# NMR Analysis of Pharmaceuticals XIV: Determination of Amyl Nitrite in Its Inhalant Dosage Form

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**Abstract** □ An NMR procedure is described for the analysis of amyl nitrite as a drug entity and in inhalant dosage forms. The choices of solvent (carbon tetrachloride) and internal standards (biphenyl or benzyl benzoate) were made with respect to stability problems and the presence of stabilizers in the formulation. The method is precise, with a standard deviation of ±0.5. The NMR results of synthetic solutions and commercial preparations were compared with those obtained by a published relative NMR procedure and a compendial titrimetric method. The results were generally satisfactory.

**Keyphrases** □ NMR spectroscopy—analysis, amyl nitrite, drug entity and inhalant dosage form □ Amyl nitrite—NMR analysis, drug entity and inhalant dosage form

Analytical procedures for amyl nitrite (I) have been complicated because of the instability of the material; a number of different decomposition products result. The various analytical approaches were summarized recently in a paper (1) describing the use of NMR in the analysis of I. The reported NMR procedure is a relative one and permits the specific measurement of I in terms of the resonance of the methylene protons alpha to the nitrite. The resonance of the two methyl groups is used as the reference area against which the $\alpha$-methylene proton area is compared. Since the latter group is affected by instability whereas the former is not, this analysis yields stability information in a relative sense but does not provide absolute measurement.

The present study extends the use of NMR for the analysis of I to yield absolute results. The choices of solvent and internal standards were made with the stability problems and the presence of stabilizers in the formulation clearly in mind. Results from the NMR measurements were compared with an independent procedure and were generally satisfactory. The NMR procedure described in this study is rapid, simple, and specific.

## EXPERIMENTAL

**Apparatus and Chemicals**—An NMR spectrometer[1] equipped with a variable temperature probe[2], having a six-turn insert, was used.

Amyl nitrite[3] (USP XVI) (stored at −2° when not in use), internal standards of benzyl benzoate[4] USP and biphenyl[5], and 0.18- and 0.30-ml commercial dosage units of amyl nitrite inhalant were used.

All chemical shifts reported are in reference to tetramethylsilane at 0 ppm.

**Preparation of I Standard Solution**—Transfer an accurately weighed quantity of approximately 5 mEq of internal standard [biphenyl (II) or benzyl benzoate (III)] into a "microflex tube"[6] and add 2–3 ml of carbon tetrachloride. Screw on the "microflex valve"[6] and the septum seal[6], thereby sealing the contents of the tube, and determine the weight of the sealed vessel.

Open the valve, introduce about 0.5 ml of I using a syringe, close the valve, and weigh the vessel when it has attained constant weight. Shake the vessel, transfer about 0.5 ml of the solution into a precision NMR tube, cap the tube, and proceed as directed under *Instrumental Measurement and Calculation.*

**Preparation of I Ampul Sample Solution**—Remove the gauze jacket and then clean, dry, and weigh an ampul containing 0.30 ml of I. (If the declared amount per ampul is 0.18 ml of I, use two ampuls.)

Place the weighed ampul into a freezer for at least 15 min[7]. When chilled, place the ampul into a 25-ml glass-stoppered erlenmeyer flask containing 4–5 mEq of accurately weighed internal standard in 1–2 ml of carbon tetrachloride.

With a glass rod, carefully break the ampul; then wash the rod with an additional 1 ml of carbon tetrachloride and immediately stopper the flask and shake. Transfer about 0.5 ml of the solution into a precision NMR tube, cap the tube, and proceed as directed under *Instrumental Measurement and Calculation.* Accurately weigh the glass fragments of the broken ampul to determine the tare weight.

**Instrumental Measurement and Calculation**—Place the tube containing the solution in an NMR spectrometer and obtain the spectrum. Integrate the triplet at 4.6 ppm (I) and the signal from the internal standard, the multiplet at 7.0–7.8 ppm (II), or the singlet at 5.3 ppm (III) (Fig. 1) at least five times and determine the average integral.

