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ASYMMETRIC INTERACTIONS AND THE SEPARATION OF OPTICAL ISOMERS BY OPTICALLY ACTIVE SORBENTS

John M. Krochta*, David R. Buss, and
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March 1971

AEC Contract No. W-7405-eng-48

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ASYMMETRIC INTERACTIONS AND THE SEPARATION OF OPTICAL ISOMERS BY OPTICALLY ACTIVE SORBENTS

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March 1971

ABSTRACT

The stereospecific molecular structure of living things has important consequences in the synthesis of biochemicals for food supplementation, pharmaceutical purposes and other biological uses. The optical isomers obtained must usually be separated, because man and other organisms can most often utilize only one of them. In this work, stereospecific sorption has been studied as an alternate method to better known procedures for separating optical isomers.

In an exploratory study, pure optical isomers of aspartic acid were found to dissolve in measurably different amounts into aqueous solutions of other amino acids, used as optically active solvents. The largest observed effect was a 1.1% difference for the aspartic acid isomers in 0.05M glutamic acid. Such differences in optical-isomer solubilities are explained by differences in binary association between solute and solvent, caused by struc-

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I. INTRODUCTION

The Asymmetry of Living Things

The asymmetric nature of most chemical materials of biological origin is a fundamental characteristic of life. By far the greater proportion of amino acids found in nature, whether as constituents of proteins or in the free state, are of L-configuration. The monosaccharides found in organisms as constituents of nucleic acids, polysaccharides like starch, disaccharides like sucrose, or in the free state, are mainly of D-configuration. Thus, the stereospecific character of the molecules that make up living things is an intrinsic "fact of life".

A major result of this stereospecific nature is that vertebrate organisms, in particular, are much better equipped for metabolism of L-amino acids and D-monosaccharides than for that of their enantiomers. This fact has an important consequence in any synthesis of biochemicals for food supplementation, pharmaceutical purposes, or other biological uses. *In vitro* syntheses result in all possible isomers in equal amounts, since from thermodynamics both isomers of a mirror-structure pair are equally probable; and kinetics provides no selectivity unless perhaps an asymmetric catalyst is used. Hence mixtures of optical isomers which result from non-biological synthesis must be separated if pure optically active forms are required.

Food Supplementation with Synthetic Amino Acids

The possibilities of large-scale food supplementation and an eventual synthetic diet are dramatic examples of the potential utility of synthetic amino acids and the need for good methods of optical-isomer separation. Table 1 lists the minimal and safe levels of the essential amino acids as determined by Rose *et al*. (15). The minimum daily requirement of essential amino acids to maintain a young human male's nitrogen balance totals 6.35 gms. In addition, Rose *et al*. (14) found that approximately 2.5 gms of nonspecific nitrogen is required for the synthesis of nonessential amino acids; in their experiments, this came principally from glycine.

Of the essential amino acids for humans, tryptophan is required in the least amount. If this amount is set arbitrarily at one and the other amino acids listed in proportion to tryptophan, a proportionality pattern of amounts of amino acids needed for best performance results. A proportionality pattern based on the amounts of amino acids listed in Table 1 is shown in Figure 1. A diet possessing an adequate supply of essential amino acids in this pattern available upon digestion (plus sufficient nitrogen for bio-synthesis of other amino acids) has by definition a biological value of 100.

No protein possesses such an ideal amino acid pattern.

Table 1. Minimal Effective and Safely Effective Daily Levels of Essential Amino Acids in the Diet of the Young Human Male (15).

	Minimal Level per Day		Safe Level per Day	
Amino Acid	Grams	Millimoles	Grams	Millimoles
L-Try	0.25	1.2	0.50	2.4
L-Phe	1.10	6.6	2.20	13.2
L-Lys	0.80	5.5	1.60	11.0
L-Thr	0.50	4.2	1.00	8.4
L-Met	1.10	7.4	2.20	14.8
L-Leu	1.10	8.4	2.20	16.8
L-Ileu	0.70	5.3	1.40	10.6
L-Val	0.80	6.8	1.60	13.6

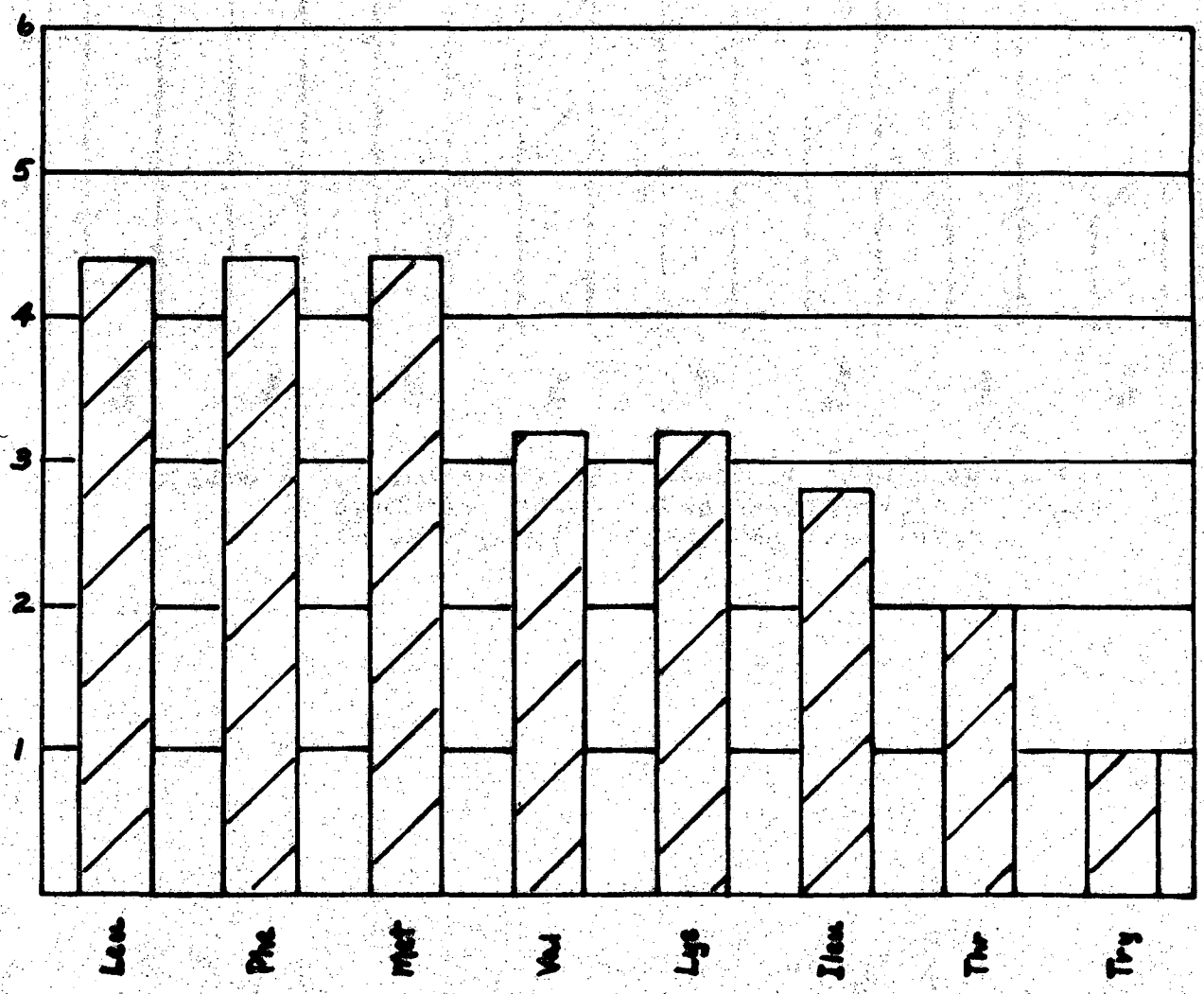


Figure 1. Proportionality Pattern of Requirements for Essential Amino Acids Expressed in Relation to Tryptophan (Calculated from Table 1.).

Nonetheless, in industrialized countries there is usually sufficient protein of high biological value to meet all requirements. Even in these countries, experiments suggest the benefit of amino acid supplementation for pregnant women (17); infants (1,2); aged men (18,3); and in postoperative feeding (12). However, it is in the technically underdeveloped countries that the need for diet supplementation is most evident. The diets of many of these countries consists almost entirely of low-protein grains: rice, corn, wheat, cassava, sorghum, millet, etc; the problem there is compounded by a deficiency of one or more essential amino acids in the protein, leading to a low biological value for it.

Amino acid profiles of the three most important grains, wheat, rice and corn, are shown in Table 2, in comparison with the reference pattern established by the Food and Agricultural Organization of the United Nations (10,11). The reference pattern conforms fairly closely to that established by Rose. Deficient amino acids in each protein are indicated by parentheses. Corn has the poorest balance. Specifically, if the same amount of corn protein were eaten that would satisfy total-nitrogen requirements in the FAO reference pattern, the body's needs for tryptophan, lysine, methionine and isoleucine would not be met. Twice as much corn would

Table 2. Amino Acid Profiles Expressed in Milligrams of Amino Acid per Gram of Nitrogen (11).

	FAO Reference Pattern	Wheat	Rice	Corn
L-Try	90	(75)	(75)	(45)
L-Phe	360	500	655	570
L-Lys	270	(155)	(210)	(170)
L-Thr	180	245	225	255
L-Met	275	280	(180)	(210)
L-Leu	305	430	525	795
L-Ileu	270	275	295	(250)
L-Val	270	280	395	330

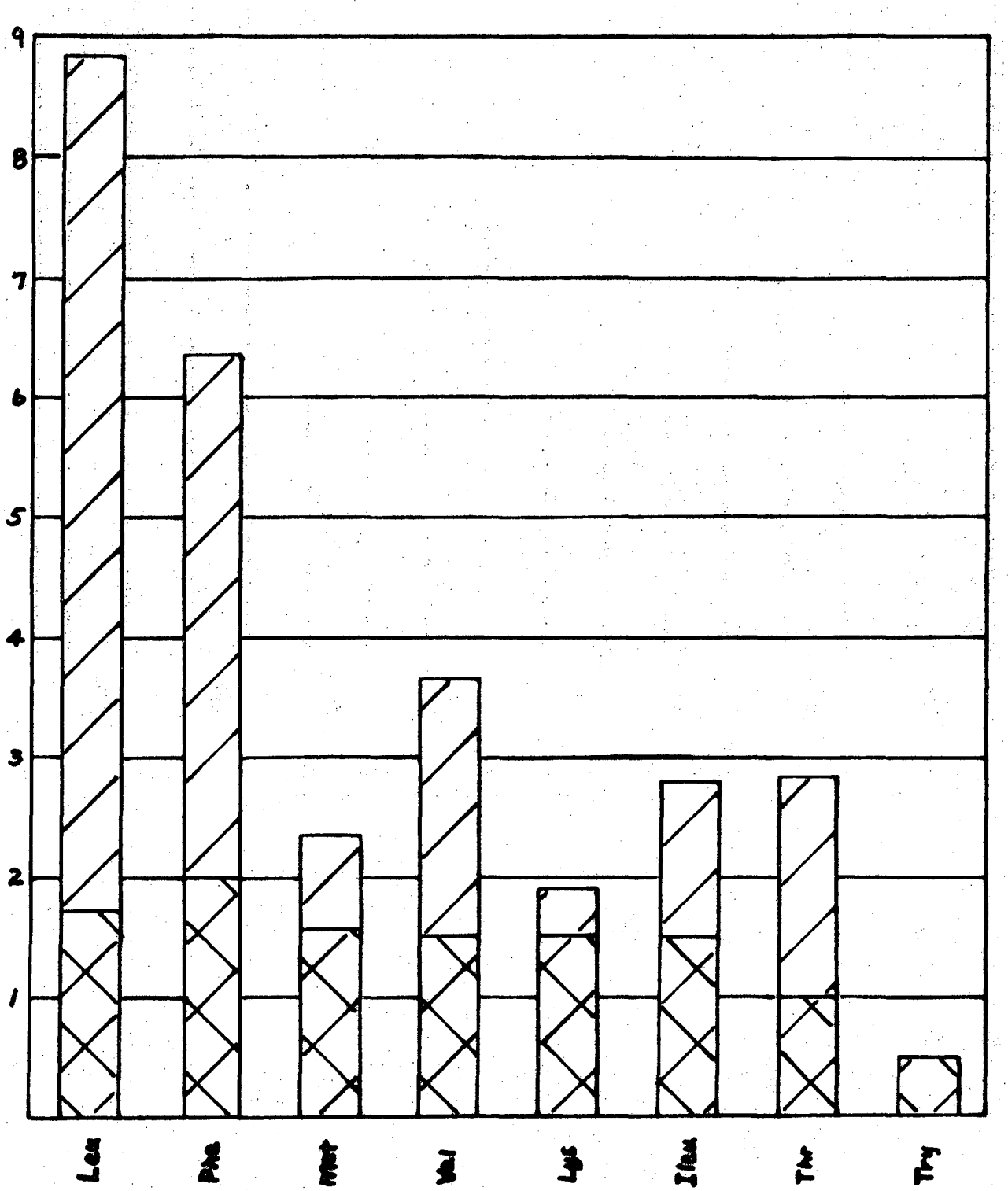


Figure 2. Proportionality Pattern for Corn Compared to 50% of FAO Reference Pattern.

have to be eaten to satisfy the tryptophan requirement. This point is illustrated in Figure 2 by comparing the proportionality pattern of corn to that of the FAO reference pattern; only that part of the amino acid pattern of corn which conforms to the reference pattern is usable by the body. All the amino acid represented by the singly ribbed part of the bar graphs is wasted, even though the body sustains a deficit of 50 percent of its amino acid needs.

The result is that the nitrogen in corn protein has a biological value of only 50.(assuming complete digestibility). That is, only 50% of the nitrogen content (or protein) in the corn can be used as a source of amino acids to support growth, and the remainder serves only as a consumable energy source like carbohydrate. Doubling the tryptophan content of corn by supplementation would raise the biological value to 63, since lysine would now be the limiting amino acid. Further supplementation by lysine could raise the biological value to 76, and by both lysine and methionine to 93.

The same analysis can be made with wheat, rice, and other foods. The point is that if dietary proteins are deficient in one or more essential amino acids, supplementation increases the nutritional value of the diet greatly out of proportion to the quantities added.

Therefore, a marked need exists for inexpensive synthetic routes to the essential amino acids (19,12). With the exception of methionine, which can by used by the body in L- or D-form, procedures allowing resolution of the resulting racemic mixture and recycle of the unusable D-isomer are needed.

A fact important to the synthesis and resolution of amino acids is that amino acid precursors or derivatives can be as useful as the amino acid itself. Thus N-acetyl-L-tryptophan is as good as an equal amount of L-tryptophan in promoting growth in the rat (9). The ethyl ester of L-tryptophan has also been found fully as effective nutritionally as the free amino acid for the growth of rats (6). The same also held true for the amide, ethylamide, diethylamide, anilide and ethylanilide of L-tryptophan (5). Hence, if N-substituted amino acids, amides or esters are formed in the course of synthesis or purification, in some cases they could be used directly as food supplements.

Table 3, reproduced from Howe (11), gives amino acid profiles for several more foodstuffs which are or may be used as major food sources. Again, the deficient amino acids are indicated by parentheses. Again tryptophan, along with lysine and methionine, is seen to be widely deficient.

Table 3. Amino Acid Profiles Expressed in Milligrams of Amino Acid per Gram of Nitrogen (11).

