RESOLUTION OF OPTICAL ISOMERS BY CRYSTALLIZATION PROCEDURES

ROBERT M. SECOR

E. I. du Pont de Nemours and Company, Wilmington, Delaware

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I. INTRODUCTION

Soon after Pasteur reported the existence of optical isomers in 1848, research efforts were in progress to discover convenient methods for separating racemic substances into their enantiomorphs. The first resolution method known (80) required removal of individual crystals of each isomer by hand, starting with the racemic mixture. This procedure was inefficient and had very limited applicability.

Later it was found that resolution could be accomplished by selective crystallization of one antipode from a solution of the racemic form (44, 45). This was accomplished by providing seed crystals of one isomer in a supersaturated solution of the racemic modification. Application of this simple, direct approach led to the discovery of many chemical compounds that could be resolved by crystallization. However, there were also many failures, where all efforts to apply the direct crystallization method resulted in no measurable degree of resolution.

It is surprising that in nearly a century since the first reported resolution by seeded crystallization, there has appeared little in the form of a theory to predict when resolution is possible by this procedure and when it is not. As a result, most investigators of the crystallization phenomenon have relied heavily on empirical approaches. The purpose of this review is to evaluate the progress that has been made on resolution by crystallization procedures and to provide a basis for further work in this field.

Phase diagrams for systems containing optical isomers will be discussed and resolution by crystallization will be described in terms of phase relations. The experimental results of resolution studies by crystallization techniques will be discussed and evaluated. Particular emphasis will be placed on techniques that comprise the separation of a multitude of crystals of the same isomer in a single operation, rather than the mechanical separation procedure, which will be treated only briefly. Principal concern will be centered on the resolution of carbon compounds containing a single element of optical asymmetry.

Familiarity with the principles of optical isomerism is assumed, since an excellent general treatment of this subject is available (107a).

II. DEFINITION OF RESOLUTION

Resolution is defined as the process whereby an optically active form of a chemical compound is separated from a racemic modification of the same chem-

ical compound. The optically active form that is obtained need not be an optically pure form, but may consist of a mixture of the *d* and *l* isomers in unequal proportions. Furthermore, resolution may be carried out with a non-racemic starting material, provided enough of the isomer initially in excess is removed to leave behind material which contains an excess of the opposite isomer.

Whenever a chemical reaction is carried out with optically inactive starting materials and in an optically inactive environment, the products are also optically inactive. When a product contains a pair of enantiomorphs, the problem of obtaining one of the isomers in pure form is that of bringing about a separation of the desired isomer from the racemic modification. The definition of resolution that has been given applies directly to this situation.

III. Phase Relations in Systems Containing Optical Isomers

A. Binary Systems

The characteristics of two-component phase diagrams consisting of a pair of optical isomers were not well defined until Roozeboom (101) studied the problem by means of the phase rule. He described many possible types of binary phase diagram for optical isomers and was the first to state correctly that the way to distinguish the three basic types of behavior (characterized by the formation of a mixture, a racemic compound and a solid solution) from one another is by means of phase relations.

Thermodynamically, in the absence of racemization, a pair of optical isomers constitutes a binary system in which the two components may crystallize together as a mixture of pure *d* and pure *l* crystals, combine to give a racemic compound or exhibit solid solution formation.

Since optical isomers are thermodynamically identical, all their binary phase diagrams are symmetrical. If the isomers form a mixture of *d* and *l* crystals when crystallizing from the melt, their phase diagram will exhibit a eutectic at the racemic composition. All compounds of optical isomers are composed of equal amounts of the two antipodes and any maxima or minima that occur on solid solution phase diagrams fall at the midpoint of the composition range. Therefore, no separations are possible when the racemic modification is subjected to phase changes since all such transitions occur congruently. The racemic form thus behaves according to the phase rule as a one-component system, regardless of whether the solid is a mixture, a compound or a solid solution.

Since the racemic composition is the state of maximum entropy, all other compositions will tend to change spontaneously in the direction of a 1:1 ratio of the isomers. If the rate of this change is zero, the system is

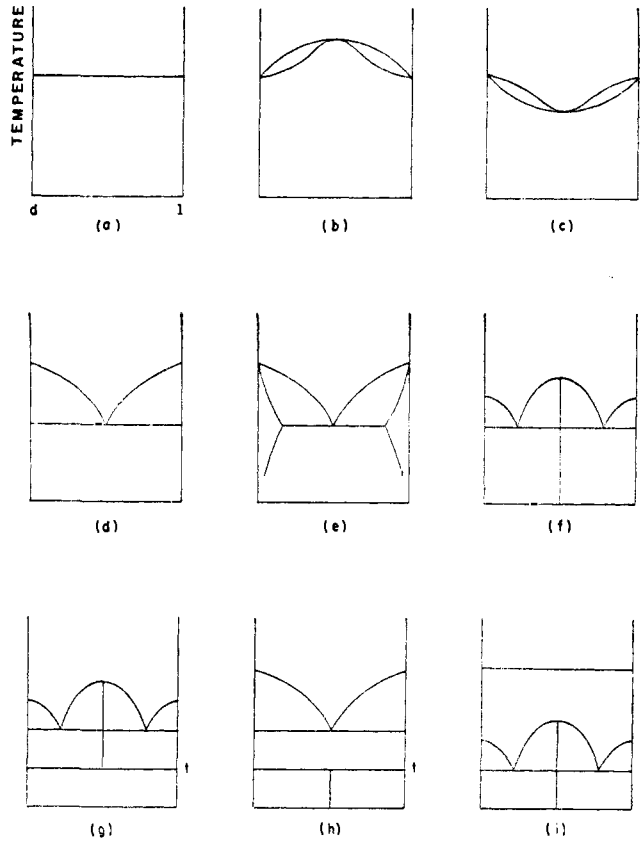

Fig. 1.—Various binary phase diagrams for optical isomers. In all cases, liquid–solid equilibrium relations are shown. In (g) (h), and (i), phase transition equilibria in the solid state are also shown.

binary. If racemization occurs at a finite rate, the system is pseudobinary. If racemization is instantaneous, the system is unary.

Nine possible binary phase diagrams for optical isomers are shown in Figure 1. The phase behavior represented by these diagrams corresponds to:

a.. Solid solution with constant melting point.
b. Solid solution with maximum melting point.
c. Solid solution with minimum melting point.
d. Mixture exhibiting simple eutectic.
e. Mixture with conjugate solid solutions.
f. Compound formation.
g. Compound decomposing below a transition temperature.
h. Compound unstable in the binary liquid system which exhibits a eutectic.
i. Compound stable below the melting point of a solid solution.