The amount of I may then be calculated as follows:

$$\% \text{ of I} = \frac{A_{\text{I}}}{A_{\text{II or III}}} \frac{E.W._{\text{I}}}{E.W._{\text{II or III}}} \frac{\text{mg of II or III}}{\text{mg of sample}} \times 100 \quad \text{(Eq. 1)}$$

where:

$A_{\text{I}}$ = integral value of the signal representing I
$A_{\text{II}}$ or $_{\text{III}}$ = integral value of the signal representing II or III
$E.W._{\text{I}}$ = formula weight of I/2 = 58.57
$E.W._{\text{II}}$ or $_{\text{III}}$ = formula weight of II/10 = 15.42 or III/2 = 106.12
mg of sample = sample weight in milligrams corrected for the stabilizer added

## RESULTS AND DISCUSSION

The choice of a particular organic solvent as an analytical medium is complicated by the instability of I. Although several decomposition products of I are possible (1), work in this laboratory indicated the presence of isoamyl alcohol (IV) only in some ampul dosage forms. As shown in Fig. 1, IV is manifest by a triplet at 3.6 ppm, identified as *e*, ascribable to the methylene protons alpha to the hydroxyl group. Even though I is very soluble in almost every organic solvent, carbon tetrachloride was the only solvent tested in which there was no noticeable decomposition of I into IV. Thus, carbon tetrachloride was used in all studies.

Generally, an internal standard is chosen on the basis of the available regions of the spectrum of the compound undergoing analysis. In this case, the choice is complicated by the need to incorporate stabilizers in the commercial ampuls; an epoxidized oil[8] and diphenylamine (V) are the compounds used today. The epoxidized oil exhibits an NMR spectrum with multiplets whose centers are at 5.2, 4.2, 3.0, 2.3, 1.5, and 1.1 ppm (with respect to tetramethylsilane). The resonance multiplet at 1.5 ppm is most intense and

[1] Varian A-60.
[2] V-6031.
[3] Matheson, Coleman and Bell, Norwood, OH 45212
[4] Merck & Co., Rahway, NJ 07065
[5] Eastman Organic Chemicals, White label.
[6] Described in Kontes New Product Bulletin No. 471, Kontes, Vineland, NJ 08360

[7] *Caution:* Although this chilling step is included partly to reduce the volatility of I, the primary reason is to guard against possible explosions when ampuls containing degraded I are broken as directed.
[8] Epoxol 9-5, Swift Chemical Co., Chicago, IL 60604

