Methoxycaffeine, on the other hand, appears to be one of the strongest binders that the authors have found to date.

Partition studies between water and cyclohexane–chloroform (1:1 by volume) have shown that $K = 32\ M^{-1}$ for tryptophan–theophylline complex and $K = 26\ M^{-1}$ for tryptophan–caffeine complex. Although these values are somewhat smaller than those determined by ORD method (35 and 30, respectively), the agreement appears reasonable if one considers the difference in concentrations employed (for partition work original concentration of each species was $1 \times 10^{-2}\ M$). It is believed that phase-solubility method will give only approximate K values for these systems because of the great solubility of tryptophan in water (soly. $= 6 \times 10^{-2}\ M$), yielding a rather complex overall system at saturation.

Experiments based on the ORD method have shown that caffeine interacts with L-tyrosine ($K = 13.4\ M^{-1}$ at 25° in water measured at 315 mμ with tyrosine concentration $= 8 \times 10^{-4}\ M$ and caffeine concentration $= 1 - 5 \times 10^{-2}\ M$). The extent of interaction measured in the same manner between caffeine and L-phenylalanine was much less ($K = 6.3\ M^{-1}$ at 25° in water measured at 320 mμ with phenylalanine concentration $= 3 \times 10^{-3}\ M$ and caffeine concentration $= 1 - 6 \times 10^{-2}\ M$). Preliminary experiments on optical rotatory dispersion studies of tryptophan indicated that tryptophan also interacted with pyrimidines and purines of biological importance.

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Keyphrases

Molecular binding determinations—optical activity measurements
Tryptophan-alkylxanthines—interaction
Complex formation—tryptophan-alkylxanthines
Stability constants—tryptophan-alkylxanthine complexes
Optical rotatory dispersion—analysis

Differential Scanning Calorimetry

Advantages and Limitations for Absolute Purity Determinations

By N. J. DeANGELIS and G. J. PAPARIELLO

A critical evaluation of the effectiveness of absolute purity determination by differential scanning calorimetry (DSC) is presented. Several pure drugs of widely differing structure have been investigated with respect to the detection of known amounts of added impurities of all types. The accuracy of purity values obtained by the DSC technique is shown to fall off rapidly below purities of 99 mole %. Experiments are described which demonstrate that impurities present in solid solution are not detected. Independent purity determinations by phase solubility and quantitative thin-layer chromatography are shown to be in good agreement with DSC values for samples that are 99% pure or better. A technique for extending the useful range of DSC purity determination to about 95% is given.

CALORIMETRIC METHODS of absolute purity determination by measurements related to melting and freezing point depressions have been successfully applied to the analysis of organic compounds that melt without decomposition for many years. However, prior to the availability of the Perkin-Elmer differential scanning calorimeter (DSC), these methods had not been widely used in pharmaceutical laboratories. With the introduction of this type of instrumentation, there has been increased interest and application of purity measurements of this type (1).

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The theoretical justification for classical calorimetric purity measurements imposes as one of its restrictions that an equilibrium exist between the solid and liquid phases (2). Hence, in measurements of this kind it has been most common to employ heating rates of 0.1° C. per hour or less in the vicinity of the melting point of the compound. This condition assures that equilibrium is established but results in a long analysis time. Unlike the above static methods, DSC utilizes a minimum rate of temperature rise of 0.625° C. per minute. In this dynamic technique one benefits from much shorter analysis time (less than 2 hr.), but under these circumstances it cannot be assumed that true solid-liquid equilibrium is always attained. The authors feel that it is necessary to investigate this latter aspect because sufficient experimental data have not been presented in the scientific literature to indicate the incidence of this occurrence and the resulting consequences. Thus, the authors have performed a comprehensive series of experiments to evaluate the DSC technique for purity measurements and to reveal its advantages and limitations.

Among the areas considered are the following: (*a*) the detection of contaminants that are either structurally similar or structurally dissimilar to the major component, (*b*) the accuracy of DSC purity determinations for various levels of impurity content, (*c*) comparison of results with other established purity techniques, (*d*) a method for extending the useful range of DSC purity determinations.

DSC TECHNIQUE

Detailed discussions of the theory of calorimetric purity determinations have already been presented (3, 4). A brief review of the theory and the details of how it is applied to the DSC method was given by Gray (5) and Reubke and Mollica (1). In this paper, the procedure will merely be outlined.