These diagrams do not represent all the possible types. There may be numerous combinations of the three basic modes of behavior, characterized by formation of a mixture, a racemic compound or a solid solution. For example, (i) is a combination of (a) and (f). The interpretation of these diagrams is straightforward and will not be given here. An ample treatment is presented in the original reference (101) and in standard sources (100). Many of the predicted binary phase

diagrams for optical isomers have been observed experimentally (1, 2, 3, 20, 27, 102, 103, 108, 109, 116, 117). A mathematical treatment of the melting and freezing curves for mixtures, compounds and solid solutions has been worked out extensively by van Laar (111, 112, 113). There are several methods available for determining the type of phase behavior (*i.e.*, mixture, racemic compound or solid solution) exhibited by a given system without determining the phase diagram (22, 23, 101, 107b).

The theory has been advanced, with modest experimental verification, that optical isomers which are highly ionized can form racemic compounds while those which do not ionize cannot (65). A related theory, also based on limited experimental evidence, states that if the molecules of one enantiomorph show a strong tendency to associate, there is also a tendency to form a racemic compound (103). These concepts must be regarded as tentative rules, not as substitutes for experimental data.

B. TERNARY SYSTEMS

Of most interest in studies of resolution by crystallization procedures are ternary systems consisting of *d*-isomer, *l*-isomer and solvent. Whereas in a binary system it is possible to represent both the temperature and composition variables in a plane, in a ternary system this requires a three-dimensional plot. Alternatively, if one is satisfied to confine consideration to a constant temperature, the ternary phase diagram may be plotted in two dimensions.

In a system consisting of *d*-isomer, *l*-isomer and optically inactive solvent, the isotherms are always symmetrical. The nature of the isotherms was worked out by Roozeboom (101) and several are described briefly by Ricci (100a). Three common isotherms are shown in Figures 2, 3, and 4. Figure 2 shows the case in which the optical isomers exist as a mixture in the solid state,

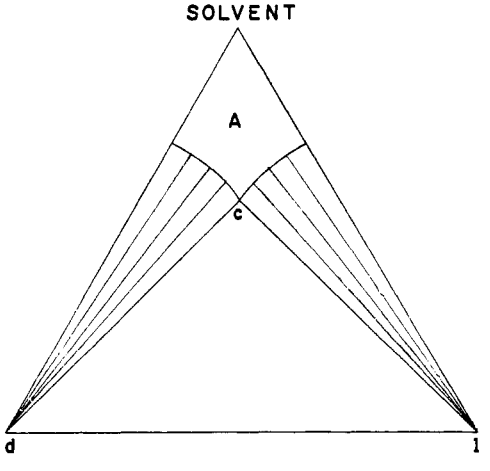

Fig. 2.—Ternary phase diagram for optical isomers forming a mixture.

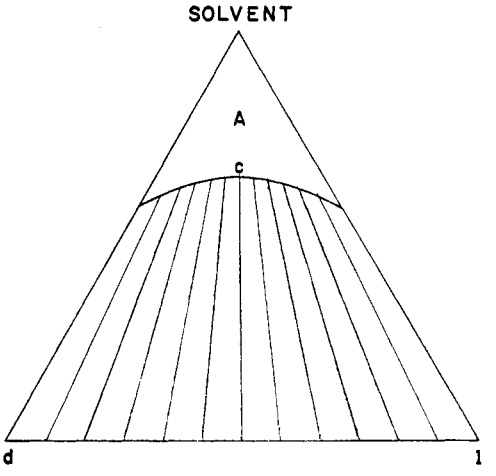

Fig. 3.—Ternary phase diagram for optical isomers forming a solid solution.

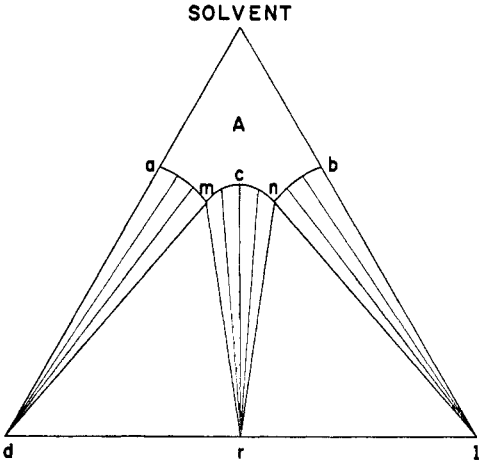

Fig. 4.—Ternary phase diagram for optical isomers forming a compound.

with the tie lines in the two-phase regions connecting the equilibrium compositions of the co-existing phases. It is apparent that the racemic mixture has the maximum solubility. The area A above the solubility curve represents unsaturated liquid compositions and the triangular area *dcl* represents three-phase compositions consisting of *d*-isomer crystals, *l*-isomer crystals and a saturated solution of composition *c*. Figure 3 shows the case in which the isomers form a solid solution. The area A represents unsaturated solutions and the remainder of the diagram is a two-phase region containing tie lines connecting compositions of solid and liquid in equilibrium with one another. The composition of minimum solubility occurs where the isomers are present in equal amounts (point *c*). Figure 4 shows the isothermal phase diagram for the case in which the enantiomorphs form a racemic compound, represented by point *r*. The solubility of the compound, represented by point *c*, may be greater or less than the solubility of the pure isomers (which have

the same solubility) represented by points *a* and *b*. Unsaturated solutions exist in the region marked A. The region bounded by the solubility curve *am* and the lines *da* and *dm* represents two-phase equilibria in which the solid phase is pure *d*-isomer and similarly, the region bounded by the solubility curve *bn* and the lines *lb* and *ln* represents two-phase equilibria in which the solid phase is pure *l*-isomer. The region bounded by the solubility curve *mcn* and the lines *rm* and *rn* represents two-phase equilibria in which the solid phase is the compound *r*. The triangular area *dmr* is a three-phase region in which the equilibrium phases are solid *d*-isomer, solid racemic compound and a saturated solution of composition *m*, while the triangular area *lnr* is a three-phase region in which the equilibrium phases are solid *l*-isomer, solid racemic compound and a saturated solution of composition *n*.

A convenient method for determining the type of phase behavior in a ternary system without obtaining the phase diagram has been described (100a).

When an optically active solvent is introduced, the ternary isotherms are no longer symmetrical and diagrams such as that shown in Figure 5 (for mixture formation) may be encountered.