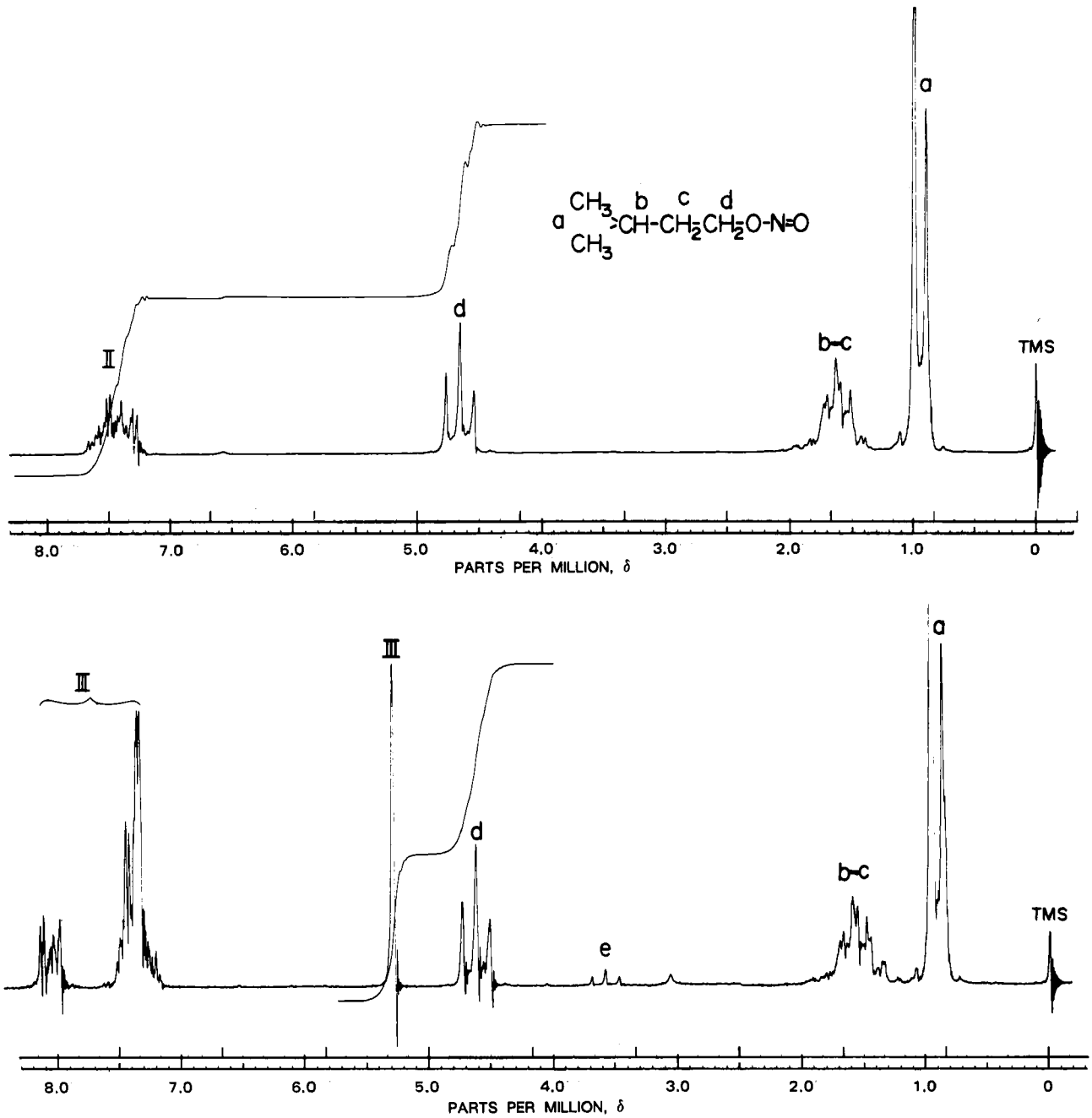


**Figure 1**—*NMR spectrum of amyl nitrite in carbon tetrachloride. Key: II, biphenyl; III, benzyl benzoate; and TMS, tetramethylsilane.*

is the only NMR signal seen when the spectrum of the sample is taken at a concentration level (2%) equivalent to that when it is used as a stabilizer in amyl nitrite. Compound V exhibits a multiplet ranging from 6.7 to 7.6 ppm, signals ascribable to aromatic protons. These data dictated the choice of internal standards.

Two internal standards, biphenyl (II) and benzyl benzoate (III), were found to be satisfactory and useful under particular circumstances. Compound II can be used whenever the nonaromatic stabilizer is present and is manifest as a multiplet in the 7.0–7.8-ppm range, ascribable to all 10 aromatic protons. Clearly, II is unsuitable when the stabilizer is V owing to the latter's NMR resonance in the aromatic region.

For all formulations, no matter what the stabilizer, a singlet at 5.3 ppm due to the two methylene protons of III is a good choice. Compound III also is manifest as a multiplet over the 7.0–8.3-ppm region, activity ascribable to the resonance of the 10 aromatic protons of the ester. The $\Delta\nu$ between the $\alpha$-methylene proton triplet of I and the methylene singlet of III is about 40 Hz. Since this finding raises some question concerning interference with the integration owing to spinning side bands, it is advisable to integrate such samples with no spinning.