The DSC technique centers around an analysis of the shape of the melting curve of the compound. Measurements of the amount of sample melted at several temperatures are made and their inverse is plotted against the temperature at which the individual fractions were measured. Theoretically, for systems that behave as ideal solutions this should represent a straight line since the following relationship can be derived (5):

$$T_s = T_0 - \frac{RT_0^2x_2}{\Delta H_f}\left(\frac{1}{F}\right) \qquad \text{(Eq. 1)}$$

where

T_s = instantaneous temperature of sample in °K.
T_0 = melting point of pure solvent in °K.
R = gas constant in calories/mole-degree
x_2 = mole fraction of solute
ΔH_f = heat of fusion of solvent in cal./mole
F = mole fraction melted at T_s

ΔH_f is directly related to the total area under the curve, T_0 is the corrected temperature of the peak of the melting curve, and F is determined by dividing the area under the curve up to the temperature T_s by the total area.

When the raw data are plotted one rarely obtains a linear relationship, and the area measurements must be mathematically adjusted to make them fit a straight line. In the absence of solid solutions, the reason for this behavior has been attributed to sensitivity limitations of the instrumentation which causes the early stages of the melt to go undetected (6).

Another approach has recently been presented by Driscoll, Duling, and Magnotta (7). In it, all samples are treated as though they form solid solutions and the equation of Randall and Lewis for such systems is applied (8). The authors have incorporated this procedure into a computer program which shortens the data reduction time and provides better accuracy.

EXPERIMENTAL

The Perkin-Elmer DSC-1B was used in all the experiments. A heating rate of 0.625° C. per minute was employed and all samples were run in a nitrogen atmosphere. Area measurements were made with a K & E compensating polar planimeter and all weighings were made with a Cahn gram electrobalance (precision ±0.1 mcg.). Sample sizes were typically in the range of 1–4 mg. The nature of the samples did not necessitate the use of the volatile sample pans and calculations were made according to the procedure outlined in the literature (5, 6).

When the dry mixing technique was used for sample preparation both the pure component and the contaminant were separately weighed into a tared sample pan and the weights of each were obtained by difference. This eliminated the possibility of sampling errors that could have been present if both components were premixed and a single aliquot taken. For preparation of samples which were mixed in solution, an organic solvent which did not adversely affect either component and yielded good crystalline material was employed.

Materials used for the major component were of analytical reference standard quality, *i.e.*, 99.8 mole % pure or greater as established by independent purity measurements. The methods employed were phase solubility and quantitative thin-layer chromatography. Chromatographically, the amount of impurity present was determined by visual comparison of the contaminant spot intensity with actual samples of the isolated impurities.

RESULTS AND DISCUSSION

Structurally Similar Impurities—Since the contaminants encountered in synthesized raw material are usually closely related to the major component,

TABLE I—DSC PURITY VALUES FOR STRUCTURALLY SIMILAR IMPURITIES

Pure Compound	Impurity	Actual Purity, Mole %	DSC Purity, Mole %
dl-13-Ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one	*dl*-13-Ethyl-17-ethynyl-3-methoxygona-2,5(10)-dien-17β-ol	99.5	99.6
		99.2	99.2
	d-Form of pure compound	99.4	99.4
		99.0	99.1
	dl-13-Ethyl-17α-ethynyl-17-hydroxygon-5(10)-en-3-one	99.5	99.5
		99.1	99.1
7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one	7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one	99.4	99.5
		99.2	99.2
	Polymorph of pure compound	99.4	99.5
		99.0	99.0
p-Ethoxyacetanilide	Acetanilide	99.5	99.4
		99.2	99.1
	p-Chloroacetanilide	99.6	99.6
		99.1	99.1
An investigational substituted carbamate	A position isomer of the pure compound	99.2	99.2
		98.8	98.9
dl-Malic acid	*l*-Form of pure compound	99.3	99.4
		98.9	99.0

the presence of this type of impurity in several different classes of compounds was investigated. Examples of position and optical isomerism, polymorphism, and common synthetic reaction contaminants were considered. These impurities were added to the drug by dry mixing and the final purity determined by the DSC method. In all cases, the purity of the starting major component was independently determined and the DSC value was in good agreement with it. The results of this study are given in Table I. The actual purity values listed in the table represent the sum of the impurities in the starting major component and the amounts of contaminant added. It is apparent that there is excellent agreement in the range considered, independent of the system investigated. This is especially significant because the nature of the impurities is such that they would be very difficult to quantitate by conventional analytical techniques.