	Peanut	Peas and Beans	Soy-bean Meal	Cotton-seed Meal	Millet	Barley Meal
L-Try	(75)	(55)	(75)	(80)	105	(75)
L-Phe	550	610	500	475	(250)	520
L-Lys	(220)	380	415	275	(150)	(225)
L-Thr	(170)	250	245	210	240	220
L-Met	(135)	(145)	(145)	(190)	(225)	(245)
L-Leu	380	505	475	355	775	420
L-Ileu	(250)	330	360	(225)	330	275
L-Val	305	330	325	290	395	295

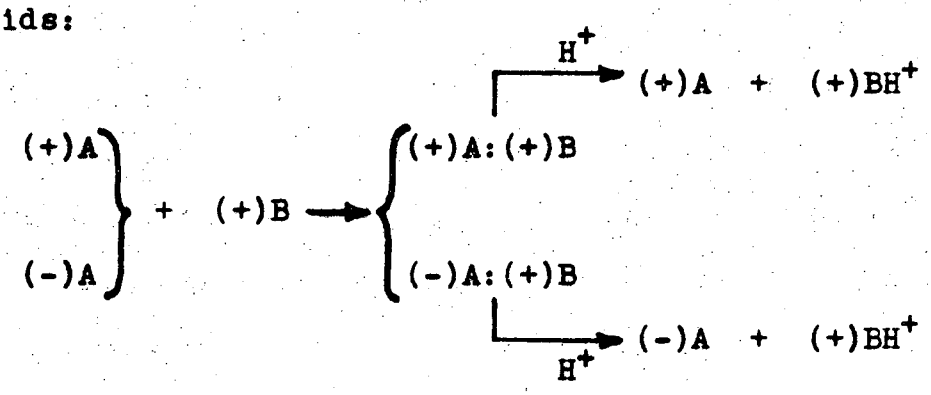
	Oat-Meal	Sor-ghum	Cass-ava	Algae	Bac-teria	Fungi	Yeast
L-Try	(75)	(70)	(40)	125	(50)	95	90
L-Phe	540	565	(255)	525	370	510	470
L-Lys	275	(125)	275	420	330	275	420
L-Thr	230	210	185	235	260	260	330
L-Met	(170)	(200)	(110)	(120)	(215)	(250)	(155)
L-Leu	450	1000	(245)	480	375	550	405
L-Ileu	305	325	(165)	290	280	(170)	330
L-Val	330	350	(240)	390	320	395	375

Present Methods for Separating Optical Isomers

Crystallization Procedures. Selective crystallization of one optical isomer from a solution of the racemic form has been accomplished for many chemical compounds (16). The essential condition here is that the racemic solid must be more soluble than the crystals of pure isomer; hence this approach fails for many compounds. Separation is made to occur by introducing a seed crystal of one of the isomers (or, in some cases, an optically pure seed crystal of a different compound) into a solution mildly supersaturated with respect to both isomers. A series of alternate D- and L- crystallizations is necessary to achieve complete separation.

Diastereoisomer-Salt Formation. The most commonly used method of optical isomer separation involves forming the salts of a pure optically active base with a racemic pair of optically active acids, or vice versa. This leads to a diastereoisomeric mixture; the two resulting compounds are no longer enantiomers, and can be separated by normal differences in solubility. The free optically active isomers can then by obtained from the salts by addition of excess acid or base. The procedure can be illustrated schematically for a pair of optically active

acids:



This method suffers many disadvantages. As the conditions necessary for a successful separation are not known beforehand, the separation of each optical-isomer pair is a new problem. The most desirable state of affairs is for the immediate separation of the diastereoisomeric salts to occur. However, crystals of the less soluble salt invariably entrain some of the more soluble salt, and the residual liquor is not easily freed of the less soluble salt. Complete purification of each salt thus usually involves one or more recrystallizations.

Biological Procedures. The use of biological procedures to resolve optical isomers ranges from employment of whole animals to enzymes. Living animals have been utilized in isolated instances by feeding or injecting a racemic amino acid to isolate one of the optical isomers in the urine. In general, however, it is the biologically active isomer that is consumed, and this method has little practical value.

Use of microorganisms is limited by several factors, notably imperfect selectivity (giving reduced recovery) and incomplete use of the most-consumed species (giving reduced purity). Consumption of the unwanted isomer eliminates the possibility of converting it to racemic mixture, and thus ultimately to the wanted form.

The use of selective oxidases to convert only one optical isomer of an amino acid pair to an α-keto acid or amine has been limited by their cost, and by the limited range of susceptible amino acids for any one such enzyme. This method is also limited since it leads to preparation of only one of the optical isomers.

Asymmetric enzymatic synthesis by selective action of a protease on N-acylated-DL-amino acids to produce a peptide bond with aniline or phenylhydrazine suffers many prodedural difficulties. This approach has been limited by the interrelated problems of solubility control, unfavorable equilibrium, nonselective action, choice of N-acyl group, pH and buffer composition.

Of all the biological procedures for the separation of optical isomers, asymmetric enzymatic hydrolysis through the action of amidases and acylases appears to have the greatest potential. Only the L-isomer of racemic amino acid esters or amides is hydrolyzed, and all products are left in solution to be separated by

differential solubility or ion exchange. Subsequent acid hydrolysis of the D-amino acid amide or acylated D-amino acid yields the free D-amino acid. Thus both optical isomers can be obtained in pure form. Large-scale production of optical isomers using this method is thus possible in principle.

Stereospecific Sorption as an Alternative Technique

In view of the difficulties encountered with the usual methods of optical isomer separation, stereospecific sorption as an alternative technique has been the focus of many studies (7). Selective sorbents, though expensive to make, offer the prospect of being reused for hundreds or even thousands of cycles. Stereospecific sorption is thus potentially more efficient, more generally applicable, and easier to scale up than crystallization and biological operations

However, use of asymmetric sorbents for separations of optical isomers has been retarded by lack of available materials and lack of understanding of how they work. Furthermore, separations achieved with most materials tried to date have given little or no separation.

The present research has included (a) study of amino acid optical isomers in aqueous solution, to determine the degree of association between these compounds and identify the cause for the stereospecific interaction

which occurs; and (b) development of two new types of asymmetric sorbents for use in separating optical isomers.

Solvent extraction using optically active solvents has also been considered as a process employing stereospecific interactions. In general terms, extraction provides higher stoichiometric capacities but smaller selectivities than sorption. Mass-transfer rates in sorption columns are often higher than in extraction columns, but not universally so. At present, there is an acute shortage of potentially effective optically active solvents. These considerations have suggested that today sorption offers greater opportunities for separating optical isomers, and therefore the present research has focused upon chromatography by either adsorption or ion exchange.

Three-Point Interaction Model

Chromatographic separations are based on a difference in affinity of the compounds being separated for the sorbent material. Because of the chemical identity of mirror-image pairs, selectivity can arise only through structural differentiation which involves some nearly pure optical isomer as the separating agent. Even then, on the molecular scale, the separating agent can only differentiate between an optical isomer and its mirror image if at least three points of intermolecular contact

(either attractive or repulsive) are involved. This concept was first recognized for enzyme reactions (13), and later for resolution of optical isomers by asymmetric sorbents (8) and solvents (4). Figure 3, illustrating the concept, shows a pair of optical isomers interacting with identical sorbent sites composed of three different chemical groups 1', 2' and 3'. For the isomer on the left, interaction respectively, of groups 1, 2, and 3 with groups 1', 2', and 3' of the sorbent site is possible. For the isomer on the right, interaction between groups 3 and 3' is impossible when isomer groups 1 and 2 are interacting respectively, with site groups 1' and 2'. Presumably the right-hand isomer can also form a 1-1', 3-3' adduct and a 2-2', 3-3' adduct; the left-hand isomer can form the same three two-point adducts (1-1', 2-2'; 1-1', 3-3'; and 2-2', 3-3'); and the strengths of these two-point adducts should be essentially the same for both isomers. But the total bonding of the left-hand isomer is stronger because it alone forms the additional three-point complex. With such differentiation in total bonding, the right-hand isomer should pass through a column of this asymmetric sorbent more quickly than the left-hand one, and a separation would be effected.

A variation of the three-point concept has been encountered in the course of this study, based upon

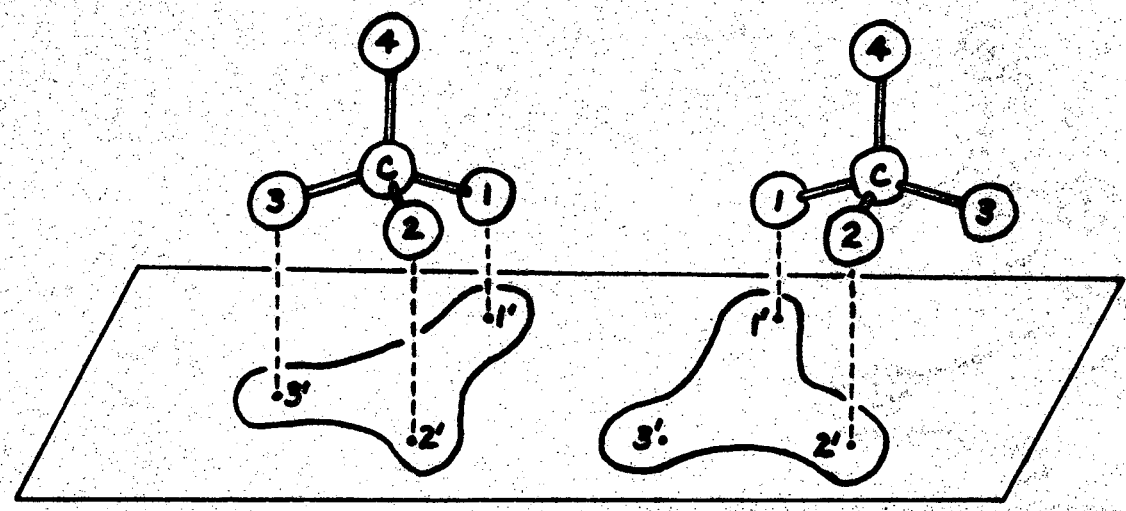


Figure 3. Demonstration That Three Points of Interaction Are Necessary for Stereoselective Sorption. Two Points of Interaction Would Not Distinguish Between the Optical Isomers.

repulsion rather than attraction. It is possible that steric interference between groups 3 and 3' could disrupt the two-point interaction of groups 1 with 1' and 2 with 2' in the left-hand case of Figure 4, but not in the right-hand case. Hence it is then the <u>left-hand</u> isomer which would pass more quickly through a column of the asymmetric sorbent.

This model has proven to be quite useful in determining the interactions leading to asymmetric association between optical isomers and separations of optical isomers on asymmetric sorbents. As experience in separating optical isomers accumulates, its value as a predictive tool will increase.

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II. INTER-DIMER AND SELF-DIMER INTERACTION CONSTANTS BETWEEN AMINO ACID OPTICAL ISOMERS

Introduction

An exploratory study on the resolution of optical isomers has included solubility measurements on pairs of pure optical isomers in optically active solvents. Due to the minimal availability of pure optically active liquids, aqueous solutions of amino acids were used. Results indicate that the isomers of aspartic acid often have different solubilities in such solutions. Diastereoisomer, formed as a collectible second phase, is not a factor in the observed selectivities; for when the optical isomers are crystallized from the optically active solvents, their equivalent weights show that they have not combined with any of the solvent.

That optical isomers should have appreciably different solubilities in optically active solvents is not surprising, but quantitative results for amino acids as extensive as these have not been reported previously. Gil-Av and coworkers (7) have resolved volatile N-trifluoroacetyl amino acid esters by stationary layers of similar nonvolatile esters in gas-liquid partition chromatography. Secor (13) reported in a review that optically active solvents have been used to resolve racemic (50-50) mixtures of optical isomers. Bowman *et al*. (2) have obtained partial resolutions of a series of organic racemates by

partioning the racemates between an aqueous phase and an optically active phase.

A statistical-thermodynamic analysis by Amaya (1) of solutions of optically active substances also indicates that optically active solvents should exhibit selective solvency for one optical isomer of a pair. From a thermodynamic viewpoint, if the solubilities of an optical isomer pair are not the same in an optically active solvent, their activity coefficients must differ also. In Amaya's model, the molecular surface is divided into a number of contact points corresponding to exposed functional groups. In order for molecular contact to favor one optical-isomer configuration over another, at least three contact points of differing composition must interact with a set of three mutually dissimilar contact points on an adjacent molecule. The strengths of such point-to-point contacts have not yet been analyzed or correlated. Nevertheless, the role of "three-point interaction" was recognized earlier by Ogston (11) for enzyme reactions, and by Dalgliesh (6) for resolution of optical isomers by paper chromatography.

The criterion of three-point interaction has been used with our systems, to determine the mechanism leading to enhanced solubility of one optical isomer over the other, and in each case tried it has led to a satisfactory

interpretation.

Solubilities of amino acids in solutions of other amino acids have been measured before, but not primarily for determining optical-isomer effects. In an early study, Cohn et al. (5) measured the solubilities of l-asparagine and of L-cystine in racemic solutions of other amino acids. More recently, Su and Shafer (17) determined the solubilities of many L-amino acids in solutions of other L-amino acids; in the case of L-arginine hydrochloride they determined solubility in a D-tryptophan solution and found it to be different from that in a L-tryptophan solution.

Interestingly, aspartic acid was not included in either of the previous studies. Data from the present study show aspartic acid to associate more strongly with various amino acids than does any other amino acid studied thus far. Furthermore, the differences in solubility of aspartic acid optical isomers in solutions of other amino acids are found to be significantly larger in some cases than the difference in solubility of L-arginine hydrochloride in solutions of L- and D-tryptophan found by Su and Shafer.

The $^{+}NH_3CH(COO^{-})$ group is common to all amino acids. In a solution of amino acids, one would expect some degree of interaction to occur between the NH_3^{+} and COO^{-} groups of one amino acid, respectively, and the COO^{-} and NH_3^{+} of

another. The dimerization constant for this quadrupole formation has been calculated from solubility data for pairs of amino acids and also from activity data for individual amino acids.

Experimental Study

Procedure. For measuring the solubilities, the following experimental procedure was followed: Excess solid amino acids and the aqueous optically active solvents were placed in glass-stoppered flasks. The flasks were mechanically shaken in a constant-temperature bath, controlled at 28.0±0.007°C, during equilibration for 12 to 24 hours. Samples were then collected by filtering the solutions under pressure into weighed test tubes. Analysis by potentiometric titration with 0.05N NaOH followed; triplicate samples agreed to within 0.3% in all cases, with 0.1% being the usual range.

Results. Because aspartic acid isomers were easily purified by crystallization from boiling water, it was used most extensively. Table 1 shows the excess solubility observed for L-aspartic acid in each of six "solvents." Negative values indicate cases where the D-isomer is more soluble.

Qualitative Structural Interpretation

Because amino acids usually have only one asymmetric

Table 1. Excess Solubilities of L-Aspartic Acid Over D-Isomer in Various Solutions at 28°C*.

Aqueous Solvent	Solubility of D-Isomer, mmoles/g solution	Excess Solubility of L-Isomer, %
Water	0.04147	
0.05M Solutions		
L-Leucine	0.04385	0.4
L-Serine	0.04394	-0.4
L-Arginine•HCl	0.04515	0.0
L-Glutamic Acid	0.04295	1.3
D-Glutamic Acid	0.04344	-0.9

*Excess solubility percentage is L-isomer solubility minus D-isomer solubility, divided by L-isomer solubility, times 100.

center, identification of the interactions causing the excess solubility of aspartic acid in the various amino acid solutions has been fairly successful. Of the amino acids used as solvents for aspartic acid, glutamic acid appears to have the greatest asymmetric interaction. Figure 1 shows the molecular structure which produces a stable three-point interaction between L-aspartic acid and L-glutamic acid, this interaction being stronger than any related effect between D-aspartic acid and L-glutamic acid. In this structure, two points of interaction occur by hydrogen bonding between the NH_3^+ of each amino acid and the head-end COO^- group of the other (quadrupole formation). (In the figures, carbon is shown by black orbitals, hydrogen by white, oxygen by dotted gray, and nitrogen by striped gray. Hydrogen bonds are shown by dashed lines.)