Thus, to summarize, III is a standard useful in the analysis of all samples whereas II is a compound that cannot be used if an aromatic stabilizer is present in the formulation. From the *Experimental* section, it is noted that the equivalent weight advantage lies with II, which means that more intense NMR absorption will be achievable per unit mass of II than per unit mass of III. Clearly, either standard may be used if the sample is a pure amyl nitrite. Fortunately, the analyst has a choice of standards. If the stabilizer is a compound other than the two discussed here, it might be necessary to choose an internal standard different from II and III.

At this point it seems appropriate to comment briefly about initial studies involving the selection of an internal standard and solvent. The NMR singlet of maleic acid (VI) at 6.3 ppm, ascribable to the resonance of the two methylene hydrogens, seemed a good choice. However, when I was analyzed with VI as the internal stan-

**Table I—Comparative Analysis of Amyl Nitrite and Its Dosage Form Using Various Spectral Areas**

| Nature of Sample | Relative Analysis $3 \times \frac{d}{a} \times 100$ | Absolute Analysis (Benzyl Benzoate Internal Standard) For Amyl Nitrite, % w/w | | | For Isoamyl Alcohol, % w/w |
|---|---|---|---|---|---|
| | | *a* | *b–c* | *d* | *e* |
| Amyl nitrite standard | 95.7 | 99.7 | 98.9 | 95.5 | — |
| with no stabilizer | 96.1 | 99.5 | — | 95.6 | — |
| | 95.0 | 100.5 | 100.1 | 95.5 | — |
| Average | 95.6 | 99.9 | 99.5 | 95.5 | — |
| *SD* | ±0.6 | ±0.5 | ±0.9 | ±0.06 | — |
| Sample with 2% (w/v) | 94.7 | 100.2 | 101.5 | 94.9 | — |
| epoxidized oil[a], | 94.4 | 99.7 | 102.1 | 94.2 | 1.5 |
| specific gravity 0.874 | 94.4 | 99.4 | 102.1 | 93.9 | 2.2 |
| Average | 94.5 | 99.8 | 101.9 | 94.3 | — |
| *SD* | ±0.2 | ±0.4 | ±0.3 | ±0.5 | — |
| Sample with 2% | 92.9 | 100.1 | 100.0 | 93.0 | 2.9 |
| (w/v) diphenylamine, | 93.1 | 99.6 | 100.2 | 92.8 | 3.7 |
| specific gravity 0.875 | 92.4 | 99.8 | 100.5 | 92.2 | 4.1 |
| Average | 92.8 | 99.8 | 100.2 | 92.7 | — |
| *SD* | ±0.4 | ±0.3 | ±0.3 | ±0.5 | — |

[a] Epoxol 9-5, Swift Chemical Co., Chicago, IL 60604

dard in deuterochloroform, definite evidence of hydrolysis of I was manifest, making rapid manipulations necessary to achieve satisfactory analytical results. Therefore, it was desirable to search for other standards and solvents.

The NMR spectrum of I offers three separate resonance signals (*a*, *b–c*, and *d* in Fig. 1) for integration and possible quantitative analysis. The assignments for the origins of the multiplets noted in Fig. 1 follow the work of Schirmer *et al.* (1). The doublet labeled *a* is ascribable to the methyl groups present in I as well as each decomposition product arising from I. This area is a good index of total I included in the original dosage form but gives no indication of intact I present at the time of analysis.

The multiplet labeled *b–c*, assigned to the β-methylene and methine protons, occurs in I and its decomposition products and has the same restricted use as doublet *a*. However, the triplet labeled *d* is ascribable to the α-methylene of I with the possible interference from overlap with a triplet ascribable to amyl nitrate, an impurity sometimes present in I at insignificant levels (1). Thus, this area presents the possibility of differential measurement of I. The work of Schirmer *et al.* (1) was based on resonance signals *a* and *d* and resulted in a relative analysis; the findings reported in this paper are absolute in nature.