IV. Phase Behavior During Resolution

In discussing or studying the separation of optical isomers, it is important to make the distinction between a separation of optically active products from the racemic form and a separation of the excess or a portion of the excess of one isomer from materials having a non-racemic composition. In the latter case a separation may be possible by allowing phase equilibrium to be attained. In the former case this is not so since, in order to accomplish resolution, the system must always be brought to a non-equilibrium condition (assuming no optically active solvents are used). The

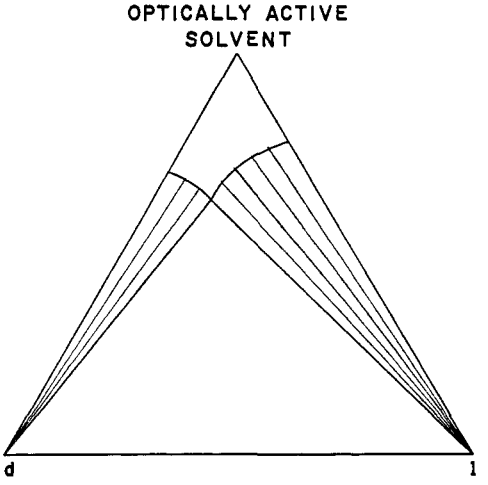

Fig. 5.—Ternary phase diagram for optical isomers forming a mixture in an optically active solvent.

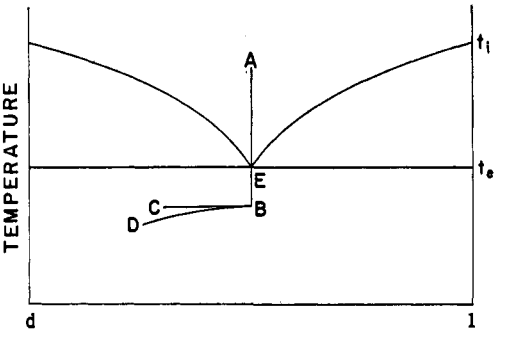

Fig. 6.—Illustration of a resolution carried out in a binary system.

success of the resolution, if it occurs at all, is therefore always dependent on the crystallization kinetics.

A. Resolution in Binary Systems

While resolution by crystallization in binary systems apparently has never been reported in the literature, it is theoretically possible to accomplish. In some instances, resolution in the binary system may be the most convenient and practical method of separation. Figure 6 illustrates such a resolution. The figure shows a phase diagram for a pair of optical isomers which form a mixture of *d* and *l* crystals, at equilibrium, for all compositions of the system. t_i is the melting point of the pure isomers and t_e is the melting point of the eutectic, which occurs at the racemic composition. To carry out a resolution, the racemic mixture is completely melted, as indicated by point A. The melt is then cooled, as represented by the line AEB. In order for resolution to be possible, the solution must be subcooled to some temperature such as that corresponding to point B, below the eutectic E. When the solution reaches the condition represented by point B, some seed crystals of *l*-isomer (for example) are added and, for the purpose of illustration, the system is maintained at constant temperature. Under these conditions it is expected that *l*-isomer will crystallize, thus causing the composition of the melt to change in the direction of the line from B to C. At some point C, the crystals are removed from the melt (by filtration, for example). The crystals would then comprise the original seed crystals plus an additional quantity of the same isomer as the seed, which crystallized from the melt. As is true of all resolutions based on crystallization, a supersaturated solution would be required. Although, in the example described, the crystallization was carried out isothermally, this condition is not a necessary requirement for resolution in a binary system. If cooling is provided after seeding, the solution composition would change with temperature along a line such as BD.

B. Resolution in Ternary Systems

Most experimental studies of resolution by crystallization have been concerned with ternary systems con-

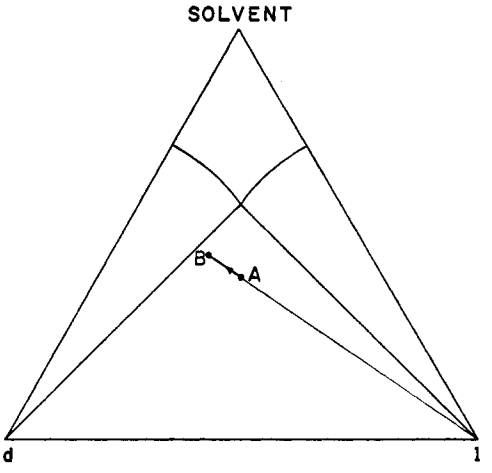

Fig. 7.—Illustration of a resolution carried out in a ternary system.

sisting of *d*-isomer, *l*-isomer and solvent. Figure 7 illustrates how a resolution might be carried out in a ternary system. Assume that the solvent (optically inactive) and the racemic form of the isomers are brought together in relative amounts corresponding to composition A and are heated to a temperature high enough to dissolve the racemic solid completely. The solution is then cooled to a temperature at which the phase diagram is that given in Figure 7. The solution is now supersaturated with respect to both the *d* and *l* isomers. Crystals of *l*-isomer (for example) are added to the solution. The crystals are suspended in the supersaturated solution by agitation. If, for example, the temperature is kept constant, *l*-isomer can crystallize from the solution, thereby causing the solution composition to move away from the *l*-isomer apex of the phase diagram along line AB. The temperature need not always be kept constant after seed addition. However, changing the temperature will change the phase diagram, and this will sometimes bring about conditions unfavorable for resolution. There are numerous variations of the resolution procedure described. These will be discussed later in the section on experimental results.

V. EXPERIMENTAL RESULTS

A. BACKGROUND

Most of the experimental studies of resolution by crystallization procedures have been attempts to resolve particular compounds, with only incidental regard for the establishment of general laws governing the phenomenon. Consequently, there has been a severe lack of critical experiments designed to obtain a fundamental understanding of resolution by crystallization. The subsequent discussion will serve to summarize the state of present knowledge in the field and to suggest areas for further research.

B. SYSTEMS STUDIED EXPERIMENTALLY

1. Sodium Ammonium dl-Tartrate

Reports of the first substance to be resolved into its optical antipodes were published by Pasteur (80, 81, 85) who, by careful sorting, manually separated the optical isomers of sodium ammonium *dl*-tartrate. When crystallized from water below 27°, sodium ammonium *dl*-tartrate produces a mixture of hemihedral crystals, which are visually distinguishable. However, above 27°, the racemic compound crystallizes. It has been shown (104, 117, 122, 123, 124) that in the binary system consisting of the two optical isomers of sodium ammonium tartrate there exists a transition point of the type shown in Figure 1(g) with the transition temperature $t = 27°$. The ternary phase diagram with water as the solvent is of the form of Figure 2 below, and Figure 4 above, 27° (100b). The abrupt change in behavior at the transition point caused some confusion among early investigators (110) since, at the time, even the equilibrium phase behavior of optical isomers was not fully understood.