However, mere two-point interaction is essentially nonselective; the precise arrangement of atoms away from the two points of contact, in either optical isomer of the solute molecule, cannot have much effect on the solvent molecule or upon the bonds between them. It is when the molecules already bound in a two-point contact swing around to develop a third contact, and find an additional energetically favored point of attachment--and only then--that the asymmetries affect the relative strength of

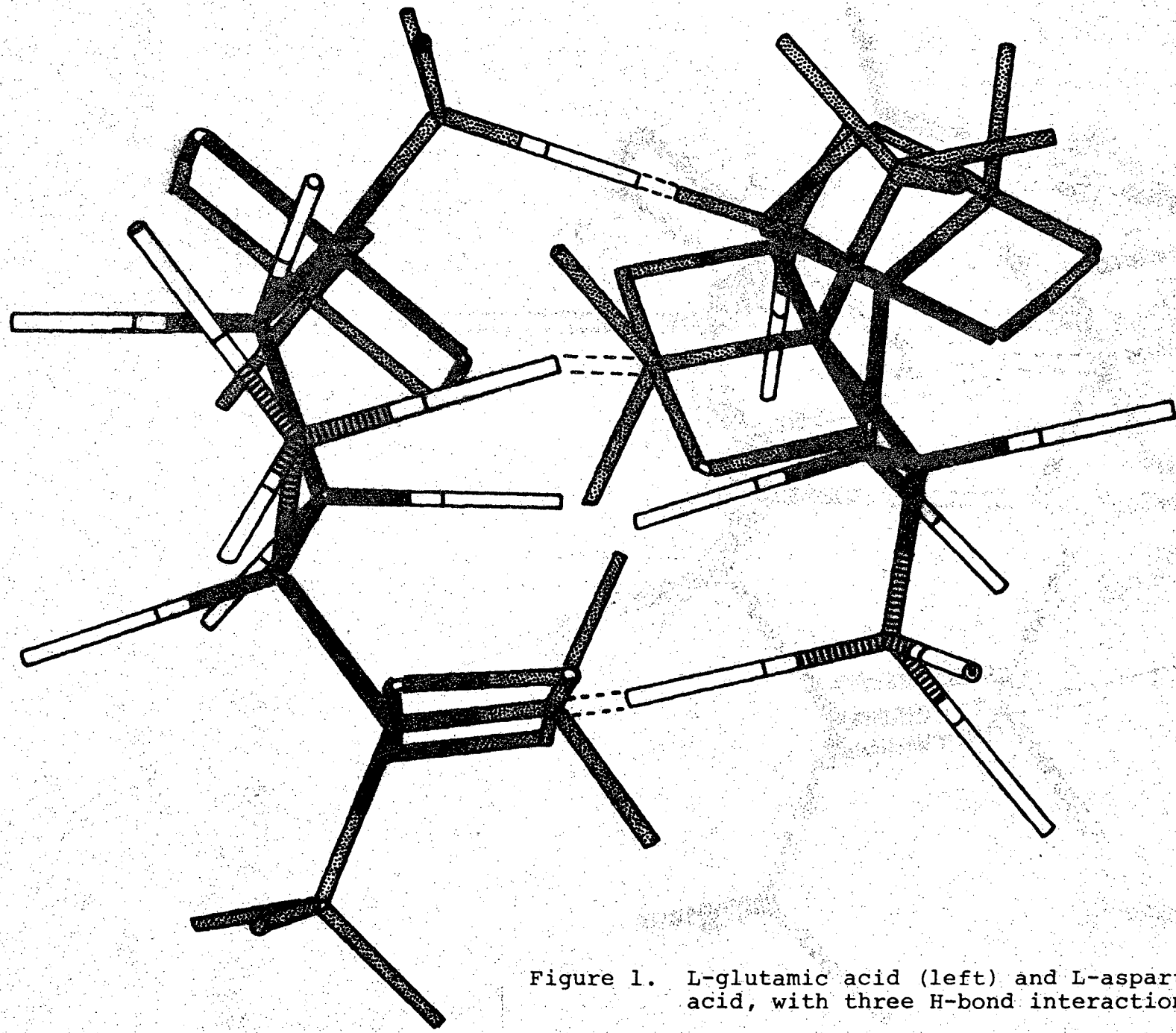

Figure 1. L-glutamic acid (left) and L-aspartic acid, with three H-bond interactions.

complex formation. A dimer bonded at three contact points will increase the equilibrium constant for forming the total dimer of that type.

In the case illustrated by Figure 1, the third point of contact occurs when the respective side chains are situated to allow hydrogen bonding between the tail carboxyl groups. Actually, Figure 1 illustrates only one of six side-chain configurations which will allow three-point contact.

Figure 2 shows the two-point interaction between L-glutamic and D-aspartic acids when hydrogen bonds occur between the NH_3^+ of each amino acid with the head-end COO^- group of the other. Here the geometry prohibits a third point of contact between any parts of the side chains of the two molecules. Nevertheless, it is possible for L-glutamic and D-aspartic acid to develop two three-point-contact complexes, and only two, by another means. In these two complexes (which differ in side-chain configuration), a hydrogen bond between NH_3^+ of L-glutamic acid and COO^- of D-aspartic acid can accompany two other points of hydrogen bonding, one between the COO^- of L-glutamic acid and the tail COOH of D-aspartic acid, and one between the NH_3^+ group of D-aspartic acid and the tail COOH of L-glutamic acid.

These interactions represent only a few of the

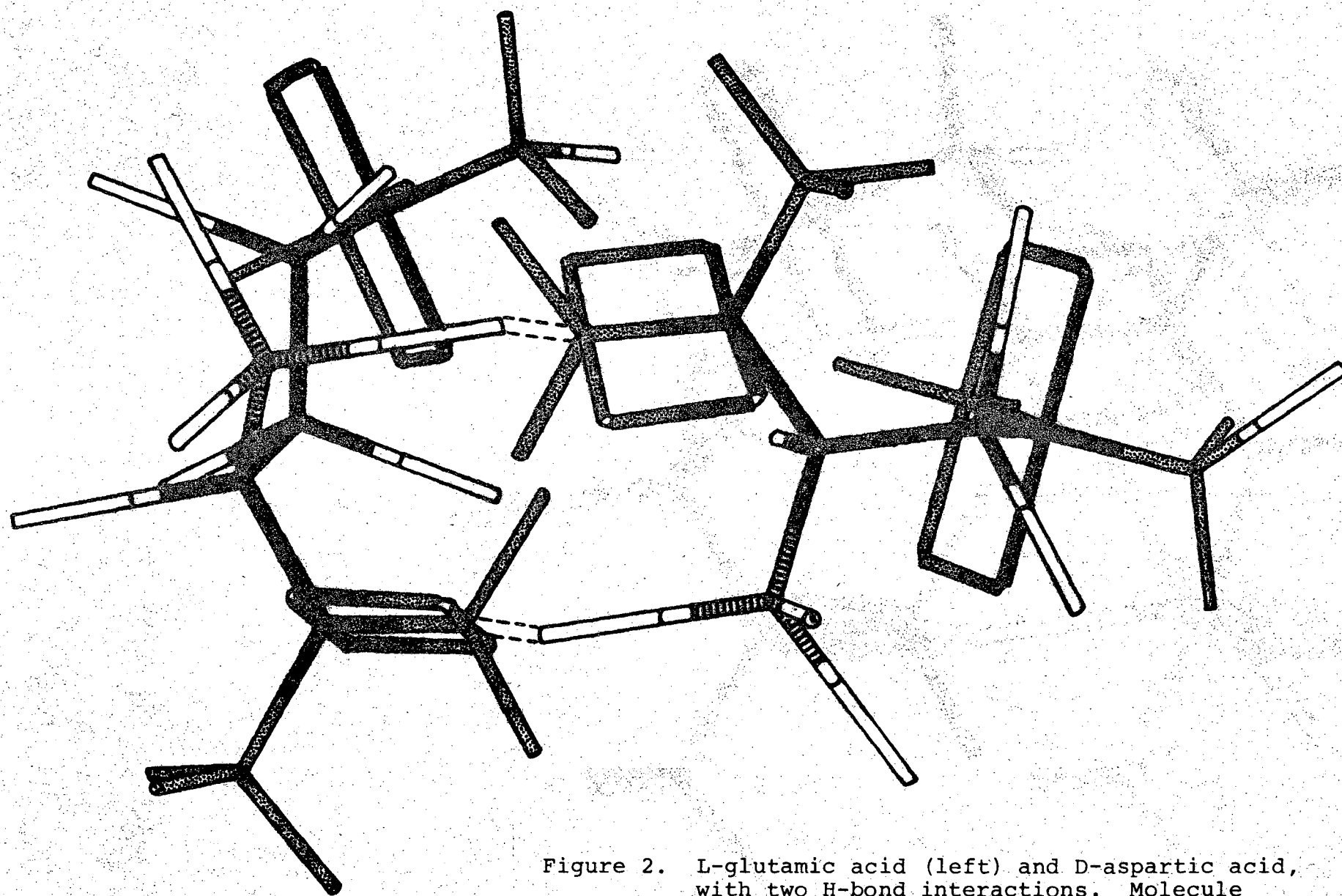

Figure 2. L-glutamic acid (left) and D-aspartic acid, with two H-bond interactions. Molecule shapes do not allow a third H-bond to form.

interactions possible between L-glutamic acid and the L- and D-aspartic acids. However, most other interactions are nonselective. Nonetheless, the modeling result that L-glutamic:L-aspartic three-point-contact complexes should form more easily than L-glutamic:D-aspartic complexes constitutes a prediction that L-aspartic acid will be more soluble than D-aspartic acid in an aqueous solution of L-glutamic acid. The experimental result is consistent with this analysis.

Figure 3 shows the three-point contact which can occur between L-aspartic acid and another amino-acid solvent, L-leucine. Two of the three contacts again occur by hydrogen bonding between the charged NH_3^+ and COO^- groups of the respective amino acids. In this case the third point of contact occurs by hydrophobic bonding between the aliphatic side chain of the L-leucine and the CH_2 group of the L-aspartic acid. However, Figure 4 shows that for L-leucine and D-aspartic acid, it is impossible to develop the third point of contact when hydrogen bonds form between the charged NH_3^+ and COO^- groups of the respective amino acids. This observation leads to the conclusion that L-aspartic acid will be more soluble than D-aspartic acid in a solution of L-leucine. Again, the experimental result is consistent with the structural model.

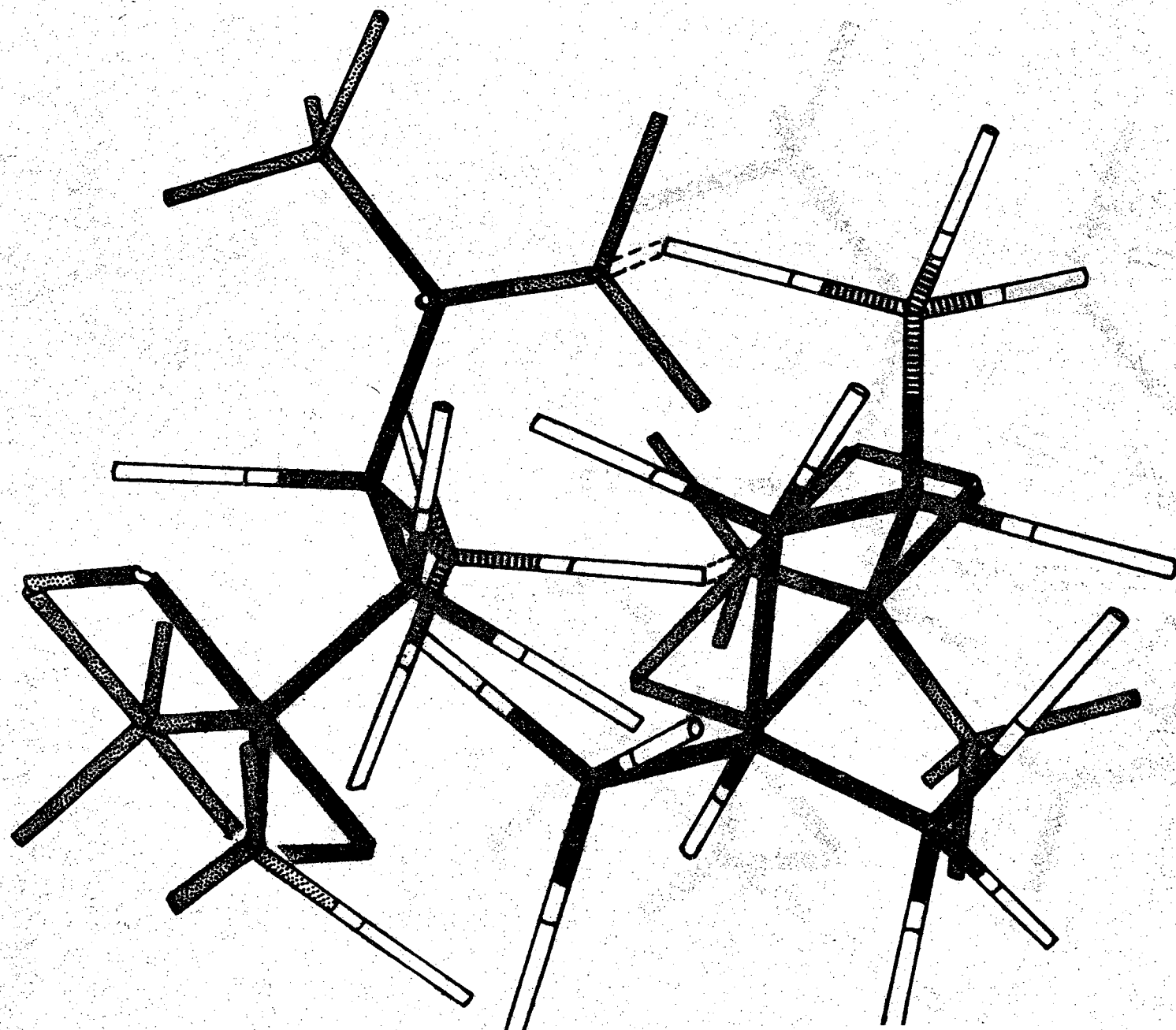

Figure 3. L-aspartic acid (left) and L-leucine, with two H-bonds and a hydrophobic bond.

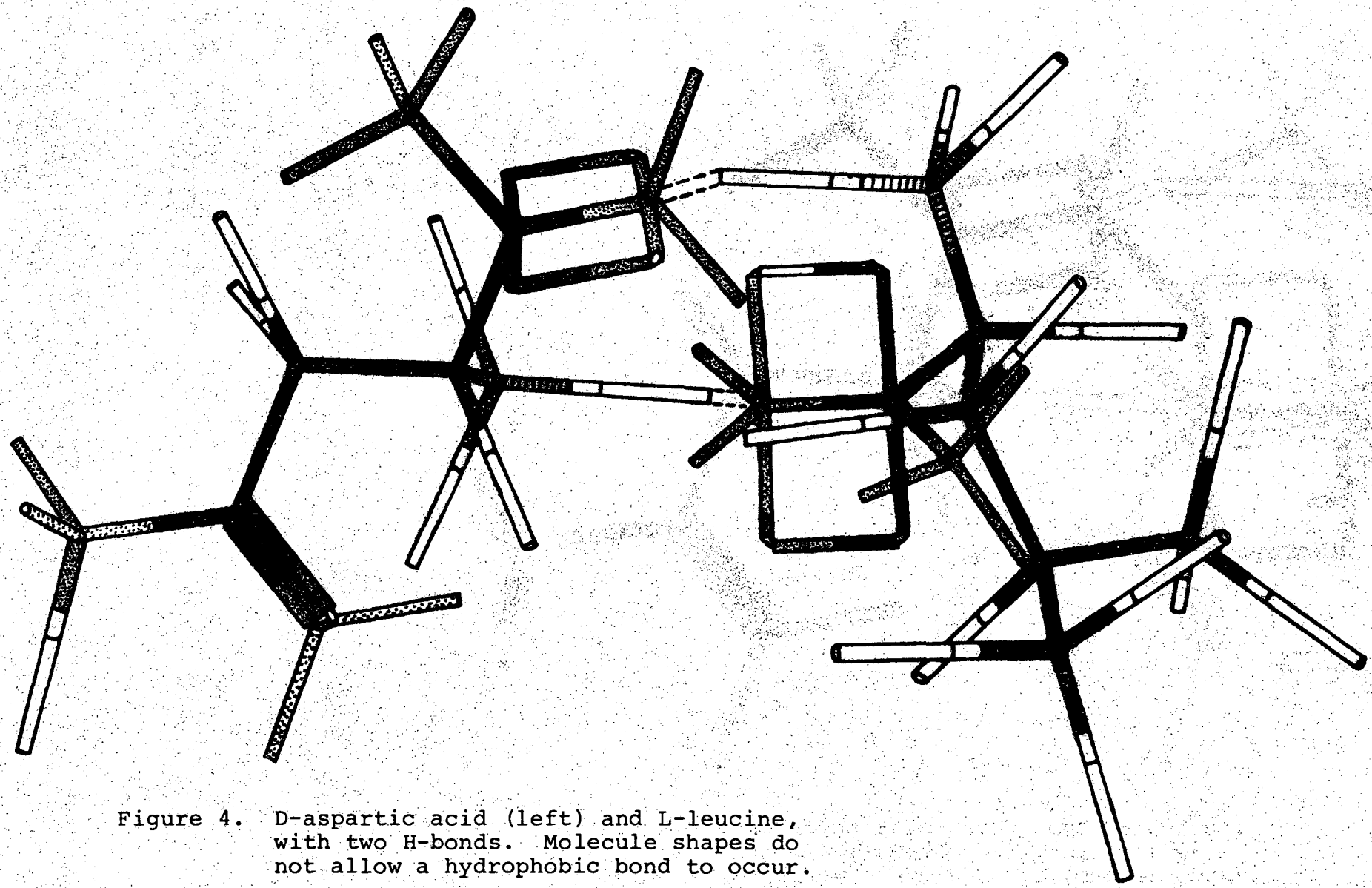

Figure 4. D-aspartic acid (left) and L-leucine, with two H-bonds. Molecule shapes do not allow a hydrophobic bond to occur.