Structurally Dissimilar Impurities—To further test the "absoluteness" of the DSC purity method, it was necessary to determine if impurities structurally different from the major component could be detected. To investigate this aspect, contaminants were dry mixed with nonrelated pure compounds and the DSC purity was determined. The results are given in Table II. With the exception of sodium chloride, lactose, and sodium acetate, the contaminants present were accurately determined.

Since the theory of calorimetric purity measurements predicts inaccuracies for systems that contain salts, the authors did not expect perfect agreement for those type samples. However, it is interesting to note that they were not detected even though present in large quantities. The behavior of lactose was not expected and indicates that either equilibrium is not rapidly established or that the two components do not form a solution. The latter point is very often neglected, but it is an important consideration since it is required that the impurity form a solution with the major component in order to be detected. It is reasonable to expect that as the major and minor component become more and more dissimilar the incidence of this effect will become more prevalent.

TABLE II—DSC PURITY VALUES FOR STRUCTURALLY DISSIMILAR IMPURITIES

Pure Compound	Impurity[a]	Actual Purity, Mole %	DSC Purity, Mole %
dl-13-Ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one	7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one	99.1	99.1
p-Ethoxyacetanilide	An investigational substituted carbamate	99.3	99.3
7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one	*N*-Acetyl-*p*-aminophenol	99.4	99.3
N-Acetyl-*p*-aminophenol	2-Methyl-2-*N*-propyl-1,3-propanediol dicarbamate	99.0	99.1
dl-13-Ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one	Lactose	98.0	99.6
	Sodium acetate	95.0	99.6
	Sodium chloride	95.0	99.8

[a] Impurity added by dry mixing.

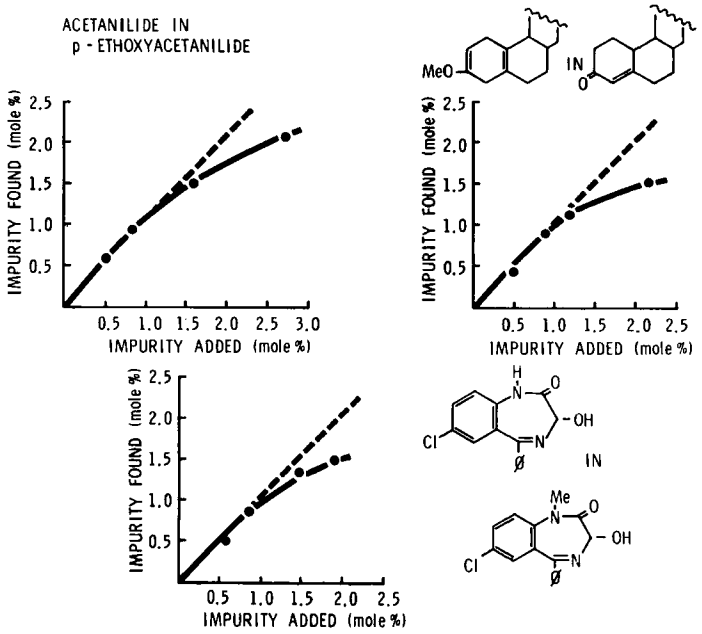

Fig. 1—Limits of accuracy. Key: —, amount of impurity as determined by DSC; - - -, actual amount of impurity present.

Accuracy Range—The range of impurity levels which can be accurately quantitated by the DSC technique (±0.1%) was also investigated by dry mixing known amounts of impurity with a pure drug. Structurally similar impurities representing 1 mole % or less of the total sample were precisely determined. In some cases, this range was increased slightly but not beyond a limit of approximately 1.5 mole %. Representative results are given in Fig. 1. Typically, the analysis of a sample containing 2 mole % impurity yielded values of near 1.5%, and approximately 2.5% for those that were actually 5% impure. It must be understood that the above results may not hold true for every system considered, because the range of applicability will primarily be determined by the ideality of the system and the rate at which equilibrium between the solid and liquid phases is attained. However, the authors have not yet encountered any system in which accurate results were obtained beyond 1.5 mole % impurity and it is indicated that DSC purity values of less than 99% are likely to be in error.

Solid Solution Effect—The use of Eq. 1 assumes the absence of solid solutions. To demonstrate the effect that their presence will have on purity measurements by the DSC method the following experiments were performed.

