fig. 2. See Table I for explanation of other symbols.

in solvent composition, it is helpful to recall that the changing solubility values along a smooth curve refer to an equilibrium between a solid phase of definite, unchanging composition and a solution of changing properties. The proportions of the variously solvated, aggregated, and charged solute species in solution vary with and are determined by the composition of the solvent. At equilibrium, the *concentration* of solute in solution, *i.e.*, the solubility, may vary as the solvent changes, but the *activity of the crystallizing molecular species* remains constant, *i.e.*, equals that of the crystalline form present. When individual solubility *versus* solvent composition curves of several available crystal forms cross one another to give a peak or cusp, a phase transition point is revealed. At such points the two solid phases represented by the separate curves have the same solubility and may therefore coexist at equilibrium. At other compositions, the respectively more soluble phase is necessarily metastable with respect to the less soluble one.

Phase Relationships in Ternary and Quaternary Solvent Mixtures—The compositions of 3-solvent mixtures in which the various crystal forms of a compound are stable can be conveniently represented by a triangular diagram such as the one presented in Fig 3*a* for cephaloglycin in water–methanol–glacial acetic acid. Each edge of the triangle was marked with the zone boundaries determined earlier in the respective binary solvent mixtures (Figs. 1*b* and 1*d*), and the positions of boundaries within the triangle were located by determining solubilities in series of appropriately chosen ternary solvent mixtures and interpreting the resulting curves in the manner described earlier (Fig. 3*b*).

Occasionally, a ternary mixture of solvents serves as a recrystallizing solvent, with a fourth miscible liquid acting as the antisolvent. The phase relationships, or stability zones, of solute crystals in such a system can be analogously represented by a triangular pyramid.

Doubly Solvated Crystals—Both cephaloglycin and cephalexin display the ability to crystallize with simultaneous stoichiometric inclusion of two solvents in the crystal lattice. The existence and identity of these phases are evident from their unique and uniform microscopic appearance, their X-ray pattern, the solubility diagram, and the various analyses of the crystals (Tables I–IV).

Crystal–Vapor Equilibria—One cause of erratic changes in the properties of powders is their physical interaction with solvent vapors. In the interests of crystal stability, therefore, the vapor composition of the environment, especially with regard to water vapor, must be given due consideration throughout the history of a powder, and the quantitative equilibrium relationships between crystal and vapor phases must be determined.

Vapor pressure *versus* composition diagrams of the kind presented for cephaloglycin–water, cephalexin–water, and cephalexin–acetonitrile (Fig. 4) delimit the vapor pressure range over which a solvated crystal is stable after isolation from the mother liquor. These diagrams also reveal the true stoichiometry of the solvated crystals.

Multiple Hydrated Forms—When a compound exists in several hydrated crystal forms at one temperature, as is clearly the case