A similar analysis has been made successfully with arginine hydrochloride and with serine, as solvents for aspartic acid.

Equilibrium Constants for Inter-Dimerization

The increase in solubility of an aspartic acid isomer (A) in a solution of a glutamic isomer (G), relative to its solubility in water, measures the extent of interspecies dimer formation. From thermodynamics, considering that activity coefficients should not change appreciably, the saturation concentration of solute monomer plus solute self-dimer should be the same (0.04147 moles/liter) in the mixed amino acid solution as in water. The molecular species present can be considered to be A, A_2, G, G_2 and AG. Then AG accounts for a reduction in concentration of G plus G_2 below the starting value (0.05 moles/liter).

Although the extent of self-dimerization is not yet known for either aspartic acid or glutamic acid, a minimum value of the equilibrium constant for inter-dimer formation, K_{AG}, can be obtained by assuming that no self-dimers (A_2, G_2) form. The solubility increase, from averaged experimental data, is 0.00200 moles/liter for L-aspartic acid in L-glutamic acid (or D in D); and 0.00153 for D-aspartic acid in L-glutamic acid (or L in D). The glutamic acid concentration, calculated

as monomer, will be reduced below 0.050 moles/liter by these amounts. These minimum values are

$K_{AG} = (AG)/(A)(G)$

$(K_{AG})_{LL} = 0.00200/(0.04147 \times 0.04800) = 1.01$ (moles/liter)$^{-1}$

$(K_{AG})_{DL} = 0.00153/(0.04147 \times 0.04847) = 0.76$ (moles/liter)$^{-1}$

If the self-dimerization constants are set at improbably large values (say, K = 3), the values of K_{LL} and K_{DL} will each increase by about 50%. However, the ratio of K_{LL} to K_{DL} remains essentially constant. Therefore this analysis indicates that the solubility increase of slightly more that 1% from D in L to L in L corresponds to a striking 33% difference (=(1.01-0.76)/0.76) in the extent of formation of inter-dimer. The use of an insoluble glutamic-acid sorbent in a chromatographic separation of racemic aspartic acid might thus show a selectivity ratio or retention-time ratio of about 1.33.

A similar approach to the data on leucine, serine and arginine hydrochloride gives the following results, similar in kind although less striking:

	Minimum K for inter-dimerization		Corresponding selectivity ratio in adsorption (K_{LL}/K_{DL})
	L-L	D-L	
Aspartic acid/Leucine	1.29	1.21	1.07
Aspartic acid/Serine	1.16	1.25	0.93
Aspartic acid/Arginine·HCl	1.92	1.92	1.00

These inter-dimerization constants provide interesting comparisons with those calculated from the data of other workers for different amino acids. The solubility data of Cohn et al. (5), duplicated in part in Table 2, give minimum inter-dimerization constants ranging from 0.56 (moles/liter)$^{-1}$ down to zero. In fact, in the case of l-asparagine, a negative solubilizing effect is observed with a solution of DL-α-aminobutyric acid. The data of Su and Shafer (17), duplicated in part in Table 3, give constants ranging from 1.59 (moles/liter)$^{-1}$ to 0.47 (moles/liter)$^{-1}$. Su and Shafer looked at many other systems, but found little or no effect compared to those reported in Table 3; they also had cases where negative solubilizing effects were observed. From a comparison of all these data, aspartic acid seems especially capable of forming dimers with other amino acids.

An interaction common to all associating amino acid pairs is that involving the NH_3^+ and COO^- of one amino aicd with the COO^- and NH_3^+, respectively, of the other. The dimerization constant for this so-called quadrupole interaction would be a piece of interesting, fundamental information. The simplest system investigated by Cohn et al. (5) was that of l-asparagine in glycine. The inter-dimer constant calculated from his results is 0.23 (moles/liter)$^{-1}$. This value should be quite close to the basic quadrupole

Table 2. Minimum Inter-Dimerization Constants Calculated from Solubility Measurements of Cohn *et al.* (5).

	l-Asparagine		L-Cystine	
	Solubility	K	Solubility	K
Aqueous Solvent	moles/ liter	(moles/ liter)$^{-1}$	moles/ liter	(moles/ liter)$^{-1}$
Water	0.184		0.000454	
0.25*M* Solutions				
Glycine	0.194	0.23	0.000518	0.56
DL-Alanine	0.188	0.09	0.000495	0.36
DL-α-Aminobutyric Acid	0.182	——	0.000474	0.18
DL-Valine			0.000454	0

Table 3. Minimum Inter-Dimerization Constants Calculated from Solubility Measurements of Su and Shafer (17).

	L-Phenylalanine		L-Tryptophan		L-Tyrosine	
	Solu-bility	K	Solu-bility	K	Solu-bility	K
Aqueous Solvent	moles/ liter	(moles/ liter)$^{-1}$	moles/ liter	(moles/ liter)$^{-1}$	moles/ liter	(moles/ liter)$^{-1}$
Water	0.16		0.07		0.0025	
0.50*M* Solutions						
L-Arg·HCl	0.195	0.47	0.11	1.24	0.0034	0.72
L-His·HCl	0.21	0.70	0.12	1.59	0.0038	1.04

interdimer-constant for amino acids. There are two pieces of supporting evidence for this belief: (a) As Cohn *et al.* point out, the data indicate that glycine-asparagine interactions are of the same strength as glycine-glycine interactions. Since glycine is the simplest amino acid, interaction between glycine molecules should result mainly from quadrupole formation. (b) The Cohn data give a value of 0.56 (moles/liter)$^{-1}$ for the glycine-cystine inter-dimer constant. Cystine has two $=C(NH_3^+)COO^-$ groups. If the calculation for the inter-dimer constant used the concentration of the $=C(NH_3^+)COO^-$ groups and not the cystine concentration, the result would be $\frac{1}{2}(0.56)$ or about 0.28. This is quite close to the 0.23 value obtained for the glycine-asparagine system.

All the amino-acid-pair systems involving aspartic acid give dimer constants greater than this basic value, and this may perhaps be attributed to the acidic side chain with its strong hydrogen-bonding potential. The system giving the largest inter-dimer constant in our work contains an amino acid with a basic side chain, arginine hydrochloride, along with aspartic acid.

The amino acid pairs in Su and Shafer's work which give large inter-dimer constants each contain an amino acid possessing a basic side chain, arginine or histidine, which also includes a significant hydrophobic section.

In some cases these molecules exert little or no influence on the solubilities of other amino acids; thereby showing the stereospecificity of amino acid side-chain interactions. For these cases, and for other cases where near-zero or negative effect on solubility was found, the quadrupole inter-dimerization may have been masked by hydration effects which will be discussed in the next section.

Equilibrium Constants for Self-Dimerization

The activity coefficients of glycine and several other amino acids have been determined by Smith and Smith (14, 15 16). Since glycine ($^{+}NH_3CH_2(COO^{-})$) is the simplest amino acid, dimerization occurring between glycine molecules was believed to result mainly from quadrupole formation. Therefore, dimerization constants for glycine were calculated from its activity coefficients.

An "ideal associated solution", in the terms of Prigogine and Defay (12), is a solution which behaves ideally when regarded as a mixture of monomer molecules and complexes. Developing the thermodynamic relationships for an ideal associated solution, Prigogine and Defay determined that the monomer and total concentration of the associating species are related by the activity coefficient. Employing as system of reference a very dilute solution of A in B, and assuming that only molecules of

A associate, the following expression holds:

$$\gamma_A = \frac{x_{A_1}}{x_{A_T}}$$

where x_{A_1} is the mole fraction of monomer and x_{A_T} is the total mole fraction of A. At the low concentrations of interest

$$\frac{x_{A_1}}{x_{A_T}} = \frac{C_{A_1}}{C_{A_T}}$$

where C_{A_1} and C_{A_T} are the monomer and total concentration in moles/liter. Since

$$C_{A_T} = C_{A_1} + 2C_{A_2}$$

where C_{A_2} is the concentration of dimer, a dimerization constant defined as

$$K = \frac{C_{A_2}}{(C_{A_1})^2} = \frac{C_{A_T}(1 - \gamma_A)}{2(\gamma_A\ C_{A_T})^2}$$

can be calculated if activity coefficients are known as a function of concentration of compound A.

The activity coefficients determined by Smith and Smith for glycine (14) are molal activity coefficients, rather than the mole-fraction activity coefficients used in the development above. Nevertheless, at the concentrations of interest the difference between these is slight.

Smith and Smith's activity coefficients for glycine, along with dimerization constants calculated from them, appear in Table 4. These constants tend to a value of

Table 4. Self-Dimerization Constants for Glycine Calculated from the Data of Smith and Smith (14).

m	C	x	γ	K
moles/ 1000 gms	moles/ liter	mole fraction		(moles/ liter)$^{-1}$
0.1	0.099	0.00180	0.980	0.104
0.2	0.198	0.00359	0.963	0.101
0.5	0.489	0.00893	0.915	0.104
1.0	0.958	0.01769	0.855	0.104

Table 5. Self-Dimerization Constants for Serine and Threonine Calculated from the Data of Smith and Smith (15).

	Serine		Threonine	
m	γ	K	γ	K
moles/ 1000 gms		(moles/ liter)$^{-1}$		(moles/ liter)$^{-1}$
0.2	0.965	0.095	0.990	0.026
0.3	0.945	0.104	0.985	0.026
0.5	0.910	0.111	0.976	0.026

0.104 (moles/liter)$^{-1}$, somewhat less than one half of the value determined for the quadrupole dimerization constant in the previous section from solubility data. A factor of two is expected, however, since symmetry of the self-dimer should halve the constant for its formation.

To explore further the significance of the activity coefficients, the self-dimerization constants for serine and threonine calculated from activity coefficients determined by Smith and Smith (15), are reported in Table 5. Serine, which possesses a fairly simple side group, has self-dimerization constants quite close to those of glycine. Threonine, which possesses an exposed CH_3 group, displays a self-dimerization constant much closer to zero.

Smith and Smith (16) also determined activity coefficients for several aliphatic amino acids- alanine, α-amino-η-butyric acid, α-amino-isobutyric acid, α-amino-η-valeric acid, and valine. Whereas glycine, serine, and threonine have activity coefficients less than one, thereby indicating association in solution, the activity coefficients for the aliphatic amino acids are greater than one. Furthermore there is a general tendency for the activity coefficient to increase with each methylene group. Aside from the solute-solute interaction which occurs in any

solution, solute-solvent interactions are also present. In the case of the aliphatic amino acids, the latter most likely predominate. As Nemethy and Scheraga have pointed out (9, 10), the presence of hydrophobic groups in an aqueous environment tends to result in greater ordering of the water molecules around the solute, with increased hydrogen bonding in the region. The resulting negative enthalpy change is more than counterbalanced by the large negative entropy change, thereby leading to a large positive free energy change. In other words, the interaction of non-polar groups with water is unfavorable because of structural (entropy) considerations. There should therefore be a tendency for non-polar groups to contact each other in aqueous solution, thereby decreasing interaction with water. Kozak et al. (8) have interpreted activity data for aqueous solutions of nonelectrolytes using both lattice and distribution-function theories. While they found that pairwise attractions seem to increase with increasing aliphatic chain length for alcohols, amines, ketones and acids, the attractive forces in the amino acids appear to rise much more slowly in a homologous series. As a result, hydration and size effects predominate, leading to activity coefficients less than one for water containing aliphatic amino acids in solution. This result ultimately gives the activity coefficients greater than

one for the aliphatic amino acids.

For those amino-acid-pair systems mentioned in the last section which showed little or no solubility enhancement (or near-zero dimerization constants), hydration effects must predominate over the quadrupole-interaction effect. Hydration of the "solvent" amino acid leaves less water available for the solute amino acid. In the extreme, this apparently results in lower solubility in certain amino acid solutions than in water.

Conclusions

These results indicate that stereospecificity towards a small molecule is not restricted to large complex molecules such as enzymes. Even single asymmetric centers can interact rather strongly with one another.

Taking all available data into account points to a probable value of about 0.23 (moles/liter)$^{-1}$ for the non-selective part of the inter-dimer equilibrium constant that results from interaction of the NH_3^+ and COO^- of one amino acid with the COO^- and NH_3^+, respectively, of a different amino acid. This means that when large inter-dimer constants are found for amino acid pairs, they must result mainly from interactions involving the remainder of the molecules.

This work has led to the study of resolving agents prepared by attaching optically active amino acids to an

insoluble matrix, in a way that preserves their zwitterionic character. Such sorbents have been synthesized successfully (16, 17), but have not yet shown the selectivity (α's of 1.07 to 1.33) that would be expected from the liquid-phase interactions.

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III. FORMYLATED AROMATIC POLYMERS AS INTERMEDIATES IN SYNTHESIS OF OPTICALLY ACTIVE SORBENTS

Introduction

Resolution of optical isomers by chromatographic techniques over asymmetric sorbents has been the subject of recent studies (2). Theoretically it should be possible to tailor-make an asymmetric sorbent capable of resolving any given enantiomorphic pair. However, present knowledge of asymmetric molecular interactions is insufficient to indicate clearly the proper structure for such sorbents. Also the known methods of resin synthesis are limited. This paper reports a new route to asymmetric resins, relatively simple and versatile, based on the use of a formylated aromatic polymer.

Amino acids attached to an insoluble matrix are desirable as sites of asymmetry. Considering the "three-point interaction" known to be necessary for distinguishing between a pair of optical isomers (6), amino acids when zwitterionic already possess two ionic sites capable of interaction. Furthermore, amino acids possess many different types of side groups. Solubility studies of amino acids in amino acid solutions show that the amino acids interact asymmetrically, presumably with the aid of ionic quadrupole interaction, but with an additional point of contact (13). Moreover, asymmetric resins containing certain amino acids have been used to resolve other racemic amino acids or mandelic acid (10,12,16,17,25,

27,28). A new synthesis has been sought because the methods used hitherto either would cause loss of zwitterionic structure or are not applicable to most amino acids.

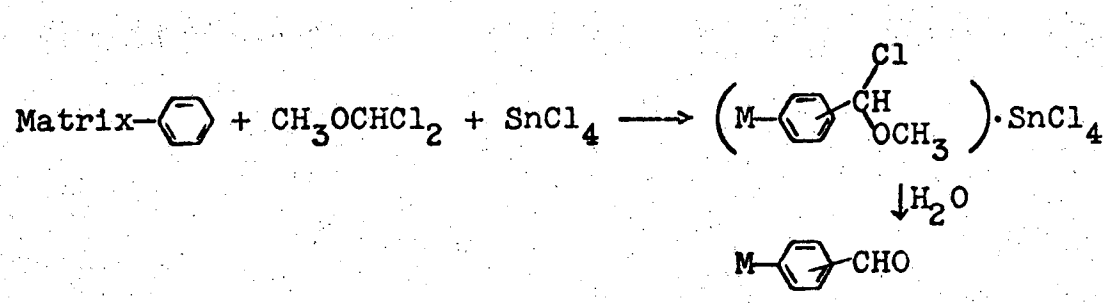
Most anion-exchange resins are prepared by reacting halomethylated polymers with the desired amine (8). Optically active ion exchangers so made from asymmetric amine compounds, including amino acids, were first reported by Rieche and Gross (23) and later improvedupon by Korshak and coworkers (11). The present authors have found halomethylated polymers to have several disadvantages. Reaction conditions may cause racemization; reactivity of sterically hindered amines may be quite low; additional crosslinking may occur by the amines reacting with more than one halomethylated group; and halomethyl may react with groups other than primary amines.

A New Synthetic Route

Imine formation and reduction offers an alternate synthetic route that can overcome these problems. Preparation of Schiff's bases in homogeneous solution from aldehydes and amines, and also their reduction to other amine compounds, is well known (15). In particular, benzaldehyde and various amino acids have been reacted and reduced to the corresponding N-benzyl derivatives in high yield (22). However, it appears that this reaction sequence has not been used previously for preparing amine-

containing polymers, primarily for want of suitable formylated polymers and of a method of reducing imine formed within the matrix.