In a study involving the quantitative NMR analysis of a substance such as I, which offers more than a single resonance structure as an analytical peak, the suitability of a particular peak for analytical use must be established. The data in Table I are results of studies with regions *a*, *b–c*, *d*, and *e* and with III as an internal standard. Values are reported after correction for the stabilizer present in the original sample weight. When the doublet *a* was used, the analyses were all very close to 100%, indicating that only I or compounds related to I were present. The values based on multiplet *b–c* also were close to 100%, with the exception of higher results for one sample where the stated stabilizer would be expected to contribute to the integrated area. The results derived from triplet *d* were lower than those observed with *a* and *b–c*, indicating some decomposition.

In the case of the ampul samples, manifestation of IV in terms of the triplet *e* permitted its measurement. The results (Table I) were much more variable than those obtained for the parent I. Two potential sources of variation may be cited. First, levels of decomposition differ in individual dosage units. And second, since *e* is manifest by an integral height of about 1–4 mm, measurement errors of these small heights contribute to the observed large variations. From these studies, the triplet designated as *d* was chosen as the analytical index of I and was used in subsequent measurements.

**Table II—Determination of Amyl Nitrite in Standard Solutions by NMR**

| Standard Solution | Internal Standard, mg | Amyl Nitrite Added, mg | Found, mg | Recovery, % w/w |
|---|---|---|---|---|
| | Biphenyl | | | |
| 1 | 72.4 | 206.6 | 196.5 | 95.1 |
| 2 | 68.1 | 200.9 | 190.5 | 94.8 |
| 3 | 64.9 | 213.8 | 203.5 | 95.2 |
| 4 | 69.6 | 475.4 | 453.1 | 95.3 |
| 5 | 72.1 | 395.1 | 374.2 | 94.7 |
| 6 | 70.5 | 300.2 | 285.2 | 95.0 |
| | | | Average | 95.0 |
| | | | *SD* | ±0.2 |
| | Benzyl benzoate | | | |
| 1 | 410.9 | 386.4 | 365.2 | 94.5 |
| 2 | 489.7 | 311.0 | 295.5 | 95.0 |
| 3 | 495.1 | 170.2 | 162.5 | 95.5 |
| 4 | 500.3 | 165.5 | 157.1 | 94.9 |
| 5 | 450.9 | 275.3 | 262.6 | 95.4 |
| 6 | 505.1 | 295.7 | 278.8 | 94.3 |
| 7 | 450.6 | 261.5 | 249.2 | 95.3 |
| | | | Average | 95.0 |
| | | | *SD* | ±0.5 |
| | | By alternative procedure (2) | | 92.5[a] |

[a] Average of three determinations.

**Table III—Comparison of NMR and Titrimetric Analysis of Amyl Nitrite in Ampuls**

| Sample with Internal Standard[a] | By NMR Procedure | | By Titrimetric Procedure | |
|---|---|---|---|---|
| Biphenyl | | | | |
| 1[b] | 332.9 | 94.2 | 320.0 | 91.0 |
| 2[b] | 331.2 | 94.7 | 318.8 | 90.6 |
| 3[b] | 330.3 | 93.9 | 314.2 | 89.8 |
| 4[b] | 334.5 | 94.7 | 318.7 | 90.5 |
| 5[b] | 332.7 | 94.6 | — | — |
| Average | — | 94.4 | — | 90.5 |
| *SD* | — | ±0.4 | — | ±0.6 |
| Benzyl benzoate | | | | |
| 1[b] | 252.4 | 91.9 | 246.8 | 89.5 |
| 2[b] | 251.7 | 92.6 | 253.2 | 90.4 |
| 3[b] | 260.4 | 92.3 | — | — |
| 4[c] | 147.2 | 91.8 | 141.9 | 88.8 |
| 5[c] | 150.3 | 93.2 | 142.7 | 88.9 |
| 6[c] | 150.2 | 91.5 | — | — |
| Average | — | 92.2 | — | 89.4 |
| *SD* | — | ±0.7 | — | ±0.8 |

[a] Sample numbers represent separate lots, and the corresponding results are obtained for an individual ampul from each lot. Results are for individual ampuls, milligrams per ampul (% w/w). The sample weight was corrected for the stabilizer. [b] 0.30 ml/ampul declared. [c] 0.18 ml/ampul declared.