Table III—X-Ray Powder Diffraction Data for Some Crystal Forms of Cephaloglycin

Cephaloglycin —Dihydrate—		Cephaloglycin —Anhydrate—		Cephaloglycin Methanolate —Hydrate—		Cephaloglycin —Formamidate—		Cephaloglycin Acetonitrile —Hydrate—		Cephaloglycin Acetic Acid —Hydrate—		Cephaloglycin 2 Acetic Acid —Solvate—	
d[a]	I/I_1[b]	d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1
16.8	1.00	9.40	1.00	10.64	1.00	10.9	1.00	20.0	1.00	10.6	1.00	11.5	1.00
11.0	0.04	8.58	0.50	9.40	0.60	9.55	0.50	16.0	0.40	9.25	0.80	9.00	0.30
9.95	0.30	7.13	0.30	7.56	0.50	8.50	0.20	11.9	1.00	7.60	0.50	7.50	0.30
8.35	0.40	6.70	0.50	6.62	0.40	7.65	0.40	9.30	0.50	6.60	0.60	6.70	0.50
7.15	0.50	5.71	0.40	5.86	0.40	6.80	0.40	8.50	0.20	5.75	0.65	5.70	0.40
6.15	0.20	5.18	0.15	5.50	0.05	5.05	0.30	7.30	0.30	5.42	0.20	5.40	0.40
5.80	0.10	4.69	1.00	5.00	0.15	5.40	0.30	6.90	0.30	4.90	0.30	4.97	0.30
5.40	0.70	4.37	0.70	4.69	1.00	4.90	0.30	5.80	0.50	4.65	1.00	4.75	0.30
4.90	0.10	4.28	0.90	4.52	0.20	4.60	1.00	5.40	0.10	4.48	0.40	4.60	0.70
4.55	0.10	3.98	0.60	4.23	0.10	4.35	0.40	5.20	0.30	4.22	0.30	4.42	0.10
4.32	0.30	3.75	0.04	4.09	0.10	4.16	0.30	4.78	0.60	4.08	0.30	4.12	0.60
4.10	0.10	3.58	0.70	3.43	0.10	3.96	0.40	4.48	0.70	3.89	0.30	3.90	0.05
4.00	0.40	3.36	0.40	3.75	0.60	3.70	0.30	4.40	0.20	3.70	0.70	3.78	0.70
3.72	0.40	3.14	0.20	3.50	0.90	3.56	0.90	4.15	0.20	3.50	1.00	3.70	0.70
3.45	0.40	3.03	0.30	3.42	0.20	3.35	0.30	3.97	0.15	3.40	0.40	3.00	0.05
3.32	0.20	2.87	0.08	3.31	0.20	3.25	0.15	3.74	0.60	3.30	0.35	3.35	0.60
3.20	0.10	2.73	0.70	3.23	0.30	3.18	0.30	3.55	0.30	3.20	0.30	3.24	0.05
3.06	0.10	2.60	0.40	3.14	0.30	2.90	0.20	3.40	0.10	3.08	0.30	3.18	0.05
2.92	0.30	2.52	0.15	3.09	0.05	2.80	0.05	3.16	0.10	2.96	0.15	3.07	0.50
2.79	0.20	2.39	0.10	2.95	0.15	2.70	0.20	2.90	0.50	2.94	0.15	2.91	0.40
2.61	0.20	2.28	0.10	2.82	0.05	2.61	0.30	2.84	0.50	2.86	0.20	2.74	0.15
2.54	0.10	2.23	0.10	2.76	0.05	2.55	0.15	2.65	0.05	2.80	0.20	2.66	0.15
2.42	0.15	2.12	0.02	2.69	0.05	2.40	0.15	2.55	0.10	2.66	0.20	2.61	0.05
2.31	0.20	2.02	0.02	2.64	0.05	2.35	0.15	2.45	0.05	2.00	0.25	2.55	0.10
2.24	0.10	1.93	0.02	2.54	0.10	2.28	0.15	2.35	0.10	2.55	0.15	2.47	0.20
2.19	0.08	1.88	0.02	2.47	0.05	2.24	0.15	2.20	0.05	2.48	0.15	2.44	0.20
2.13	0.08	1.68	0.02	2.42	0.10	2.15	0.10	2.12	0.15	2.45	0.15	2.40	0.05
2.08	0.20			2.30	0.30	2.07	0.05	1.80	0.10	2.34	0.10	2.30	0.30
1.97	0.06			2.23	0.20	1.90	0.05			2.26	0.20	2.24	0.30
1.94	0.06			2.18	0.20					2.22	0.20	2.16	0.10
1.92	0.04			2.11	0.15					2.17	0.30	2.11	0.20
				1.99	0.15					2.08	0.30	2.04	0.05
				1.95	0.05					1.98	0.10	1.90	0.05
				1.88	0.10					1.96	0.10	1.85	0.20
				1.81	0.05					1.86	0.20		
				1.67	0.05					1.54	0.10		
				1.59	0.05					1.52	0.10		

[a] Interplanar spacing in A. U. [b] Relative intensity, visual estimation. Radiation; Cu/Ni. Norelco DeBye-Scherrer camera.