<u>Formylation of Polystyrene</u>. A process for formylation similar to the common chloromethylation (21) of styrene-divinylbenzene copolymer was developed (1), using dichloromethyl methyl ether (DMME) in place of the monochloro compound. DMME has also been used to formylate aromatic monomeric compounds in homogeneous solution (24). In the present process the copolymer beads are alkylated with DMME, using anhydrous $SnCl_4$ as a relatively mild Friedel-Crafts catalyst, after having first been swollen in methylene chloride. Hydrolysis of the intermediate complex proceeds smoothly and yields a brilliant yellow formylated resin with conversions up to 85% based upon the polymer:



The effect of various solvents on swelling an 85% formylated styrene-divinylbenzene resin is shown in Table 1. The results indicate that little, if any, additional cross-linking is produced by formylation. The beads swell slowly

Table 1. Solvent-Swelling at 25° C of Formylated Polystyrene (4% divinylbenzene, 150-200 mesh, 6.5 meq. CHO per g.)

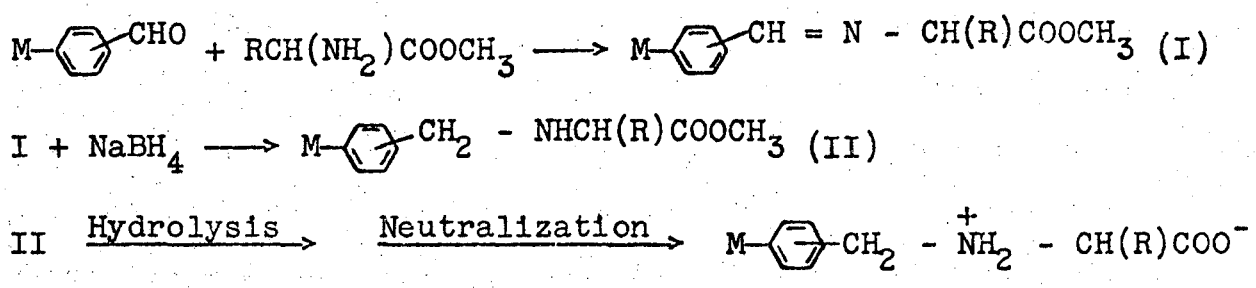
Solvent	Relative Volume of Beads to Dry Beads at 25 C
$CHCl_3$	2.4
$C_2H_4Cl_2$	2.4
Benzyl Alcohol	2.1
Dioxane	2.1
Dimethyl Formamide	2.1
Acetone	2.0
Benzene-Methanol (1:1)	2.0
Benzene	1.9
Dimethyl Sulfoxide	1.7
Methanol	1.5
i-Propanol	1.4
N-Propanol	1.4
Water	1.3

in alipathic alcohols, and do not reach the indicated relative volumes unless they are first swollen with another solvent and then rinsed with the alcohol.

Other methods for preparing a formylated resin, some of which were tried, appeared unsuitable. Polymerization of formylstyrene monomer yields a product incapable of swelling in solvents such as chloroform (29), due evidently to crosslinking side reactions. Another long reaction sequence which was considered involves polylithium styrene (3). Oxidation of either chloromethylated or hydroxymethylated styrene-divinylbenzene by dimethyl sulfoxide (19), or KOCL (18) was rejected because of multiple products, or because reactants could not penetrate into the matrix. Certain Friedel-Crafts reactions also proved unsatisfactory: the Gatterman-Koch reaction and its modifications (20) called for gaseous reactants and high-pressure conditions unsuitable in small-scale preparation, and gave the possibility of undesirable side reactions; alkylation experiments with $CHCl_3$ and several active metallic-chloride catalysts in the usual solvents also failed.

<u>Synthesis of Amino Acid Resin</u>. The scheme developed for attaching amino acids to an insoluble polymeric matrix while preserving their zwitterionic character consists of three steps (1): imine formation by reacting the formylated beads with an amino acid ester, reduction of the imine with $NaBH_4$ which is soluble in various solvents and hence

can penetrate the matrix, and hydrolysis of the attached ester, as follows:



Resins containing L-tyrosine, L-aspartic acid, and L-glutamic acid have been prepared. Tyrosine can only be attached to a resin matrix in this way, because of its active phenolic hydroxyl group.

The results of a series of syntheses are shown in Table 2, along with the conditions used for the imine formation. The first three syntheses reveal that ionic amino acid derivatives do not react as readily with the formylated beads as does the uncharged one, an ester. Two factors probably are responsible -- the insolubility of ionic derivatives, and Donnan exclusion of charged species from a bead once some charge is attached. Lysine has the additional problem of crosslinking the matrix since it is a diamine. Thus, the use of amino acid esters or amides seems the best approach, although esters may condense to form cyclic anhydrides or peptides especially at elevated temperatures (7).

$NaBH_4$ was chosen as the reducing agent because it is soluble, can penetrate the matrix, and does not reduce the

Table 2. Synthesis of Amino Acid Resins with Formylated Polystyrene
(4% divinylbenzene, 150-200 mesh, capacities as shown)

Amino Acids (mmoles)	Salts Added (mmoles)	Solvent (ml.)	Formylated Beads, g. (mmoles CHO)	Temp. of Reaction	Time (hrs.)	% Conversion	Notes.
L-Lysine·HCl (6.03)	LiOH (5.9)	BzOH (25)	0.50 (3.25)	60°C	3	4.1	Amino acid only sparingly soluble.
L-Tyrosine (6.02)	LiOH (5.9)	BzOH (25)	0.50 (3.25)	54°C	3	1.6	Amino acid salt not very soluble.
L-Tyrosine Me Ester·HCl (6.0)	LiOH (5.9)	BzOH (25)	0.50 (3.25)	58°C	3	48.8	Ester soluble.
L-Tyrosine Me Ester·HCl (6.1)	LiOH (5.9)	Benzene (20) MeOH (5)	0.50 (2.70)	49-57°C	8	70.8	Ester soluble, two phase liquid system initially, soln. darkened during run.
L-Tyrosine Me Ester·HCl (6.1)	LiOH (5.9)	Benzene (20) MeOH (5)	0.50 (2.70)	29°C	8	53.4	Same as above except soln did not turn dark.
L-Aspartic Acid Dimethyl Ester·HCl (6.1)	LiOH (5.9)	Benzene (20) MeOH (5)	0.50 (2.70)	25°C	12	43.5	Ester precipitated, did not appear very soluble.
L-Tyrosine Me Ester·HCl (51.0)	LiOH (50.0)	MeOH (20) Benzene (16)	8.00 (43.0)	25°C	36	90	Ester partially precipitated initially but disappeared during reaction.
L-Glutamic Acid Dimethyl Ester·HCl (8.0)	LiOH (7.0)	Benzene (25) Methanol (10)	0.50 (3.30)	25°C	23	31	Ester completely soluble.

ester group as $LiAlH_4$ would (15). The literature indicates that $NaBH_4$ can reduce esters to alcohols (4), but tests on the L-tyrosine resin indicated that the ester was not reduced and that hydrolysis was complete. The imine beads (I) are brilliant yellow like the starting formylated beads, and both become colorless upon complete reduction. After reduction and hydrolysis the amino acid content, and CHO conversion, are determined. The cause of the brilliant yellow color of the beads, and the extent of side reactions such as crosslinking during formylation, have not been investigated. However they are not appreciably harmful, as indicated by the swelling data of Table 1 and by the high reactivity shown in Table 2.

Separations

Resolution of DL-Mandelic Acid

(a) Frontal and Elution Analysis. Because of its good solubility in many solvents, large optical rotaion of its isomers, and ease of detection, DL-mandelic acid was used in initial experiments to test the resolving ability of the amino acid resins. Table 3 shows the results of frontal plus elution analysis on DL-mandelic acid in methanol, using a column containing 14.6 gm of the L-tyrosine resin and measuring 1 cm in diameter by 36 cm long. The column was first preconditioned by washing with 0.15M ammonium chloroacetate in methanol. A solution of 0.15M (22.8 mg/ml).

Table 3. Resolution of DL-Mandelic Acid on L-Tyrosine Resin in Methanol

Fraction	ml	Mandelic Acid mgm/ml	Rotation, α_D l = 4 cm	Resolution D/L
1-2	32	0	0.0000	
3	15	0.2	0.0000	
4	15	6.2	+0.0055	0.974
5	14	14.0	+0.0055	0.989
6	14	15.9	+0.0040	0.992
7	13	17.2	+0.0025	0.995
8 ←	15	18.7	+0.0025	0.996
9	15	25.6	+0.0020	0.997
10	15	29.1	+0.0025	0.997
11	15	24.8	+0.0000	1.000
12	15	18.3	-0.0040	1.007
13	15	12.6	-0.0055	1.016
14	15	7.2	-0.0045	1.022
15	15	3.9	-0.0030	1.025
16	15	2.0	-0.0015	1.026
17	15	0.9	-0.0010	1.046
18	15	0.4	-0.0010	1.105
19	15	0.2	-0.0005	1.105
20	15	0	0.0000	

← indicates switch to 0.15M ammonium chloroacetate solution from 0.15M (22.8 mgm/ml) DL-mandelic acid feed.

DL-mandelic acid was then fed to the column at a rate of 2.5 ml/hour. As the column outlet concentration approached the mandelic acid feed concentration, the column feed was switched to 0.15M ammonium chloroacetate elutant. Samples were collected at the outlet of the column with an automatic fraction collector, and individual fractions were analyzed for their total mandelic acid content and optical rotation. Concentrations of mandelic acid were measured by UV absorption at 257.4 mμ with a Bechman Model DU-2 Spectrophotometer. Optical rotation measurements were performed using a Bendix Automatic Polarimeter, Model 143A, with a 4 cm cell. Based on a $[\alpha]_D$ of +153° for L(+)-mandelic acid, calculations show that fractions with isomer ratios as high as 1.105 occurred.

Table 4 shows the results of frontal plus elution analysis on DL-mandelic acid in methanol, using a column containing 4.0 gm of the L-glutamic acid resin and measuring 0.85 cm in diameter by 18 cm long. In this case, preconditioning and elution were performed with a 0.15M ammonium chloroacetate-chloroacetic acid methanolic buffer (pH=3.22)* at the pI of glutamic acid. Flow rate for the run was 3 ml/hour. Fractions with isomer ratios up to 1.025 occurred.

*pH's reported for methanol solutions are calculated using the acid dissociation constants given for aqueous solutions.

Table 4. Resolution of DL-Mandelic Acid on L-Glutamic Acid Resin in Methanol

Fraction	ml	Mandelic Acid mgm/ml	Rotation, α_D l = 4 cm	Resolution D/L
1-2	30	0	0.0000	
3	15	7.8	+0.0035	0.985
4	15	13.0	+0.0020	0.994
5	15	16.7	+0.0005	0.998
6	15	21.0	0.0000	1.000
7-8	25	22.8	0.0000	1.000
← 9	15	28.1	0.0000	1.000
10	15	22.6	0.0000	1.000
11	15	20.8	-0.0005	1.001
12	15	8.2	-0.0055	1.025
13	15	0	0.0000	

← indicates switch to 0.15M ammonium chloroacetate-chloroacetic acid buffer (pH = 3.2) from 0.15M (22.8 mgm/ml) DL-mandelic acid feed.

A frontal and elution analysis was also made on DL-mandelic acid, using a column containing 14.9 gm of the L-aspartic acid resin and measuring 1 cm in diameter by 39 cm long. Preconditioning and elution were performed with a 0.15M ammonium chloroacetate-chloroacetic acid methanolic buffer (pH=2.77)* at the pI of aspartic acid. Although the L-aspartic acid resin showed some slight preference for D-mandelic acid, virtually no separation occurred.

(b) Interpretation Using "Three-Point-Interaction" Model. If amounts of resin and resin capacity are taken into consideration, the amino acid resins are effective in separating DL-mandelic acid in the order: L-tyrosine, L-glutamic acid, L-aspartic acid. The three-point-interaction model can be used to explain why this order of effectiveness occurs. Molecular models show that when the $-NH_3^+$ and $-COO^-$ of the L-amino acid on the resin interact, respectively, with the $-COO^-$ group and -OH group of D-mandelic acid, no further interaction between the two molecules is likely to occur. However, when the L-amino acid on the resin interacts in the same way with L-mandelic acid, the possibility for interference between the phenyl groups of L-mandelic acid and the side group of the amino acid can be seen for certain molecular configurations. In this situation, rather than having a third point of interaction enhancing the

*pH's reported for methanol solutions are calculated using the acid dissociation constants given for aqueous solutions.

association of one optical isomer over the other with the optically active sorbent, a third point of interaction interferes with the formation of two points of contact in one case.

Thus, for each of the L-amino acid resins, the D-mandelic acid is sorbed more strongly and L-mandelic acid passes through the column more quickly. The L-tyrosine resin has the greatest selectivity, presumably because of the large side group of tyrosine. The small side group of aspartic acid, in turn, explains the relative ineffectiveness of the L-aspartic acid resin.

(c) Chromatograms: L-Tyrosine Resin. A feed of 10 ml of 0.15M DL-ammonium mandelate in a methanolic solution of 0.05M ammonium chloroacetate was charged to a column containing 14.6 gm of L-tyrosine resin and measuring 1 cm in diameter by 36 cm long. This column was first preconditioned by washing with 0.05M ammonium chloroacetate solution, and a solution of this composition was used to elute the ammonium mandelate from the column. Feeding and elution were both performed at a flow rate of 2.25 ml/hour, and the results obtained are shown in Figure 1.

Effluent fractions had isomer ratios of up to 1.095. Neutralized L(+)-mandelic acid was found to have an $[\alpha]_D$ of +95.

Chromatograms were also obtained by charging 0.15M solutions of DL-mandelic acid in methanol to the L-tyrosine column. Subsequent elution with methanol

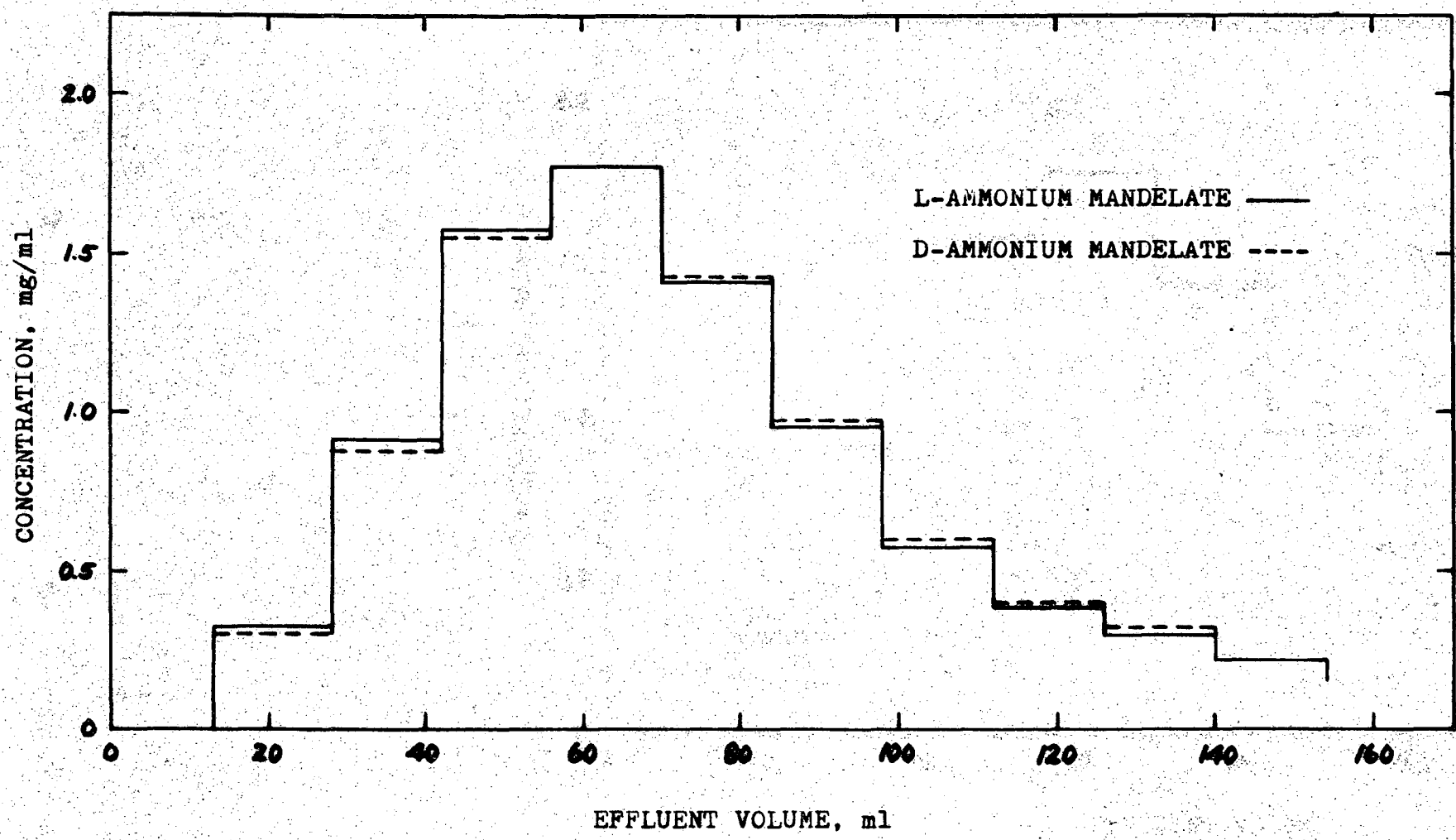


Figure 1. Resolution of DL-Ammonium Mandelate on L-Tyrosine Resin in Methanol with 0.05M Ammonium Chloroacetate Elution.

solutions of 0.05M ammonium acetate, 0.05M chloroacetic acid, or 0.05M ammonium chloroacetate-chloroacetic acid buffer (pH=2.85) all gave very unsymmetrical chromatograms, longer solute retention, and smaller resolution than for ammonium mandelite. Attempts to separate DL-mandelic acid on the L-tyrosine resin with aqueous-phase preconditioning, feed, and elution caused the racemic acid to go straight through the column with no appreciable retention whatever.