The analysis of a series of I standard solutions containing either II or III as an internal standard is presented in Table II. Where both internal standards were used, the results were the same, indicating that both standards were satisfactory. Furthermore, the results show that the overall procedure is very reproducible.

Table III presents the analytical results, corrected for the weight of stabilizer present, obtained when I ampul dosage forms were analyzed by the proposed NMR method. When the epoxidized oil was the stabilizer, II was used as the internal standard; when V was the stabilizer, III was the internal standard. The analytical values indicate good reproducibility for each of the two series of samples. Comparison of the NMR method with the titrimetric procedure (2), involving reaction of all nitrites with chlorate ion followed by the determination of the chloride formed, indicates that the classical titration method yields lower results. It is not possible to reproduce by calculation comparable results for the same lot from the data in Table III since each analytical result represents the analysis of a different ampul (with its own sample weight) from the indicated lot.

The problems involved in the analysis of I in ampuls have been evident from the standpoint of specificity. The NMR procedure described here uses a property that allows the absolute measurement of I in the presence of decomposition products. Furthermore, this specificity is achieved without any evidence of decomposition during the measurement. The analytical results indicate that this NMR method is precise.

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# Utilization of an Enantiomer as a Solution to a Pharmaceutical Problem: Application to Solubilization of 1,2-Di(4-piperazine-2,6-dione)propane

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**Abstract** □ An enantiomer of the cytotoxic agent (±)-1,2-di(4-piperazine-2,6-dione)propane [(±)-I] (ICRF 159) was utilized to overcome a solubility problem in the preparation of a solution suitable for intravenous use. The enantiomers were about five times more soluble and melted at about 40° lower than the racemic compound. This study appears to be the first reported instance in which the difference in the physical properties of a racemic compound and its enantiomers was utilized to improve a pharmaceutical formulation. The expected differences in the physical properties of racemic solids and their corresponding enantiomers are discussed briefly in relation to the three racemic modifications known to exist.

**Keyphrases** □ Enantiomers—physical properties compared to racemic substance, potential use in pharmaceutical formulations □ 1,2-Di(4-piperazine-2,6-dione)propane—solubilization of enantiomers compared to racemate, potential use in intravenous formulations □ Solubilization—enantiomers compared to racemates, pharmaceutical formulations

Some advantages of using various crystalline modifications such as polymorphs, hydrates, and other solvates in improving pharmaceutical formulations of drugs are well documented (1–5). An additional type of crystalline modification may be encountered when the drug molecules possess an asymmetric or optically active center. Although the employment of optically active compounds in pharmaceutical formulations is not new, their past and present usage has been due largely to one enantiomer exhibiting a quantitatively or qualitatively different biological activity than the corresponding racemic compound (6, 7). Examples of such drugs include epinephrine, ephedrine, hyoscyamine, and dextropropoxyphene (8).

The chemical and physical properties such as melting behavior, IR spectra, and solubility of crystalline racemic substances and their enantiomeric components have been well studied (9–15). The combination of these different physical properties with the stereochemical requirements of biological systems is an approach that has been largely overlooked as a means of formulation improvement.

## BACKGROUND

In the case of solid crystalline compounds, the intercrystalline forces between molecules may be greatly affected by only a minor change in the crystal geometry (11, 13, 16). The intercrystalline forces between the two like (+ and + or − and −) enantiomer molecules are the same (16). Therefore, the physical properties of a pair of pure crystalline enantiomers are identical except for the direction in which they rotate plane polarized light. However, the intercrystalline forces between opposite (+ and −) enantiomer molecules are usually very different than those between like enantiomer molecules. Such differences may give rise to different solid-state physical properties, and the nature and magnitude of these differences between enantiomers and the corresponding racemic material are dependent upon the relative strength of the intermolecular forces in the crystal. Enantiomeric systems may fall into one of three possible types (11, 16), and the solubility behavior as a function of the composition for each of the three cases is shown in Fig. 1.