Table IV—X-Ray Powder Diffraction Data[a] for Some Crystal Forms of Cephalexin

Cephalexin Dihydrate		Cephalexin Monohydrate		Cephalexin Diacetonitrilate		Cephalexin Formamidate		Cephalexin Methanolate		Cephalexin Acetonitrile Hydrate	
d	I/I_1	*d*	I/I_1	*d*	I/I_1	*d*	I/I_1	*d*	I/I_1	*d*	I/I_1
14.3	0.20	15.15	0.40	11.18	0.05	10.4	1.00	10.8	0.80	9.50	1.00
13.0	1.00	11.85	1.00	9.81	1.00	9.80	0.40	8.55	0.50	8.60	0.05
11.1	0.15	11.00	0.30	8.84	0.50	9.00	0.05	7.60	0.50	7.20	0.10
9.65	0.20	9.36	0.20	6.96	0.40	8.00	0.05	7.05	0.50	6.70	0.40
7.43	0.50	8.55	0.50	6.55	0.30	6.90	0.50	6.55	0.50	5.76	0.60
7.28	0.10	7.86	0.50	5.64	0.60	6.15	0.20	6.00	0.60	5.25	0.40
6.68	0.50	6.89	0.20	5.40	0.10	5.61	0.15	5.35	0.40	4.76	0.70
5.80	0.80	5.98	0.40	4.79	0.50	5.25	0.60	5.00	0.05	4.60	0.30
5.44	0.20	5.39	1.00	4.64	0.60	5.00	0.10	4.85	1.00	4.35	0.60
5.29	0.40	4.97	0.50	4.39	0.30	4.75	0.70	4.65	1.00	4.25	0.30
4.94	0.60	4.76	0.40	4.19	1.00	4.38	0.90	4.11	1.00	3.96	0.60
4.47	0.15	4.57	0.40	4.03	0.30	4.08	0.70	3.98	1.00	3.80	0.10
4.21	0.90	4.39	0.60	3.66	0.15	3.80	0.50	3.89	0.30	3.55	0.50
3.97	0.70	4.22	0.60	3.61	0.10	3.00	0.60	3.76	0.40	3.35	0.50
3.79	0.60	4.00	0.70	3.41	0.40	3.42	0.50	3.53	0.30	3.18	0.10
3.54	0.50	3.86	0.70	3.28	0.30	3.26	0.50	3.28	0.55	3.12	0.20
3.39	0.15	3.60	0.80	3.12	0.30	3.10	0.30	3.08	0.15	2.99	0.30
3.27	0.30	3.46	0.30	3.06	0.05	2.95	0.40	2.95	0.30	2.90	0.20
3.15	0.40	3.24	0.60	2.90	0.15	2.23	0.40	2.87	0.20	2.75	0.30
3.02	0.40	3.10	0.60	2.80	0.20	2.75	0.30	2.76	0.20	2.63	0.45
2.92	0.50	2.98	0.40	2.77	0.15	2.68	0.20	2.05	0.40	2.55	0.20
2.78	0.30	2.90	0.60	2.66	0.15	2.60	0.30	2.57	0.20	2.36	0.20
2.64	0.20	2.81	0.40	2.59	0.20	2.50	0.20	2.54	0.20	2.30	0.45
2.55	0.15	2.73	0.20	2.50	0.05	2.37	0.30	2.45	0.20	2.24	0.10
2.45	0.10	2.68	0.40	2.46	0.10	2.28	0.40	2.36	0.20	2.12	0.50
2.34	0.15	2.63	0.10	2.37	0.20	2.22	0.15	2.32	0.20	1.90	0.05
2.26	0.10	2.47	0.30	2.31	0.05	2.15	0.15	2.17	0.15	1.81	0.10
2.15	0.05	2.41	0.15	2.25	0.15	2.11	0.15	1.99	0.05	1.68	0.15
2.09	0.10	2.31	0.30	2.16	0.15	2.02	0.05	1.89	0.05		
2.01	0.05	2.25	0.30	2.10	0.05	1.97	0.02				
1.98	0.05	2.12	0.10	1.97	0.02	1.93	0.02				
1.94	0.02	2.09	0.05	1.89	0.10	1.87	0.10				
1.86	0.02	2.01	0.02	1.85	0.10	1.72	0.05				
1.81	0.02	1.93	0.05	1.80	0.10	1.70	0.05				
1.77	0.02	1.87	0.05	1.67	0.02	1.67	0.05				
		1.85	0.05	1.53	0.02						
		1.82	0.10								
		1.72	0.05								
		1.66	0.02								
		1.62	0.02								