L-Glutamic Acid Resin. A charge of 5 ml of 0.15M mandelic acid in methanolic solution buffered with 0.05 M ammonium chloroacetate-chloroacetic acid buffer (pH=3.0) was fed to a column containing 4.0 gm of L-glutamic acid resin, measuring 0.85 cm in diameter by 17 cm long. Column preconditioning and elution were both carried out with the methanolic buffer solution, at a flow rate of 2.25 ml/hr. As is indicated in Figure 2, fractions showed isomer ratios up to 1.055.

A similar chromatogram was obtained in aqueous solution using somewhat different conditions. A column containing 4.0 gm of the resin and measuring 0.85 cm in diameter by 13 cm long was first preconditioned by washing with water. 5 ml of 0.15M DL-mandelic acid in water was then fed to the column at a flow rate of 7.5 ml/hr, followed by elution with water at the same flow rate. After 130 ml, no mandelic acid had yet appeared. The

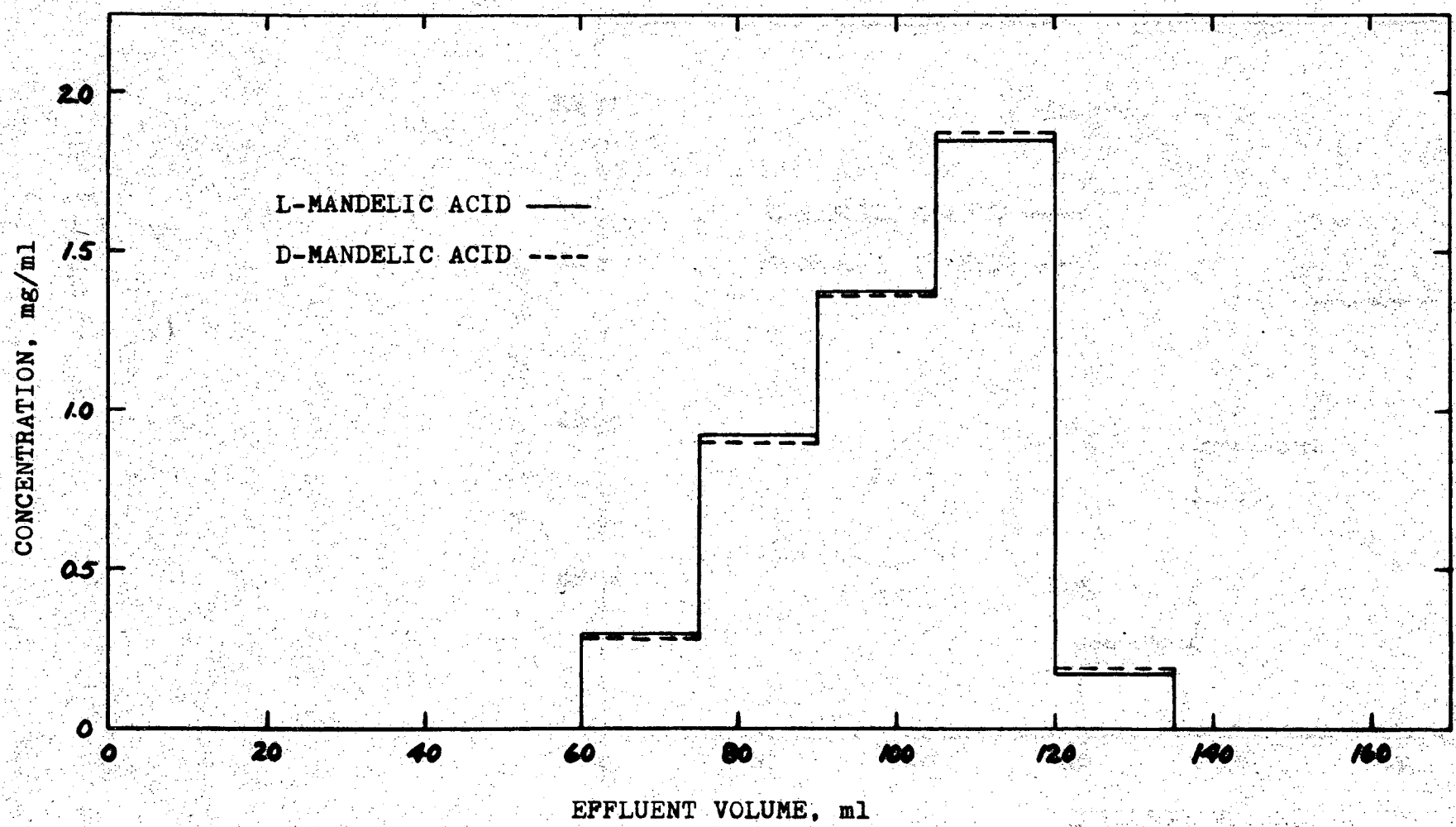


Figure 2. Resolution of DL-Mandelic Acid on L-Glutamic Acid Resin in Methanol with 0.05M Ammonium Chloroactate-Chloroacetic Acid Buffer Elution (Effective pH=3.0).

elutant was then switched to 0.10M sodium chloroacetate-chloroacetic acid buffer (pH=3.22). The resulting chromatogram, shown in Figure 3, has isomer ratios as high as 1.07.

Trial Resolution of Amino Acids

Several attempts at resolving amino acids on the L-tyrosine and L-glutamic acid resins showed virtually no tendency for amino acids to sorb onto the resins, and no separations. Frontal analyses on methanolic solutions of DL-phenylalanine, DL-tryptophan and DL-tryptophan·HCl using the L-tyrosine resin indicated no sample hold-up and no separation. The same results were obtained with frontal analyses on aqueous solutions of DL-phenylalanine, DL-tryptophan and DL-arginine·HCl. Solubility work (13, 26) had shown L-tyrosine and L-arginine·HCl to have an inter-dimerization constant of 0.72 $(\text{moles/liter})^{-1}$ in water. Therefore, the lack of affinity of arginine·HCl for the L-tyrosine resin was surprising.

Similar results were obtained using the L-glutamic acid resin. A frontal analysis on DL-phenylalanine in water showed no sorbtion or separation. DL-aspartic acid was found to sorb onto the resin to a degree, but no separation could be detected. Solubility work (13) had shown the inter-dimerization constant of L-glutamic acid with L-aspartic acid to be 1.01 $(\text{moles/liter})^{-1}$ and the constant for L-glutamic acid with D-aspartic acid to be

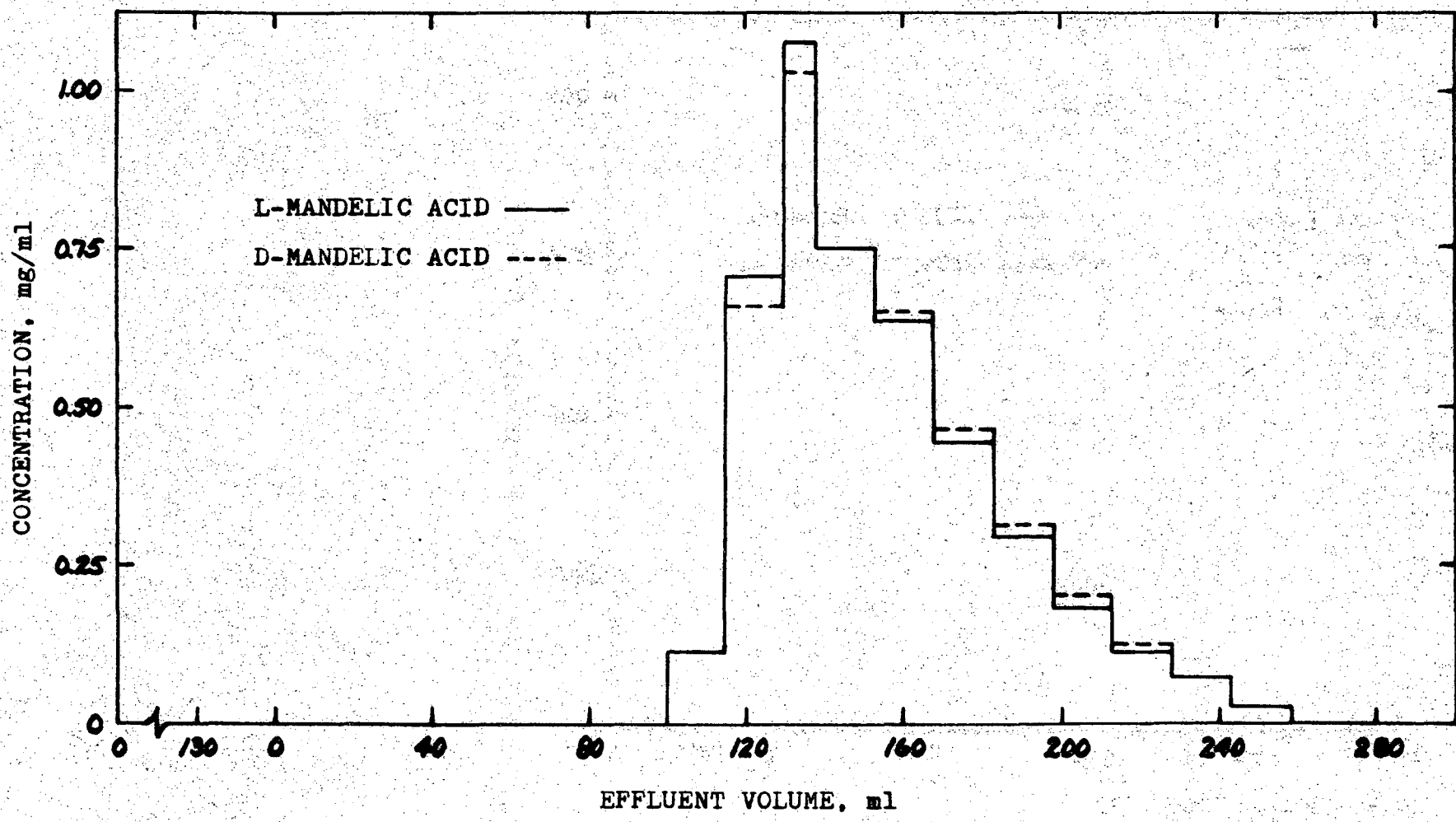


Figure 3. Resolution of DL-Mandelic Acid on L-Glutamic Acid Resin with Water, then 0.01M Sodium Chloroacetate-Chloroacetic Acid Buffer Elution (pH=3.2).

0.76 (moles/liter)$^{-1}$. It was therefore hoped that use of an insoluble glutamic acid sorbent in a chromatographic separation of racemic aspartic acid might show a selectivity ratio of (1.01/0.76)=1.33. Finally, a frontal analysis on DL-lysine·HCl using the L-glutamic acid resin showed neither sorption nor separation. This was surprising in view of the complementary base-acid characteristics of the amino acid pair lysine-glutamic acid.

Trial Resolution of N-Acetyl-DL-Phenylalanine

Because of the relative success in separating the optical isomers of DL-mandelic acid, the possibility of resolving N-acylated amino acids was explored. A frontal analysis on a methanolic solution of 0.15M N-acetyl-DL-phenylalanine using the L-tyrosine resin showed relatively good sorption, but no separation could be detected.

Conclusions and Discussion

A new route to synthetic sorbents has been developed based on the use of formylated aromatic polymers, and has been applied to making optically active sorbents containing amino acids which are otherwise difficult to prepare. In principle, any primary amine may be attached to the formylated polymers, first forming an imine and then reducing it to the corresponding attached secondary amine.

This work has shown that amino acids attached to a crosslinked polystyrene resin in a manner which preserves their zwitterionic structure do not in general sorb or

resolve amino acid optical isomers. This type of asymmetric sorbent does show promise for the resolution of optical isomers which are true acids. Because of the zwitterionic character of the material, it is also likely true bases can be sorbed and basic optical isomers resolved on these amino acid resins.

Certain solubility studies of amino acids in amino acid solutions (5, 26) indicate that interaction between amino acids in aqueous solution can be negligible. However, sorption and resolution were anticipated at least for DL-aspartic acid on the L-glutamic acid (13) resin and DL-arginine•HCl on the L-tyrosine resin (26). Attachment of amino acids to a polystyrene resin most likely alters the amino acid configurations and certainly provides an environment different from an aqueous solution. Either or both could explain the lack of effectiveness experienced with the resins. Providing a more polar resin structure, somewhat in the range of the lower alcohols, might improve the chances of producing the needed configurations.

Of all the other amino-acid containing resins which have been synthesized and used successfully to partially resolve amino acid optical isomers, only one contained an amino acid in the zwitterionic form. The L-cysteine containing resin synthesized by Roberts and Haigh (25) achieved a small partial resolution of DL-methionine, but a large column was used and breakthrough was rapid. Other amino-acid

containing resins used in successful partial resolutions of amino acid optical isomers incorporated the amino acid in such a way that it acted as a true acid or else as a nonionic molecule. The most dramatic results were achieved by Suda et al. (27).who achieved 90% resolution of basic amino acids (DL-lysine and DL-ornithine) on their acid-type resin prepared by the copolymerization of ethyl-N-acryloyl-L-pyroglutamate and divinylbenzene. These results and the good resolutions of N-substituted amino acids (true acids) obtained on chitosan (weak base ion exchanger) obtained in our laboratory (14) point to the use of asymmetric acid-like resins to resolve basic optical isomers or vice-versa. This might require substitution on the optical isomers as in the case of N-substituted amino acids.

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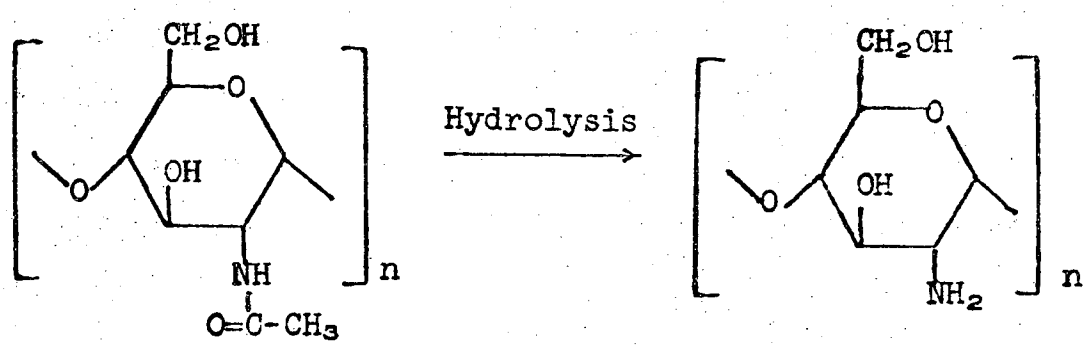
IV. CROSSLINKED CHITOSAN AS A SELECTIVE ASYMMETRIC ANION EXCHANGER

Introduction

In recent years, polysaccharides such as cellulose and dextran have been modified into a number of useful adsorbents and ion exchangers (10). With the goal of preparing an asymmetric ion exchanger, chitin or poly-(N-acetyl D-glucoseamine) has been hydrolyzed into chitosan of poly(D-glucosamine), a weak base:



To increase the useful capacity of the chitosan resin and to preserve its structure in weak acid solutions, a method of crosslinking chitosan has also been developed, and the product has been shown to be an effective chromatographic agent.