(*a*) Varying concentrations of different types of impurities were mixed with *p*-ethoxyacetanilide in chloroform solution. Aliquots of these solutions where pipeted into the DSC sample pan with a microliter syringe and then recrystallized rapidly by evaporation. This same procedure was used

TABLE III—EFFECT OF SOLID SOLUTIONS

		Method of Recrystallization			
		Fast Evaporation		Controlled Process	
Pure Compound	Impurity	Actual Purity, Mole %	DSC Purity, Mole %	Actual Purity, Mole %	DSC Purity, Mole %
p-Ethoxyacetanilide	*p*-Chloroacetanilide	99.6	99.9	99.5	99.5
		98.9	99.8	99.3	99.2
		98.4	99.5	98.9	98.9
	Acetanilide	99.4	99.7	99.7	99.7
		98.9	99.5	99.4	99.4
		98.4	99.3	99.0	99.1
	An investigational substituted carbamate	99.5	99.6	—	—
		99.0	99.1	—	—
		98.5	98.7	—	—
dl-13-Ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one	*dl*-13-Ethyl-17-ethynyl-3-methoxygona-2,5(10)-dien-17β-ol	99.5	99.8	99.5	99.5
		99.0	99.6	99.2	99.2
		98.5	99.3	98.8	98.9
	7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one	99.6	99.7	—	—
		99.2	99.2	—	—
		98.8	99.0	—	—

TABLE IV—COMPARATIVE PURITY[a] RESULTS BY OTHER METHODS

Sample	DSC	Phase Solubility	TLC
dl-13-Ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one			
1	99.4	99.3	99.2
2	99.6	99.5	99.5
3	99.8	99.6	99.7
4	99.8	100	99.8
5	99.1	99.2	99.2
An Investigational Substituted Carbamate			
Sample	DSC	NMR[b]	TLC
1	99.7	>99	>99.5
2	99.5	>99	99.5
3	98.6	98.2	98
4	97.4	95.4	95–96
5	96.5	94.4	<95

[a] All purity values in mole %. [b] Impurity not detectable by NMR if it does not exceed 1%.

for a system whose major component was *dl*-13-ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one. These samples, when analyzed by the DSC method, gave poor results if the contaminants were closely related in size and structure to the major component. For impurities that were dissimilar, the agreement was equivalent to that obtained by dry mixing. The results are summarized in Table III.

The above results are not surprising, because where the compounds were closely related and then rapidly recrystallized, migration of one component into the crystal lattice of the other is likely to occur. In the resulting solid solution, the contaminant is either in part or in whole dissolved in the solid phase of the major component. Hence Eq. 1 must be modified by the addition of a distribution coefficient to take this into account. Mastrangelo and Dornte (9) have derived a method for the analysis of systems which form solid solutions but Driscoll *et al.* (7) have shown that this treatment is not applicable to the DSC technique when they actually occur. The results verify this conclusion. The reason for this behavior is most likely due to the nonequilibrium operating condition. Unfortunately, one does not know *a priori* if a solid solution exists, and this information is not obtainable from the DSC curve.

(*b*) The systems described above for which inaccurate results were obtained were recrystallized under controlled conditions to simulate actual synthetic reaction processes. This was done to determine if the solid solution limitation would be prevalent in production-recrystallized material. The combination of *dl*-13-ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one and *dl*-13-ethyl-17-ethynyl-3-methoxygona-2,5(10)-dien-17β-ol was slowly recrystallized from ethyl acetate by the addition of isooctane. The *p*-ethoxyacetanilide systems were dissolved in ethyl alcohol and precipitated by dilution with water. DSC purity determinations on the above samples were in good agreement with theory as seen in Table III.

It is true that there will always be uncertainty in the purity values obtained on samples for which the nature of the contaminants and the history of the sample is not known. However, it has been demonstrated in this laboratory, at least for two systems, that DSC analysis of production material is not limited by solid solutions. The justification for this statement is based on the excellent agreement obtained with independent purity determinations as will be shown below.

TABLE V—DSC PURITY BY DILUTION TECHNIQUE

Compound	Actual Purity, Mole %	DSC Purity— Before Dilution, Mole %	DSC Purity— After Dilution, Mole %
dl-13-Ethyl-17α-ethynyl-17-hydroxygon-4-en-3-one	95.5	97.4	95.5
p-Ethoxyacetanilide	96.0	97.6	96.2
2-Methyl-2-propyl-1,3-propanediol dicarbamate	97.0	97.8	97.2
7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one	96.5	97.6	96.4

Comparative Results—Comparative purity results by the phase solubility technique, which has been generally accepted as the standard method for determining absolute purity, and quantitative TLC were found to be in good agreement with DSC purity values on production material which was 99 mole % pure or better. This is shown in Table IV. At the same time it is apparent from the NMR and TLC results on the carbamate that impurities of 2 mole % or more are not accurately determined. This is in agreement with findings on the dry mixed samples.