Gernez (44, 45) found that a supersaturated solution of sodium ammonium *dl*-tartrate would deposit only dextrorotatory crystals when a crystal of the dextro tartrate was in contact with it and only levorotatory crystals when a crystal of the levo tartrate was in contact with it. Although no quantitative data were given, this work was the first reported resolution based on seeding a supersaturated solution and led to similar work with other chemical compounds.

By crystallization of sodium ammonium *dl*-tartrate from water, Kipping and Pope (63) were able to prepare nearly pure deposits of the dextrorotatory form by permitting part of the water to evaporate in open beakers. However, resolution was attributed to the inoculation of the solutions by crystals of the dextrorotatory isomer present in the laboratory atmosphere.

Kipping and Pope (62) also resolved sodium ammonium *dl*-tartrate by fractional crystallization of the racemic form from aqueous solutions of glucose or fructose. At first (62) the investigators suggested that the separations were accomplished by means of the asymmetric influence of the optically active solvents employed. However, they later stated (63) that the separations could have been brought about by optically active crystals entering the solutions from the laboratory dust.

Ostromisslensky (78) found that sodium ammonium *dl*-tartrate could be resolved by seeding a supersaturated solution of the salt with optically active crystals of another substance. Seed crystals used successfully for this purpose were *l*-asparagine and many optically active alkali salts of tartaric and malic acids. Seeding a supersaturated solution of sodium ammonium *dl*-tartrate with *l*-asparagine brought about crystallization

of the *d*-tartrate while the *l*-tartrate remained in solution. Each of the seed materials reported to bring about resolution is believed to be isomorphous with the isomer of sodium ammonium tartrate it induced to crystallize.

2. DL-*Threonine*

The resolution of DL-threonine by crystallization was accomplished by Amiard, Joly, and Velluz (5, 8, 11, 13, 118). Some of the resolutions were carried out in the usual manner, by seeding supersaturated solutions (118). However, other resolutions were accomplished somewhat differently. These (5) were started with solutions of threonine in water containing an excess of one or the other isomer at 80°. The solutions were cooled to 30° and when crystallization started they were further cooled to 20°. After allowing crystallization to proceed for one hour, the excess isomer plus an additional quantity of the same isomer crystallized from the solution. The crystals obtained were optically impure, but a single recrystallization produced optically pure product in amount, on the average, about two-thirds greater than the excess of isomer employed initially. No seed crystals were added to the solution. However, the procedure of using an excess of one isomer in the initial solution is equivalent to adding seed crystals, as is shown schematically in Figure 8. Initially, a solution of composition P (which, for illustration, contains an excess of *l*-isomer) is prepared at an elevated temperature and is cooled to temperature t_1, at which the phase diagram is given by the dashed lines. The solution is maintained at t_1 until crystallization occurs. Since at t_1 the point P is in a two-phase region in which the equilibrium phases are pure *l*-isomer and a saturated solution, allowing the system to remain at t_1 for a sufficient length of time will always result in the formation of pure *l*-isomer crystals in the solution. When this occurs, the system is cooled to temperature t_2, at which the phase diagram is given by the solid lines in Figure 8. The existing seed crystals then bring about further crystallization of the same isomer and resolution occurs when the solution becomes, in this case, dextrorotatory. During crystallization the solution composition changes along the straight line from P to Q. Several variations of this procedure have been described for threonine (13).

A technique of alternate *d* and *l* resolutions, to be described later, was employed. In eleven resolutions, about 60 per cent of the racemic starting material was resolved into the pure isomers, while 33 per cent was recovered in unresolved form (5).

A continuous process for resolution of DL-threonine by seeded crystallization in a fluidized bed has been described (4, 55).

3. DL-*Threo-1-(p-nitrophenyl)-2-aminopropane-1,3-diol*

Amiard, Joly, and Velluz (6, 7, 8, 9, 10, 12, 119) carried out the resolution, by crystallization, of DL-threo-1-(*p*-nitrophenyl)-2-aminopropane-1,3-diol, an intermediate in the synthesis of the antibiotic chloramphenicol. In this study, the investigators employed a technique of alternate *d* and *l* resolutions, also used in the resolution of DL-threonine (5, 8, 118) and DL-glutamic acid (73). The procedure is illustrated by Figure 9. Consider a resolution system of composition M initially in equilibrium at temperature t_1, at which the phase diagram is given by the dashed lines. The system, as described, consists of a saturated solution. A resolution is carried out by cooling to t_2, at which the phase diagram is given by the solid lines, and seeding (with *l*-isomer in this case) at some temperature between t_1 and t_2. The composition of the solution moves to point N as *l*-isomer crystallizes from the solution. The *l*-isomer crystals are then filtered off. Solid racemic material is added to the filtrate until the gross composition reaches point P. The mixture is heated to t_1, or above, and allowed to come to equilibrium. At P

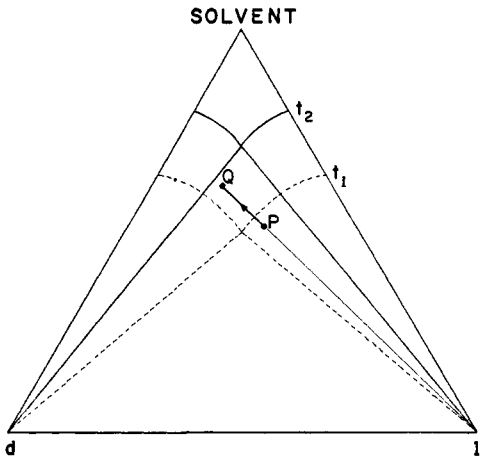

Fig. 8.—Illustration of a resolution accomplished without the addition of seed crystals.