Several excellent reviews on chitin cover its wide distribution in nature, its physical and chemical properties, and its chemical derivatives and their uses (3, 7, 15). Chitin occurs in animals, especially arthropods, as a component of complex chemical systems involving large

percentages of protein and also calcium carbonate. Consequently, the chitin commercially available has had extensive chemical treatment to remove these dissimilar materials. Chitin must therefore be considered a variable material with properties dependent upon its source and subsequent treatment.

Conversion of Chitin to Chitosan

Pretreatment. The chitin used, Eastman technical grade, had the appearance of curled shavings from a tough plastic, with a uniform light yellow color and a faint amine odor. The flakes ranged in size from about 1 cm square to fine powder, but averaged about 0.5 cm square. In order to make the material more homogeneous for packing columns, the chitin was dry-ground in a Waring Blendor, then sieved into narrow size ranges. Wet-grinding in water or glycerine proved slower and gave a less uniform product.

Hydrolysis. Conflicting results of previous studies on the base-catalyzed hydrolysis of chitin are believed to reflect variations in its source and pretreatment (8, 9). Two different types of interchain bonding occur, involving N-acetyl groups, with roughly half these groups entering into each type of bond (1); one type is strongly resistant to hydrolysis. In the present study, the chitin had an initial exchange capacity of 0.09 meq/gm, about 2%

of the ultimate value. Hydrolysis proceeded quite rapidly to about 3.0 meq/gm chitin, which is far short of the theoretical ultimate capacity (4.9 meq/gm), but then continued to increase more slowly.

Kinetic studies of hydrolysis were performed batchwise by adding 7 ml of 50 wt-% aqueous NaOH to 250-mgm samples of 80-100 mesh chitin in 15-ml test tubes, placing the tubes for various times into a covered thermostated water bath controlled to ± 0.5°C. Reaction was stopped by pouring the sample into 25 ml of distilled water. Washing and rinsing by decantation several times with distilled water followed. Finally, 0.5N KCl was added, and base capacity was determined by potentiometric titration with 1N HCl.

Table 1 gives observed initial rates of hydrolysis for chitin at 50, 60, and 70°C and the calculated pseudo-first-order coefficients (k_1) for the Type 1 (rapidly hydrolyzing) N-acetyl groups.

The slope of conversion-time curves, at large times, provided an estimate of the initial first-order rate coefficient for Type 2 hydrolysis at 100°C: $k_2 = 3.3 \times 10^{-3}$ min^{-1}.

The best hydrolysis result was 72% (4.2 meq/gm chitosan), obtained in 50% NaOH at 100 C for 1.5 hours. For a larger-scale preparation, 120 ml of 50 wt-% aqueous NaOH and 20.0 gm of 100-200 mesh chitin were mixed at 100°C in a 250-ml

Table 1. Initial Rates of Hydrolysis of 80-100 Mesh Chitin at Various Temperatures in 50 Wt-% Aqueous NaOH

Temperature (°C)	Initial Rate (meq/gm chitin/hr)	$k_1 \times 10^3$ (min^{-1})
50	0.350	1.95
60	1.30	5.73
70	2.85	15.8
90	18.0 [a]	100.0 [a]
100	42.6 [a]	237.0 [a]

[a]Extrapolated, assuming activation energy constant at 25.4 kcal/mole.

flask. After stirring the mixture for 30 minutes, the reaction was stopped by pouring it into 500 ml of water. The product was water-washed several times, then filtered on a large sintered-glass Buchner funnel where further water-washings followed. Finally the chitosan was dehydrated by acetone rinses, and air-dried to a fine white powdery material. The yield was 19.0 gm of chitosan, with a capacity of 3.15 meq/gm.

Crosslinking. Considerable swelling and dissolution of chitosan was observed in weak-acid solutions for materials with capacities much above 1.2 meq/gm. This result, due to the amine groups freed by hydrolysis, is similar to observations by Porath on cellulose-type ion exchangers (12). Thus, at high capacities, the interchain hydrogen bonding in chitosan must be supplemented by chemical crosslinking in order to hold the matrix together.

A known technique for crosslinking starch with epichlorohydrin (6) was adapted for treating chitosan, producing a very low nominal percentage of crosslinking but giving greatly improved properties. To a 125-ml flask was added, with stirring, 12.0 gm of chitosan (capacity 3.15 meq/gm, 67 mmoles glucose units) and 90 ml of 1.5M Na_2SO_4 containing 16 mmoles of NaOH. Next, 10 ml of 1.5M Na_2SO_4 containing 0.78 mmoles of

epichlorohydrin and 8 mmoles of NaOH were added, and reaction at room temperature was allowed to proceed for 20 hours. Washing and rinsing on a Buchner funnel followed, first using water and then acetone. Air-drying yielded 11.5 gm of product insoluble in weak acid; nominal cross-linking was 1.1%, based on the number of epichlorohydrin molecules per glucose unit.

A 1%-crosslinked material and a 4%-crosslinked material prepared under similar conditions showed the same swollen density to within 10%; each occupied about 30% more volume than the same weight of uncrosslinked and unhydrolyzed material, and had an exchange capacity near 3.15 meq/gm. The crosslinked chitosan product viewed under a microscope has the same dry appearance as the hydrolyzed chitosan before epichlorohydrin treatment.

Apparent Ionization Constant of Chitosan. Chitosan is a weak base. Titration curves for a low-capacity (0.93 meq/gm) uncrosslinked chitosan, in distilled water and in 0.1 and 0.5N KCl, showed an extreme dependence of external pH on ionic strength. The apparent pK at the 50% conversion point, as corrected for salt content and Donnan uptake (5), was 5.4. It thus becomes apparent that chitosan will only be an effective anion exchanger at pH values below about 7; in alkaline solutions it

will function as a cellulose-like sorbent.

Separations

Chromatographic Separation of Aspartic and Glutamic Acid. As a test of its ion-exchange properties, 1% cross-linked chitosan was used in a trial separation of aspartic and glutamic acids (2 μmoles each). Figure1shows the elution, by 0.05M sodium chloroacetate-chloroacetic acid aqueous buffer at pH 3.1, of these two amino acids, at a flow rate of 30 ml/hr. A column 14.5 cm long and 1.0 cm in diameter, having a capacity of 3.15 meq/gm (0.24 meq/cm^3), was used. Analysis was performed by a standard ninhydrin procedure (4). The sharp separation obtained suggests that chitosan may exhibit selectivities towards proteins and related macromolecules at least as good as, and perhaps better than, commercial cellulosic sorbents.

Resolution of DL-Mandelic Acid. A primary objective in preparing crosslinked chitosan was to test it for chromatographic resolution of racemic acids. Because of its good solubility in many solvents, large optical rotation for individual isomers, and ease of detection, DL-mandelic acid was used in initial experiments. In the first of these 5 ml of a 0.15M DL-mandelic acid (22.8 mgm/ml) solution in methanol was charged to a column of chitosan 30 cm long and 1.0 cm in diameter, which contained 4.0 gm of a 1%-crosslinked chitosan

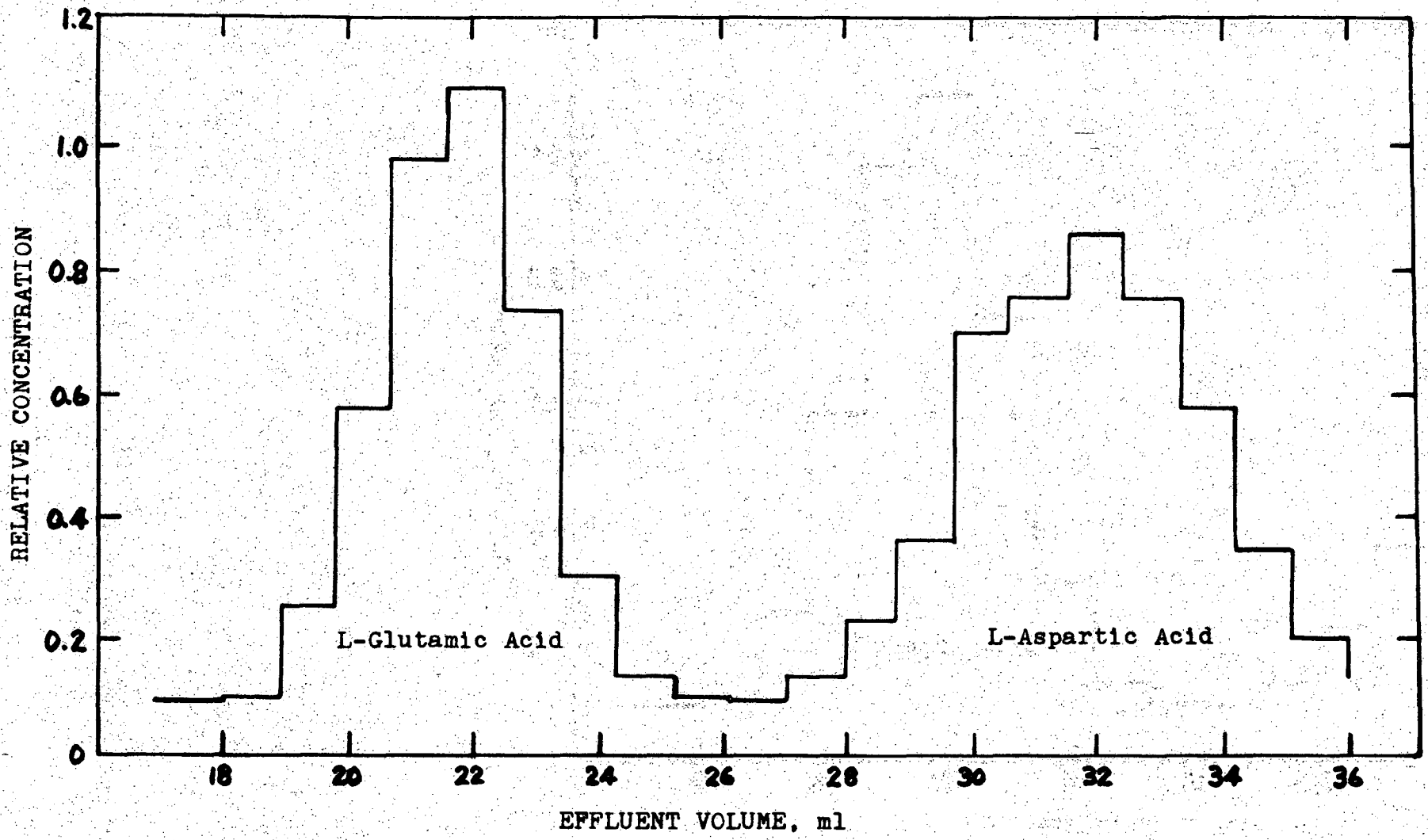


Figure 1. Separation of L-Aspartic Acid and L-Glutamic Acid

with a capacity of 3.15 meq/gm. Column preconditioning and elution were accomplished with a 0.01M solution of chloroacetic acid in methanol (pH = 1.93)* at a rate of 6 ml/hr. The results are shown in Figure 2. Optical rotations of the fractions were measured in a 4-cm cell of a Bendix Model 143A Automatic Polarimeter, and the excess isomer concentration was calculated by using a known $[\alpha]_D$ of 153° for L(+)-mandelic acid in methanol. The total mandelic acid concentration was measured by observing optical density at 257.4 mμ with a Beckman DU-2 spectrophotometer. Chromatogram fractions with isomer ratios ranging from 1.12 to 1.98 were found. Very similar results were obtained with the 4-% cross-linked chitosan.

In a second experiment on the same column, preconditioning and elution were performed with a 0.10M sodium acetate-acetic acid buffer in methanol of pH 4.5.* The methanolic mandelic acid feed was partially neutralized (82%) to a pH of 4.5 by adding NaOH to a concentration level of 0.123M. (At this neutralization, the $[\alpha]_D$ of L(+)-mandelic acid was found to be 105°). The sample residence time was substantially increased, and the sorbent selectivity was actually reversed, implying

*pH's reported for methanol solutions are calculated using acid dissociation constants reported for aqueous solutions.

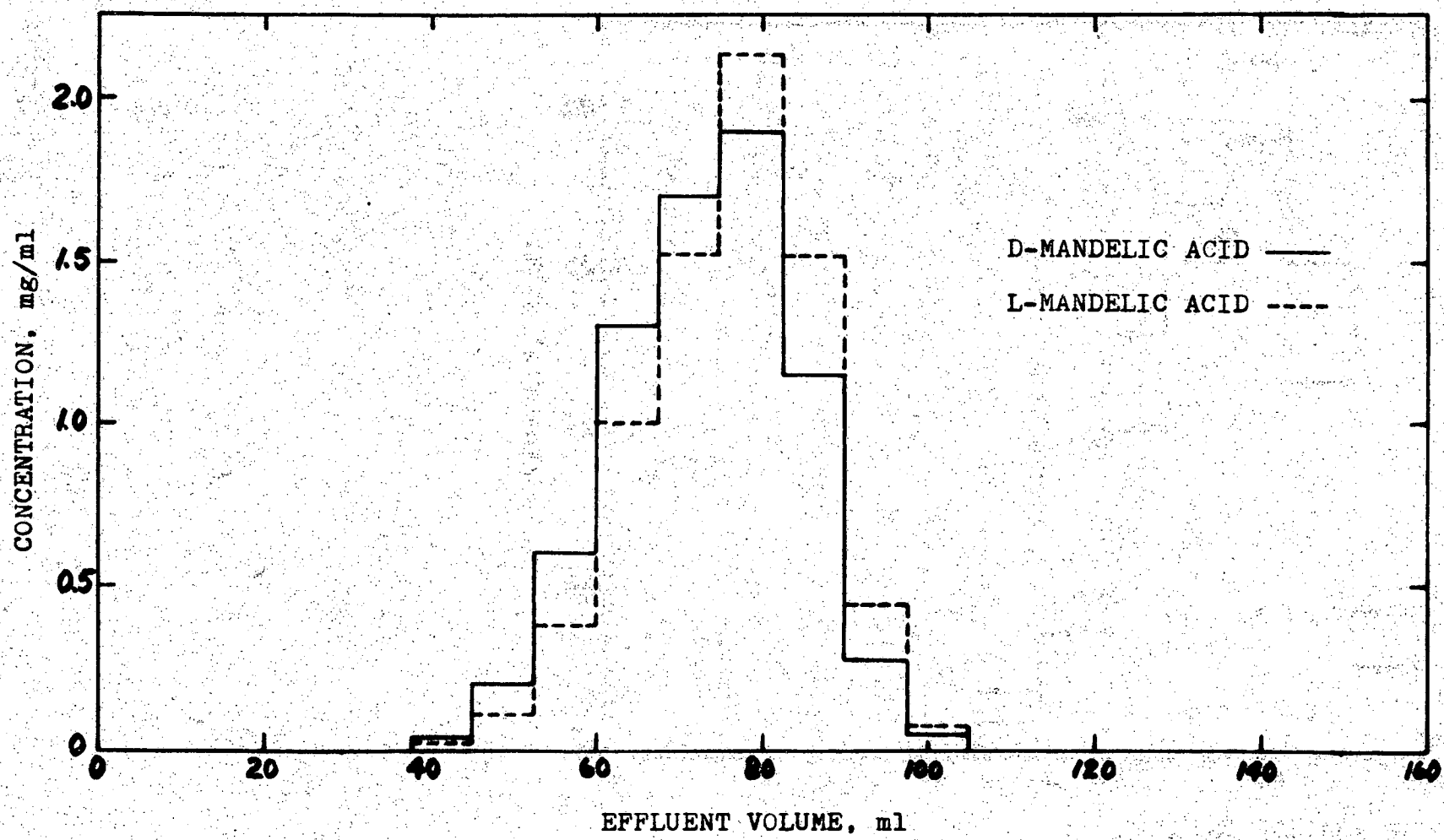


Figure 2. Resolution of DL-Mandelic Acid in Methanol with 0.01M Chloroacetic Acid Elution (Effective pH = 1.93).

that the separation is now dominated by the behavior of a different form (possibly the bimandelate). Nonetheless, the degree of separation was notably reduced, as shown in Figure 3.