Extending Accuracy Range—To circumvent this problem of inaccurate DSC results for samples more than 1 mole % impure, the following method is applicable.

Mix in known proportions the sample whose purity is to be determined with a known pure sample (>99.5%) of the same compound. Adjust the amounts so that the final purity of the mixture is between 99% and 99.5% pure. The ratios to be used can be estimated from the initial DSC results of the nondiluted sample. Determine the DSC purity of the mixture and from this information, along with the known weights and purity of the diluent, algebraically calculate the values for the sample in question.

Using this technique, the results shown in Table V are obtained.

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Keyphrases

Differential scanning calorimetry—analysis
Purity determination, drugs—differential scanning calorimetry
Solid solution effect—purity determination
TLC—analysis confirmation
NMR spectroscopy—analysis confirmation

Pharmaceutical Heterogeneous Systems II

Study of Hydrolysis of Aspirin in Combination with Fatty Acid Tablet Lubricants

By H. V. MAULDING, M. A. ZOGLIO, and E. J. JOHNSTON

Stearic acid USP, in combination with aspirin in tablets, capsules, and powder mixes increases the rate of salicylic acid formation over pure aspirin controls. The breakdown of aspirin in combination with stearic acid USP and reagent grade stearic and palmitic acids has been studied with regard to the relative suitability of the fatty acids in question as tablet lubricants. Commercial stearic acid was found to have a marked effect on acceleration of salicylic acid formation as compared to reagent palmitic and stearic acids which were 95% + pure (gas chromatography). In powders and tablets with a constant aspirin:fatty acid ratio (20:1) the degree of aspirin degradation reaches a maximum when the two reagent acids are mixed together at approximately the same molar ratio as found in stearic acid USP. This maximum closely parallels the minimum on the melting point curve for mixtures of stearic and palmitic acids.

IT HAS BEEN observed that the commonly used tablet lubricant, stearic acid USP, accelerates aspirin decomposition and salicylic acid formation upon combination with pure aspirin in tablets, powders, and capsules (1). This deleterious effect has been well documented in this laboratory (2). It was thought that by varying the purity of the stearic acids used, one might note a difference in the degree of acetylsalicylic acid degradation as a result of either chemical and/or physical effects of the fatty acid lubricants. For this comparison aspirin powder mixes and tablets containing food grade stearic acid[1] USP and reagent grade stearic and palmitic acids (Fisher Chemical Co.) were compared.

EXPERIMENTAL

Free Salicylic Acid Determination (3)—A sample of powder or tablet (crushed in a mortar) equivalent to 200 mg. of aspirin was dissolved in 10 ml. of water-saturated chloroform with agitation (1 min.). This solution was then poured onto a column containing 8 g. of acid-washed diatomaceous earth[2] previously mixed with 8 ml. of 2% ferric chloride. The column was eluted with water-saturated chloroform (about 50 ml.) to remove the aspirin. The purple complex was eluted into a volumetric flask with 10% acetic acid in chloroform (10 ml.) followed by 1% acetic acid in water-saturated chloroform to remove the complex. The concentration of salicylic acid was determined by measuring the absorbance of the solution at 310 mμ.

Melting Point Determination—Exactly weighed quantities of reagent steric and palmitic acids (Fisher) were mixed and heated to melting on a water bath. The mixtures were stirred and allowed to cool overnight. The congealed mixtures were crushed, mixed intimately, and melting points run on a Thomas-Hoover melting point apparatus with a temperature increase of 1°/min.[3]

Gas Chromatography—*Esterification Procedure*—One gram of fatty acid was transferred to a 125-ml. conical flask, and 30 ml. of BF_3-methanol reagent, 14% w/v (Applied Science Laboratories, Inc., State College, Pa.) added. The flask was fitted with an air condenser and the reactants refluxed on a steam bath for at least 0.5 hr. The reactants were cooled at room temperature, 20 ml. of petroleum ether added, the flask swirled, and the mixture

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[1] Emeresol 6332, Emery Ind. Inc.

[2] Celite 545, Johns-Manville, New York, N. Y.

[3] All melting points are uncorrected.