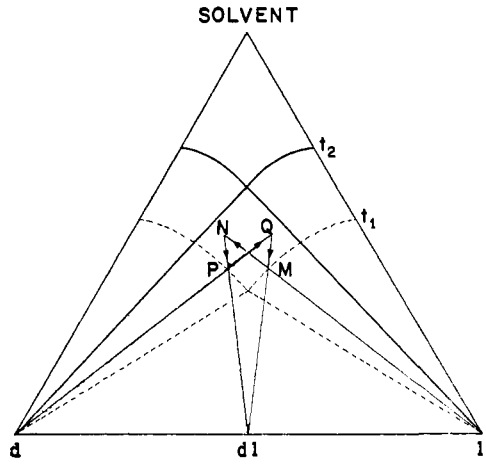

Fig. 9.—Illustration of alternate *d* and *l* resolutions.

a *d*-rich solution is obtained, which is then cooled to t_2 and seeded with *d*-isomer at some temperature between t_1 and t_2. As *d*-isomer crystallizes, the solution composition changes from P to Q. The *d*-isomer crystals are then filtered off. Then solid racemic material is added to the filtrate until the gross composition is at point M. The mixture is heated to t_1 or higher in order to dissolve the solids and the entire cycle is repeated indefinitely. As described in the case of DL-threonine, seed crystals can be generated in the solution, in which case seed addition is omitted.

In a single cycle, using the procedure of alternate resolutions, with cooling from 80 to 20° and 100 cc. of water as the solvent, these results were obtained for DL-threo-1-(*p*-nitrophenyl)-2-aminopropane-1,3-diol:

	Starting solution, g.		Crystallized product, g.	
	D	L	D	L
D-resolution	6	5	1.9	...
L-resolution	5.1	6	...	2.1

4. DL-*Histidine Monohydrochloride*

The resolution of DL-histidine monohydrochloride by crystallization was demonstrated by Duschinsky (34, 35), who separated the isomers by seeding supersaturated aqueous solutions containing an excess of one or the other antipode. The results are important because they are the only data available on the successful resolution of a racemic compound by crystallization.

Duschinsky determined the ternary phase diagrams for the optical isomers of histidine monohydrochloride and water at 20.2, 30.5 and 40.4°. It was found that at each of these temperatures the antipodes form a racemic compound. Although no measurements were given for temperatures above 40.4°, Duschinsky stated that he believed there was a transition point from compound formation to mixture formation between 40 and 55°. The example given for resolution of DL-histidine monohydrochloride (35) comprises preparing an aqueous solution at 65° containing the antipodes in a ratio of 2:1 (added in the form of 2 parts DL and 1 part D or L), cooling, seeding with crystals of the antipode present in excess, further cooling to 20° and filtering. A crystallization time of 15 minutes was used. The net gain of resolved isomer in each resolution was about 22 per cent of that isomer present in the DL-histidine monohydrochloride employed initially. Alternate D and L resolutions were carried out indefinitely in a manner similar to that described previously. The presence in the initial solution of an excess of the isomer being crystallized was found to be important for the successful functioning of the resolution procedure.

The results were interpreted (34, 35) as a demonstration of the resolution of a racemic compound. Although the initiation of crystallization by seed addition took place above the highest temperature at which the phase diagram was determined, it is possible to show that the racemic composition must have been crossed in a region where compound formation prevails, at equilibrium. This observation is rather unusual since in no other instance have data been presented to prove that any racemic compound has been resolved by crystallization.

It has been suggested (35) that in the case of compound formation, with a phase diagram such as that shown in Figure 4, the two solubility curves for the pure isomers can be extended into the region of compound formation to describe the upper limits of the degree of resolution that can be attained in such a system.

5. DL-*Glutamic Acid*

Since the solubility of glutamic acid in water is fairly low (2 per cent at 25° (76)), most procedures for its resolution in aqueous media are based on the use of solutions appreciably below or above the pH at the isoelectric point. These solutions are prepared by dissolving the glutamic acid in aqueous acids or bases. In such solutions the solubility of glutamic acid is much greater than in water.

Ogawa and Akashi (72, 73, 74) studied the resolution of DL-glutamic acid in solutions of acids and bases. In order to avoid crystallization of racemic glutamic acid, both the rate of crystallization and the degree of resolution had to be limited. These observations are a direct consequence of the tendency of the non-seeded isomer to crystallize when in a state of supersaturation. Resolution was accomplished by seeding supersaturated solutions of glutamic acid containing 0.6 to 10 equivalents of mineral acid or 0.6 to 1 equivalent of base per mole of DL-glutamic acid. Cooling, partial neutralization and evaporation were all found suitable for bringing about supersaturation. In addition, a new method of maintaining supersaturation was described. DL-glutamic acid monohydrate was found to be a stable phase only below about 22°, which is the transition point to anhydrous DL-glutamic acid. By providing solid glutamic acid monohydrate in the resolution vessel while either isomer was being crystallized above 22°, the monohydrate dissolved, since it was not a stable phase, and replenished the supply of racemic glutamic acid in solution. However, the isomer being crystallized was then contaminated with DL-glutamic acid monohydrate, which was subsequently removed by crystallization to obtain optically pure glutamic acid. In the absence of solid glutamic acid monohydrate, the usual seeding procedure yielded crystals having optical purities in the range of 99 to 100 per cent. In most cases, the resolutions were accomplished with crystallization times of several hours.

Purvis (50, 52, 91, 93, 94) found that DL-glutamic acid could be resolved in aqueous media in much shorter times than those used by Ogawa and Akashi. To prepare the supersaturated solution required, racemic

glutamic acid was dissolved in either acidic or basic solution and then brought to the isoelectric point by neutralization of the acid or base. Since the solubility of the amino acid is a minimum at this pH, it is possible to produce supersaturated solutions by this procedure.

Balmat (15, 16, 17, 18, 33) studied the resolution of DL-glutamic acid in aqueous solutions of sodium hydroxide. By varying the relative amounts of the amino acid and the base, it was concluded that 0.72 to 0.74 mole of sodium hydroxide per mole of DL-glutamic acid gave optimum results. Below this range high optical purities were obtained but at reduced yields. Above, both the degree of resolution and the optical purity of the product crystals decreased. It was found important to use seed crystals of extremely high optical purity since small amounts of the opposite enantiomorph of the desired isomer had a marked detrimental effect on the optical purity of the product. In one example cited, in which 0.737 mole of sodium hydroxide per mole of DL-glutamic acid was used, 27.8 per cent of the L-glutamic acid present in the original solution was crystallized in a single resolution, with a crystallization time after seeding of 15 minutes. A similar study has been reported for the resolution of DL-glutamic acid in aqueous solutions of potassium hydroxide (19, 32).