[a] See Table III for explanation of symbols and experimental conditions.

with cephalexin (Fig. 4*b*), the relative humidity *versus* composition diagram indicates the exact relative humidity that marks the transition from one form to another. Thus, cephalexin crystallized from water at room temperature is separated from solution as the dihydrate but converts to the monohydrate when the relative humidity is below 70%.

The Desolvated Crystal—Cephalexin·2 acetonitrile (Tables II and IV) serves as an example of what the authors believe to be a widely occurring but often undetected or improperly described case in powder technology, *viz.*—the "desolvated" crystal. This crystal is solvated stoichiometrically while in equilibrium with saturated solution; but when isolated and dried under ordinary conditions, it loses all except small mole fractions of solvent from the crystal lattice without thereby converting to another form (Fig. 4*c*). It is in this condition that the compound is commonly analyzed for the first time. When subsequently exposed to ambient atmospheres, the desolvated crystal takes up limited amounts of moisture, the water molecules presumably occupying lattice vacancies left by the original solvent. This water can be removed by drying or it can be displaced by exposing the crystal to vapor of the original solvent. In the latter case, the crystal returns to its native stoichiometric composition. All these changes may occur reversibly and without significant changes in the lattice, as evidenced by the X-ray powder diffraction pattern.[5] It is, therefore, proper in such cases to refer to a "desolvated" crystal, *e.g.*, "desolvated acetonitrilate crystal," "desolvated methanolate crystal," *etc.* This is necessary to differentiate it from a truly unsolvated unique structure (anhydrate), which would have different physicochemical properties despite having a chemical composition virtually identical to that of the desolvated crystal.

The authors have also observed the partial desolvation of the doubly solvated crystals listed in Tables I and II, but have not studied these more complex vapor–composition relationships.

The proper handling of solvated crystals to assure the maintenance of their stoichiometry for purposes of analysis should be evident from this discussion. Such crystals should always be isolated from the mother liquor and blotted free of liquid. To avoid exposure to atmospheres differing from that above the saturated mother liquor, well-sealed containers with a minimum of void space should be used for storage. Temperature changes should also be avoided.

Mixtures and Achievement of Equilibrium—While the results in this study lend themselves particularly well to interpretation by equilibrium principles, the authors realize that in studying other systems, kinetic barriers may be encountered that prevent the practical achievement of equilibrium, leading to highly variable mixtures.

Failure to observe equilibrium in laboratory experiments should, therefore, be regarded as highly pertinent to everyday industrial product variability. To be sure, it is sometimes desirable to deliberately make nonequilibrium forms, or even to reproducibly halt crystal transformations at a given stage. In any event, it is necessary to characterize all the available crystal forms to evaluate properly the role of crystal transformations in a pharmaceutical process.

[5] In some cases of crystal desolvation, the diffraction intensities of one or two planes are particularly sensitive to the solvent content of the crystals; in other cases, the loss of solvent is accompanied by a general loss of diffraction sharpness or by slight changes in the unit cell constants (2). However, the X-ray powder diffraction pattern retains its value in identifying the unique origin of the solid phase.