The resolution of DL-glutamic acid by means of optically active solvents is described by Purvis (49, 51, 89, 90, 92, 95). This type of separation method seldom has been mentioned in the literature. In the present instance, the crystallization mechanism was not established. However, some discussion of basic principles is in order. Experimentally, crystallization was allowed to occur from solutions of DL-glutamic acid containing an optically active form of another alpha-aminocarboxylic acid, such as L-aspartic acid or L-leucine, with the aim of attaining equilibrium. While the products were optically impure and were contaminated with the optically active compound added initially, the results leave no doubt that resolution was accomplished. There are two possible mechanisms by which resolution could have occurred: (1) The optically active compound added initially crystallized and acted as seed for one of the isomers of glutamic acid; or (2) The isomers of glutamic acid behave sufficiently differently in the optically active solvents employed that the observed phase separations occurred in equilibrium relations. There appears to be no way of determining, in the absence of additional data, which of these mechanisms prevailed. The first possibility is essentially no different from the usual resolution by crystallization. The second possibility is illustrated in Figures 10 and 11, which are intended to demonstrate the principles involved rather than represent specific systems. The case in which the isomers form a mixture is shown in Figure 10, while the case of compound formation is

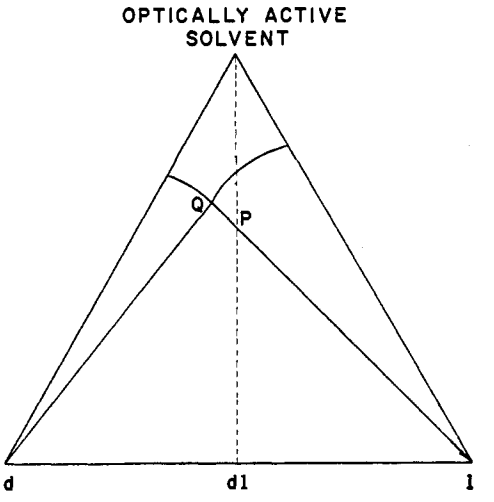

Fig. 10.—Illustration of resolution, by crystallization, of the racemic modification from an optically active solvent when the optical isomers form a mixture.

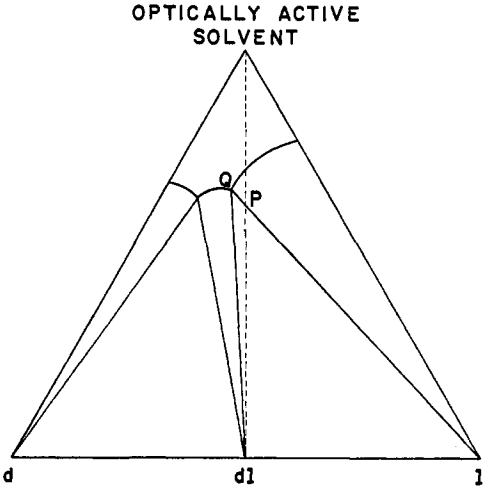

Fig. 11.—Illustration of resolution, by crystallization, of the racemic modification from an optically active solvent when the optical isomers form a racemic compound.

shown in Figure 11. A resolution could be carried out in either case by allowing a system of composition P to come to equilibrium. The coexisting phases then would be a solution of composition Q and crystals of *l*-isomer.

Fike (39) has selectively crystallized one isomer of glutamic acid from a supersaturated aqueous solution of the racemic modification containing a gamma ester of glutamic acid in the opposite optically active form. The dissolved ester suppresses the crystallization of the glutamic acid isomer having the same optical configuration as itself and therefore permits crystallization of the opposite isomer of glutamic acid in a state of high optical purity. Optically pure gamma methyl glutamate was particularly effective in preventing crystallization of the corresponding isomer of glutamic acid. It was found desirable to add a quantity of ester at least equal to the number of moles of the glutamic acid

isomer to be retained in solution during resolution. Crystallization times of 12 to 48 hours were required. However, in a single resolution, 67 per cent of the D-glutamic acid was crystallized from racemic glutamic acid in 99 per cent optical purity when 1.2 grams of gamma-methyl L-glutamate per gram of L-glutamic acid were dissolved in the original solution. Crystallization of the glutamic acid isomer having a configuration opposite to that of the gamma ester employed proceeds without the addition of seed crystals. Apparently the gamma ester creates a solubility difference between the glutamic acid enantiomorphs with the result that the enantiomorph having the same optical configuration as the gamma ester has the greater solubility.

Integrated processes for resolution of DL-glutamic acid are described by Purvis (96) and Fike (40). The former has studied the effect of time on the degree of resolution R, defined by

$$R = (W - 2A - S)/B$$

where W is the total weight of crystallized solids, A is the weight of the non-seeded enantiomorph in the crystallized solids, S is the weight of seed crystals added to the solution and B is the weight of the seeded enantiomorph in the original racemate. In one example, the degree of resolution reached 26.0 per cent at 30 minutes after seeding and remained constant until 60 minutes after seeding. However, at 80 minutes the degree or resolution had dropped to 10.7 per cent as a result of crystallization of the non-seeded enantiomorph.

A continuous process for resolution of DL-glutamic acid by seeded crystallization in a fluidized bed has been described (4, 55).

6. DL-*Glutamic Acid Hydrochloride*

Ogawa, Akashi, Sato, and Yamamoto (75) carried out the resolution of DL-glutamic acid hydrochloride by suspending seed plates in supersaturated solutions of the racemic modification. The seed plates were prepared by allowing crystals of the pure isomers to form from solutions of the respective isomers, on the surfaces of separate plates made of any convenient, corrosion-resistant material to which the crystals would adhere. Plates made of brick, unglazed ceramic material and polyvinyl chloride were used. By inserting a single plate having crystals of one isomer on its surface into a supersaturated solution of the racemic form, crystals of the same isomer as that on the plate formed on the surfaces of the existing crystals. When a series of plates was placed in the solution, some having D-isomer and others L-isomer on their surfaces there occurred preferential crystallization of the enantiomorphs on the respective plates. The crude products were optically impure, but were purified by recrystallization.

Resolution also has been accomplished in the usual manner, by seeding with crystals of one isomer (50, 52, 74, 94). However, in some cases, instead of preparing a supersaturated solution by cooling, concentrated hydrochloric acid was added to a solution of DL-glutamic acid hydrochloride. Since the solubility of the latter decreases with increasing hydrogen chloride concentration, a supersaturated condition was created.

Dowling (31, 54) has developed a resolution method based on seeding a supersaturated solution with crystals of one isomer that have a mesh size greater than some definite value. The dried product crystals are screened and the fraction having mesh sizes larger than the mesh size of the seed contains the resolved product. The smaller crystals are largely the other enantiomorph. This resolution method circumvents the requirement of keeping one isomer in solution while it is in a state of supersaturation. The larger fraction, although optically impure, can be purified by crystallization (37). If optically impure products are obtained in a resolution, it is often easy to devise a purification method based on crystallization if ternary phase diagrams are available.

Resolution also has been carried out by seeding aqueous solutions of DL-glutamic acid containing hydrochloric and sulfuric acids with crystals of one isomer of glutamic acid hydrochloride (38). Products of high optical purity were obtained directly.

Integrated processes for resolution of DL-glutamic acid hydrochloride are described by Purvis (96) and Fike (40). Brief experimental studies of the variables time, proportion of seed crystals and degree of supersaturation are reported (96).

7. *dl-Adrenaline*

Resolution of *dl*-adrenaline was accomplished by Gero (46), without seeding, by partially neutralizing a solution of 55 per cent *l*- and 45 per cent *d*-adrenaline in dilute hydrochloric acid. The adrenaline that precipitated was about 85 per cent *l*- 15 per cent *d*-adrenaline. The filtrate (which contained an excess of the *d* form) on further neutralization yielded a precipitate containing 77 per cent *d*- and 23 per cent *l*-adrenaline. This resolution procedure is the same, in principle, as that described for threonine in Figure 8. In the case of adrenaline, however, supersaturation was created by partial neutralization rather than by cooling. A similar procedure was used by Calzavara (25). A very unusual case reported for the resolution of *dl*-adrenaline was described by Darmois (28), in which the same neutralization procedure was employed, but with racemic adrenaline. The crystallized adrenaline was always slightly optically active but was sometimes levorotatory and sometimes dextrorotatory. The direction of rotation could not be predicted. The possibility of inoculation from the atmosphere was in-

vestigated experimentally and was eliminated. No explanation was offered for the behavior observed.

8. DL-*Asparagine*

The first recorded resolution of DL-asparagine based on crystallization consisted of mechanical sorting of the crystals, which are hemihedral and visually distinguishable (86). DL-Asparagine was also resolved by seeding its supersaturated aqueous solutions with single crystals of glycine (78), which of course does not contain an asymmetric atom. Some single crystals of glycine induced crystallization of D- and others L-asparagine. The investigator could not predict which asparagine enantiomorph would crystallize. Resolution was attributed to the existence of optical activity due to the crystal structure of glycine. Since glycine crystals do not possess hemihedral facets, the asymmetric crystal structure cannot be ascertained visually.

9. *dl-Dilactyldiamide*

A brief note (47) indicates that *dl*-dilactyldiamide crystallizes from water as a mixture of *d* and *l* crystals in the vicinity of room temperature. Resolution was accomplished by removing individual crystals from the mixture with a pair of pincers and testing them, one by one, in a polarimeter to determine the sign of rotation, since there are no hemihedral facets. Later it was reported (120) that a transition point of the type shown in Figure 1(h) with $t = 35°$ exists for dilactyldiamide. In the binary system at equilibrium a mixture of *d* and *l* crystals is stable above 35° and the racemic compound is stable below. The ternary phase diagrams are of the types shown in Figure 2 above and Figure 4 below 35°. A description was given of a resolution carried out above 35° by seeding a supersaturated solution of *dl*-dilactyldiamide with crystals of either isomer. It was also claimed that resolution could be carried out below 35° because of the existence of "false equilibrium" but no description of the procedure was given nor were any data presented.

10. *Zinc Ammonium dl-Lactate*

Racemic lactic acid has been resolved by converting it to the zinc ammonium double salt, dissolving the latter in water and seeding with either of the optically active forms of the salt (87, 88). The *d* or *l* forms of the salt were found to produce aqueous solutions which were markedly supersaturated. Resolution was incomplete and optically impure products were obtained. Small variations in the amount of water present influenced the optical purity and quantity of the salt which crystallized. Dilution beyond a certain point gave large amounts of the racemic crystals while concentration stopped crystallization entirely, apparently because of high viscosity of the solutions. However, an intermediate range of concentrations gave a mixture of racemic and optically active crystals. Alternate crystallizations of the *d* and *l* salts were carried out in the manner described previously. The crystallization times in most cases were twelve hours or more, reflecting the influence of viscosity on the rate of crystallization.

11. *Alkali Metal Hydrogen dl-Tartrates*

It was found that resolution occurred to a very small degree when racemic sodium, potassium, rubidium or cesium hydrogen *dl*-tartrate was crystallized from a solution of either *d*- or *l*-malic acid (68, 69, 70). Since no seed crystals were added, resolution presumably occurred as a result of the asymmetric influence of the medium, that is, as a result of the difference in solubility of the *d*- and *l*-tartrates in the optically active solutions of malic acid. In an attempt to find out if acids other than malic would induce a measurable degree of resolution, fifteen other optically active acids were tried, all without success.

12. *Other Systems*

Recently, in the course of verifying van't Hoff's prediction that an unsymmetrically substituted cumulene should exhibit optical isomerism when the number of cumulative double bonds is even (114), it was shown that racemic 1,5-di-*p*-chlorophenyl-1,5-di-*t*-butylpentatetraene can be resolved by crystallization (71). This is an example of the resolution of a compound containing no individual asymmetric atom, but which exhibits optical isomerism because of the asymmetric structure of the molecule itself (107c).

Resolution of *dl*-5,5-phenylethylhydantoin by seeded crystallization in methyl alcohol, ethyl alcohol, or their aqueous solutions was recently reported (26). This appears to be the only case known of the resolution by crystallization of a chemical compound containing an asymmetric carbon atom as one member of a ring. The usual procedure was used, with a small excess of the seed isomer dissolved in the initial solution. Solvents in which 5,5-phenylethylhydantoin is sparingly soluble were found to be most suitable as resolution media.

An excellent example of the usefulness of the alternate *d* and *l* resolution technique has been reported for DL-threo-1-*p*-methylmercaptophenyl-2-aminopropane-1,3-diol (66). Forty-six crops of crystals were obtained by seeding alternately with the *d* and *l* forms and adding make-up racemic material between successive resolutions.

A specially designed crystallizer has been used for the resolution of *dl*-methadone (6-dimethylamino-4,4-diphenyl-3-heptanone) by simultaneous crystallization of both isomers on their respective seed crystals from a solution of the racemic form (125). This procedure avoids the necessity for maintaining one isomer in a

state of much greater supersaturation than the other, but requires special provisions for keeping the crystallized isomers separated.