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Influence of Several Autonomic Drugs on Sodium Nitroprusside and Oxotremorine-Induced Hypothermia in Immature and Mature Mice

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Abstract □ Sodium nitroprusside and oxotremorine each produced body temperature depression that was independent of age. Atropine inhibited oxotremorine hypothermia in both age groups, but was ineffective in modifying thermal responses to nitroprusside in both age categories. Pilocarpine administration did not alter oxotremorine activity at either age level, while nitroprusside hypothermia was enhanced and partially reversed, respectively, in immature and mature mice. Nicotine and tetraethylammonium chloride were unable to modify hypothermia produced by oxotremorine and nitroferricyanide in adult mice. Nicotine enhanced nitroprusside hypothermia in 10-day-old mice, while temperature depression due to oxotremorine was unaffected in the same age group. Administration of tetraethylammonium chloride to immature animals treated with oxotremorine and nitroprusside resulted in greater temperature depression. Chlorpromazine, which produced no change in oxotremorine or nitroprusside hypothermia in 10-day-old mice, partially blocked oxotremorine-induced hypothermia in mature animals; the weak parasympatholytic phenothiazine produced no significant difference in hypothermia when given prior to nitroprusside in the adult group.

Keyphrases □ Hypothermia, mice—sodium nitroprusside, oxotremorine induced □ Oxotremorine, sodium nitroprusside-induced hypothermia—autonomic drugs effect □ Autonomic drugs effect—oxotremorine, sodium nitroprusside-induced hypothermia □ Age of mice, effect—induced hypothermia

The mammalian body "thermostat," because of its vast complexity, is susceptible to the action of various drugs and agents, particularly those that mimic or interfere with neurotransmitter substances. Oxotremorine, the active metabolite of tremorine, was shown to produce hypothermia in rodents through a central cholinergic mechanism (1, 2).

Age has been shown to modify the effects of certain centrally acting drugs in rats (3, 4). These animals are born functionally immature with poorly developed nervous systems. Bagdon and Mann (5) demonstrated the age factor in mice with the drug chlorpromazine. The hypothermia normally seen in mature mice in response to chlorpromazine administration was reversed in immature animals to marked hyperthermia.

During routine screening procedures in this laboratory, it was discovered that sodium nitroprusside induces a pronounced fall in the body temperature of mice. It was the purpose of this investigation, therefore, to compare the hypothermia caused by oxotremorine with that induced by sodium nitroprusside with respect to alteration by several autonomic drugs in order to ascertain the mechanism of action and to observe the effects of age on the thermic response.

EXPERIMENTAL

Young (10-day-old) and adult male mice (1398) of the Huntingdon Farms (HTF) strain were utilized in this investigation.

The drugs employed were as follows: oxotremorine,[1] sodium nitroprusside,[2] atropine sulfate,[3] pilocarpine nitrate,[3] nicotine,[4] tetraethylammonium chloride,[5] and chlorpromazine hydrochloride.[6] All solutions were freshly prepared with sterile water distilled in the laboratory.

A model 43 Telethermometer equipped with a No. 402 probe[7] was used for obtaining oral and rectal temperatures. The animals were kept at a constant environmental temperature of 23–24° for 24 hr. prior to and including the time of the experimental course.

All injections were given as a fixed dose in a volume of 0.05 ml. for immature mice and 0.25 ml. for mature mice. Calculation of doses for the immature mice was based upon an average weight of 4.5 g. obtained from preliminary experiments in this laboratory. Mature male mice, weighing from 20 to 25 g., received doses calculated on the basis of 22.5 g./mouse.

The agonists with their concentration and dose were: oxotremorine (0.0045%; 0.5 mg./kg.) and sodium nitroprusside (0.045%; 5 mg./kg.).

[1] Nutritional Biochemicals Corp.
[2] Baker Chemical Co., Phillipsburg, N. J.
[3] Merck & Co., Inc., Rahway, N. J.
[4] Eastman Organic Chemicals, Rochester, N. Y.
[5] Etamon, Parke, Davis & Company, Detroit, Mich.
[6] Thorazine, Smith Kline & French Laboratories, Philadelphia, Pa.
[7] Yellow Springs Instrument Co.