Recently, the racemic forms of 2,3-dibromobutane-1,4-diol and bis-(4-pyridyl)glycol were resolved into their antipodes by crystallization from diisopropyl *d*-tartrate (67) by means of the difference in solubility of the enantiomorphs in the asymmetric medium. The separations obtained in a single crystallization were small, principally because optically impure products were obtained. However, in the case of 2,3-dibromobutane-1,4-diol, repeated recrystallization from the optically active solvent raised the optical purity considerably.

Resolution of monoammonium DL-glutamate has been accomplished by seeding solutions of the racemic form with crystals of the monohydrate of either isomer (31, 53, 54, 74). In one case (53) 21 per cent of the monoammonium L-glutamate in the original solution was crystallized in 50 minutes, giving crystals of monoammonium L-glutamate monohydrate having an optical purity of 98 per cent. Under resolution conditions which produced optically impure products, addition of sufficient water to dissolve the racemic impurity was employed for purification (74). Decreasing the crystal size of the seed increased both the rate and the degree of resolution (53).

Ammonium hydrogen *dl*-malate forms a mixture above 73° and is resolvable above the transition temperature (61, 82, 83, 84, 115). The binary phase diagram is of the type shown in Figure 1(h) with $t = 73°$. The ternary phase diagram with water is of the type shown in Figure 2 above, and Figure 4 below, 73°.

The resolution of ammonium *dl*-molybdomalate has been studied by crystallization of a solution containing a large excess of the levorotatory isomer (29). However, whether or not optically active material was crystallized from a solution of the racemic composition is not clear. The crystals were found to be deposited as a mixture, although the temperature range over which this occurred was not given.

DL-Aspartic acid has been resolved by crystallization from solutions containing one optical isomer of glutamic acid (49, 51, 89, 90, 95). No seed crystals were added. The products were optically impure and also contained some glutamic acid. The earlier discussion of resolution of DL-glutamic acid in optically active solvents applies here as well.

Resolution of zinc DL-glutamate was accomplished by seeding an aqueous solution of DL-glutamic acid containing sodium hydroxide and zinc sulfate with crystals of zinc L-glutamate dihydrate (77). The filtrate was seeded with zinc D-glutamate dihydrate to obtained the D-isomer.

The resolution of *dl*-atropine sulfate was accomplished by crystallization from alcohol (14). No seed crystals were intentionally added to the solution. The resolution was attributed to inoculation by seed crystals present in the laboratory atmosphere.

When crystallized from ether, *dl*-isohydrobenzoin forms hemihedral crystals (42) which can be separated manually (36, 79). It was found that the crystals obtained in this manner were optically impure (98), possibly because of the tendency of the enantiomorphous crystals to form twins when crystallized from ether (97). The pure enantiomorphs have been obtained directly by crystallizing the racemic modification from ethyl acetate or chloroform (97, 99).

dl-Gulonic acid lactone has been resolved by crystallizing it from water at room temperature and mechanically sorting the crystals, which possess hemihedral facets (41).

The complex salt $[Co(C_2O_4)_3]K_3 \cdot 3.5H_2O$ was found to form a racemic compound below 13° and a mixture above (57). The crystals formed above the transition temperature were separated manually. Since no hemihedral facets were formed, the crystals had to be tested individually to determine the direction of rotation.

Various coördination compounds of cobalt and chromium have been resolved by several modifications of the usual crystallization techniques (121).

Brief or incidental studies of the resolution of several other compounds (21, 24, 43, 59, 60, 64, 126) are cited in the treatises of Grignard (30) and Houben (105). The latter two references also contain brief discussions of some of the earlier work on resolution by crystallization methods. A number of cases in which attempted resolutions were unsuccessful have been briefly discussed (48).

VI. DISCUSSION

While the crystallization procedure for resolution is usually easy to apply, the separation obtained in a single crystallization usually is far from complete. In some instances this is a consequence of unfavorable solubility relationships. In others, crystallization of the nonseeded isomer or of a racemic compound along with the desired isomer substantially reduces the degree of separation. However, by repeated crystallizations, as in the method of alternate *d* and *l* resolutions, an essentially complete separation frequently can be attained. The only losses encountered are mechanical in nature. In most cases, however, the products are not optically pure and require further treatment, such as recrystallization, before the pure isomers can be obtained.

Aside from the requirement of a supersaturated solution, little is known about the conditions under which resolution is possible. There are several cases of exhaustive attempts at resolution that have been completely unsuccessful. It has been suggested (105, 120) that resolution can be accomplished only when the antipodes form a mechanical mixture, as indicated by

the phase diagram. While many cases are known of resolutions occurring when the antipodes form a mixture at equilibrium, the resolution of a racemic compound by Duschinsky (34, 35) is in contradiction to the theory. Furthermore, it has been observed that the complex tri-(cyclopentylenediammino)cobaltic perchlorate deposits crystals of the antipodes from solutions of the racemic form at temperatures where the racemic compound is the stable phase (56, 58). Additional data on other racemic compounds would be useful to confirm the finding that they can be resolved by crystallization.

One would expect resolution to be more likely when the enantiomorphs exist as a mixture at equilibrium. However, the crystallization procedure for resolution requires that equilibrium *not* be attained and it is therefore to be expected that any rule based on equilibrium phase behavior will have exceptions.

It has been suggested (121) that resolution is only possible when the solubility of each of the pure isomers is less than that of the racemic modification. This criterion therefore includes as conditions under which resolution is possible all cases of mixture formation and some cases of compound formation. This concept has not been well tested, however.

No case is known in which a substance forming a solid solution has been resolved by crystallization.

It has been shown that seeding an optically active supersaturated solution containing an excess of the isomer not seeded can result in crystallization of the seed isomer from the solution even though it is present in lower concentration than the unseeded antipode (106). It is apparent, therefore, that crystallization can be initiated in a non-equilibrium direction despite the greater concentration of the non-seeded isomer.

Although a wide variety of chemical compounds have been resolved by crystallization, it is not yet possible to predict whether or not resolution of a given racemic modification is possible. At present, each pair of optical isomers must be studied experimentally to determine whether selective crystallization can be accomplished. This situation exists in part because quantitative experimental resolution studies carried out under accurately controlled conditions are scarce. Such studies could be carried out profitably for substances exhibiting each of the three types of phase behavior as well as for single substances in the vicinity of transition points.

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Note: Each of the following groups of references constitutes a set of corresponding (equivalent) patents: 6,7,9,10,12; 15, 16,17,18,33; 19,32; 31,54; 49,51,89,90,92,95; 50,52,91,93,94.