A more marked change of preconditioner and elutant to a 0.01M ammonium chloride-ammonia buffer in methanol, with pH 9.3, together with a change of feed to DL-ammonium mandelate in methanol, resulted in a very small sample residence time and no separation. At this high pH, the amine group of the chitosan is no longer protonated, and its ion-exchange properties are lost.

Aqueous solution was found to give a smaller separation, using otherwise the conditions of Figure 2, with a 4%-crosslinked chitosan. In this case, 4 gm of the chitosan occupied a column 1 cm in diameter and 49 cm long. Chromatogram fractions were observed to have isomer ratios ranging from 1.06 up to 1.27, as shown in Figure 4. Apparently the relative importance of selective interactions which discriminate between the isomers of mandelic acid is diminished by shifting from methanol to water solvent.

The resolving ability of chitosan is compared to that of a cellulose-like material in Figure 5. Conditions of this run were identical to those of Figure 4, except that the column packing was changed to Sephadex G-50M,

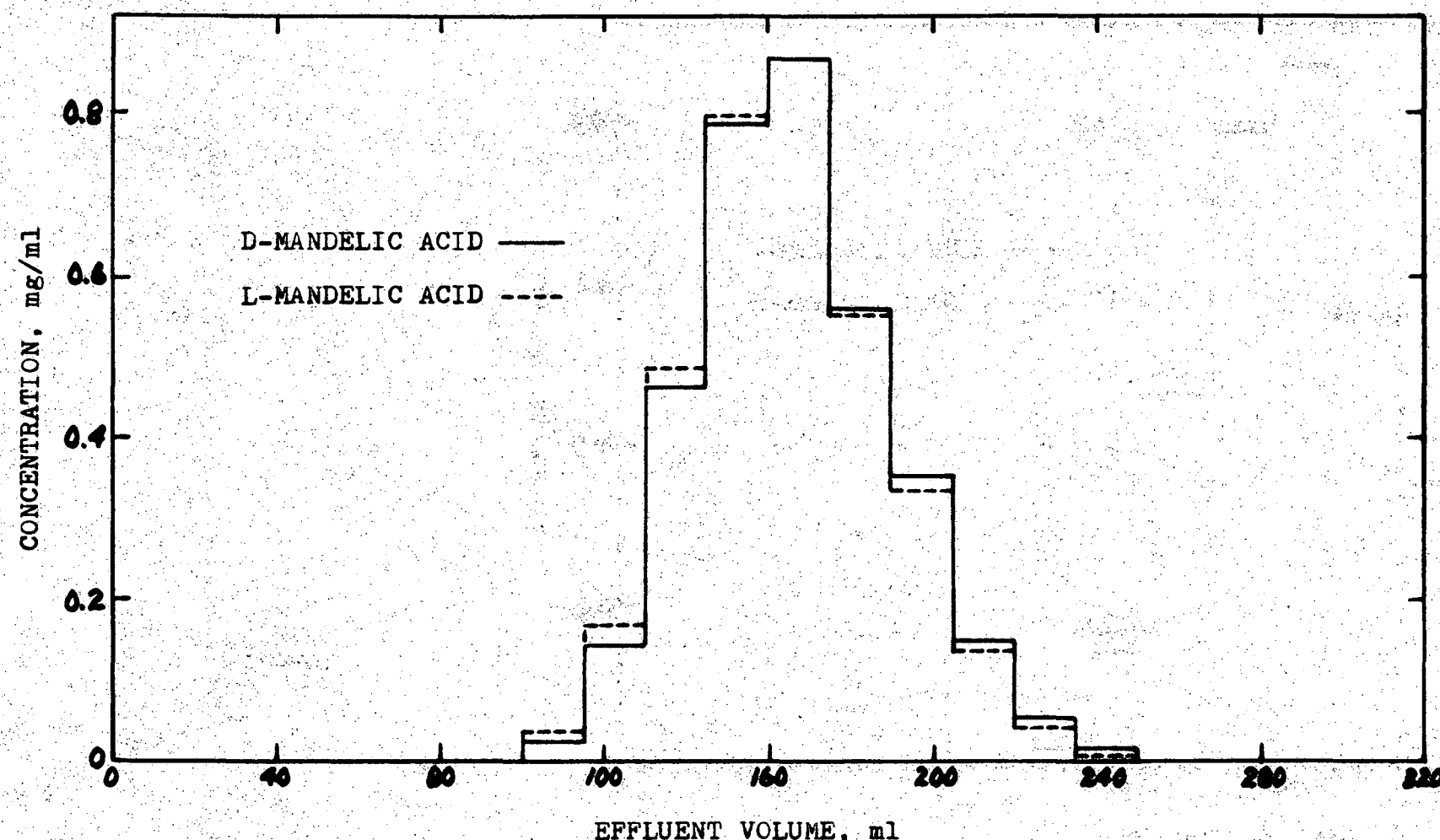


Figure 3. Resolution of DL-Mandelic Acid in Methanol with 0.10M Sodium Acetate-Acetic Acid Buffer Elution (Effective pH = 4.5).

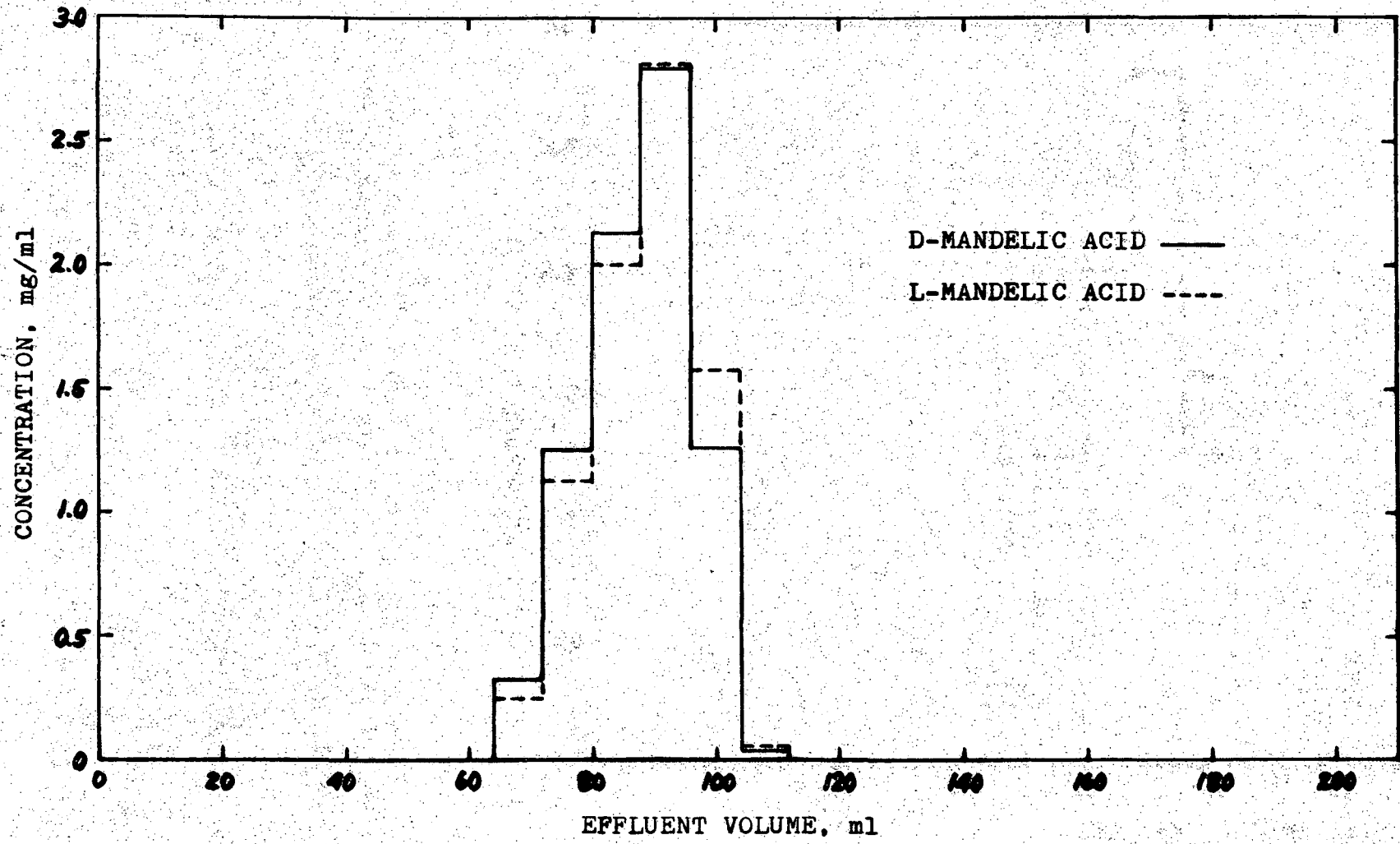


Figure 4. Resolution of DL-Mandelic Acid in Water with 0.10M Chloroacetic Acid Elution (pH = 1.93).

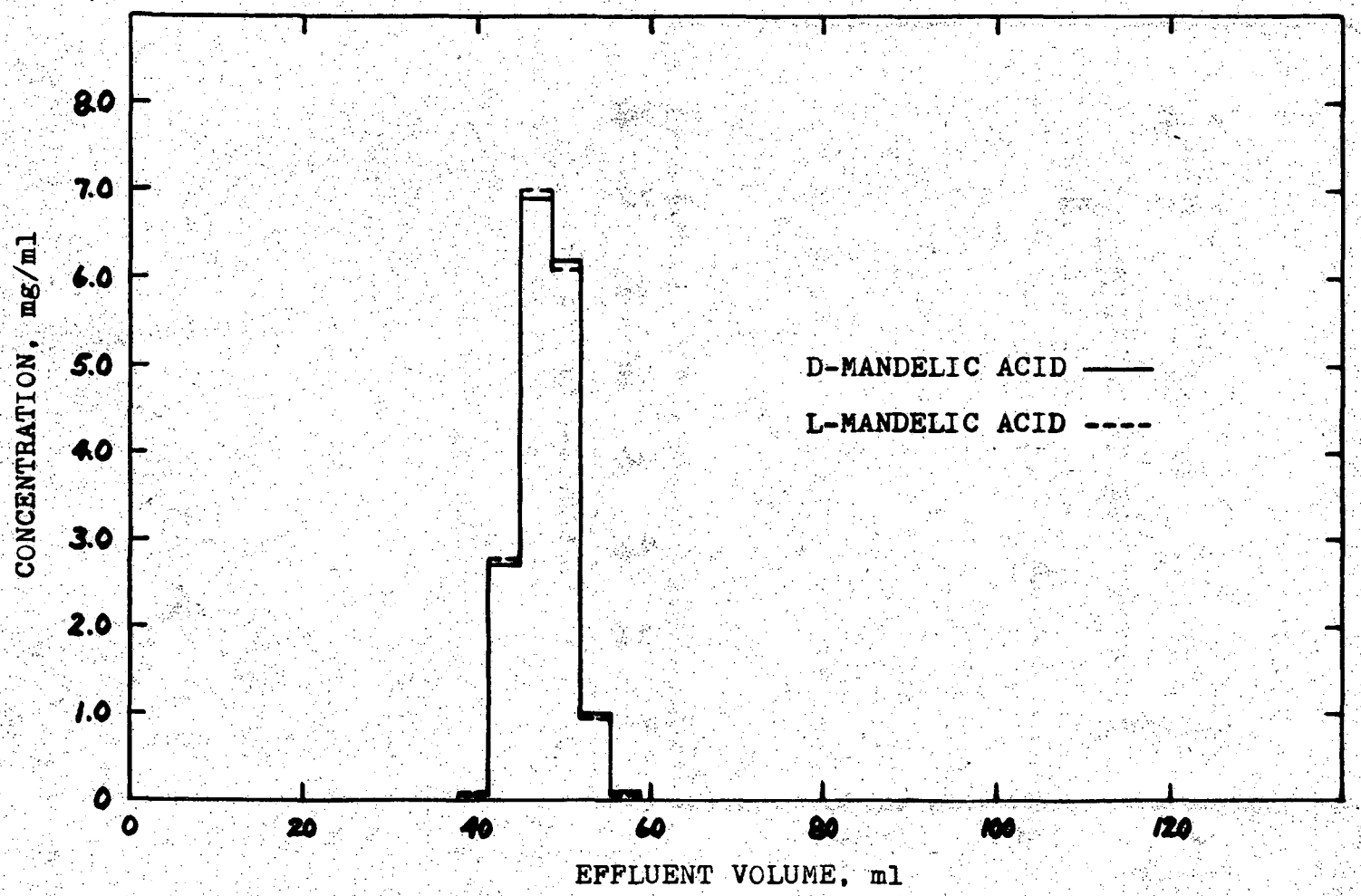


Figure 5. Resolution of DL-Mandelic Acid in Water on Sephadex G-50M with 0.10M Chloroacetic Acid Elution.

a crosslinked polymer of glucose. Both sample residence time and separation were greatly reduced. The recovered fractions were found to have isomer ratios from 1.01 to 1.04.

Trial Resolutions of Amino Acids. Attempts to resolve the optical isomers of amino acids on columns of chitosan met with only slight success. Runs in methanol solution showed detectable separation, but few amino acids are appreciably soluble in methanol. The results of a frontal analysis of DL-tryptophan on a column of 1%-crosslinked chitosan containing 4 gm of material and measuring 1 cm in diameter by 28 cm long, with a flow rate of 6 ml/hr, are given in Table 2. Although tryptophan holdup on the column is quite small, a partial separation was indicated in the first and last several samples containing tryptophan. An identical run using DL-phenylalanine showed very small sample hold-up on the column, and a smaller partial resolution. Analyses for these runs were again accomplished with a combination of optical rotation and optical density measurements.

A frontal analysis of phenylalanine on chitosan in water solution showed no phenylalanine hold-up on the material and no separation. Frontal analysis of an acidic amino acid , glutamic acid, showed significant sorption onto chitosan, but no detectable resolution.

Table 2. Resolution of DL-Tryptophan on Chitosan in Methanol

Fraction	ml	Tryptophan meq/ml	Rotation, α_D l = 4 cm	Resolution L/D
1-3	36	0	0.0000	
4	13	0.0018	-0.0015	1.53
5	13	0.0030	-0.0005	1.12
6-8	40	0.0030	0.0000	1.00
← 9-10	26	0.0030	0.0000	1.00
11	14	0.0029	+0.0005	0.91
12	14	0.0008	+0.0010	0.50
13	13	0.0001	0.0000	
14	13	0.0000	0.0000	

← indicates switch to 0.01M sodium acetate-acetic acid buffer (pH = 4.5) from 0.003M DL-tryptophan in 0.01M acetate buffer feed solution.

Resolution of N-Substituted Amino Acids. Because of the good partition and appreciable resolutions obtained with mandelic acid on relatively small columns of chitosan, it appeared that N-substitution of amino acids, thereby removing their amphoteric behavior and converting them into true acids, might lead to resolution. Several runs were therefore made with N-acetyl-DL-phenylalanine and one with N-acetyl-DL-tryptophan. Figure 6 shows the result of feeding a 5 ml solution of 0.15M (31 mgm/ml) N-acetyl-DL-phenylalanine in methanol into 5.8 gm of 1%-crosslinked chitosan, in a column 1 cm in diameter by 27 cm long. Column preconditioning and elution were accomplished with 0.10M chloroacetic acid (pH = 1.93) in methanol at a feed rate of 6 ml/hr. Once again, optical rotation and optical density measurements were used in analysis; $[\alpha]_D$ for N-acetyl-L-phenylalanine was determined as 40°. Although the N-acetyl-phenylalanine had a short retention time, the chromatogram of Figure 6 shows that fractions were isolated having isomer ratios from 1.10 to 1.33.

A similar run with 0.15M N-acetyl-DL-tryptophan (37 mgm/ml) as feed gave rather similar results, as shown in Figure 7. ($[\alpha]_D$ of N-acetyl-L-tryptophan was found to be 25°). The larger side group of tryptophan seems to have resulted in a broader chromatogram; fractions with

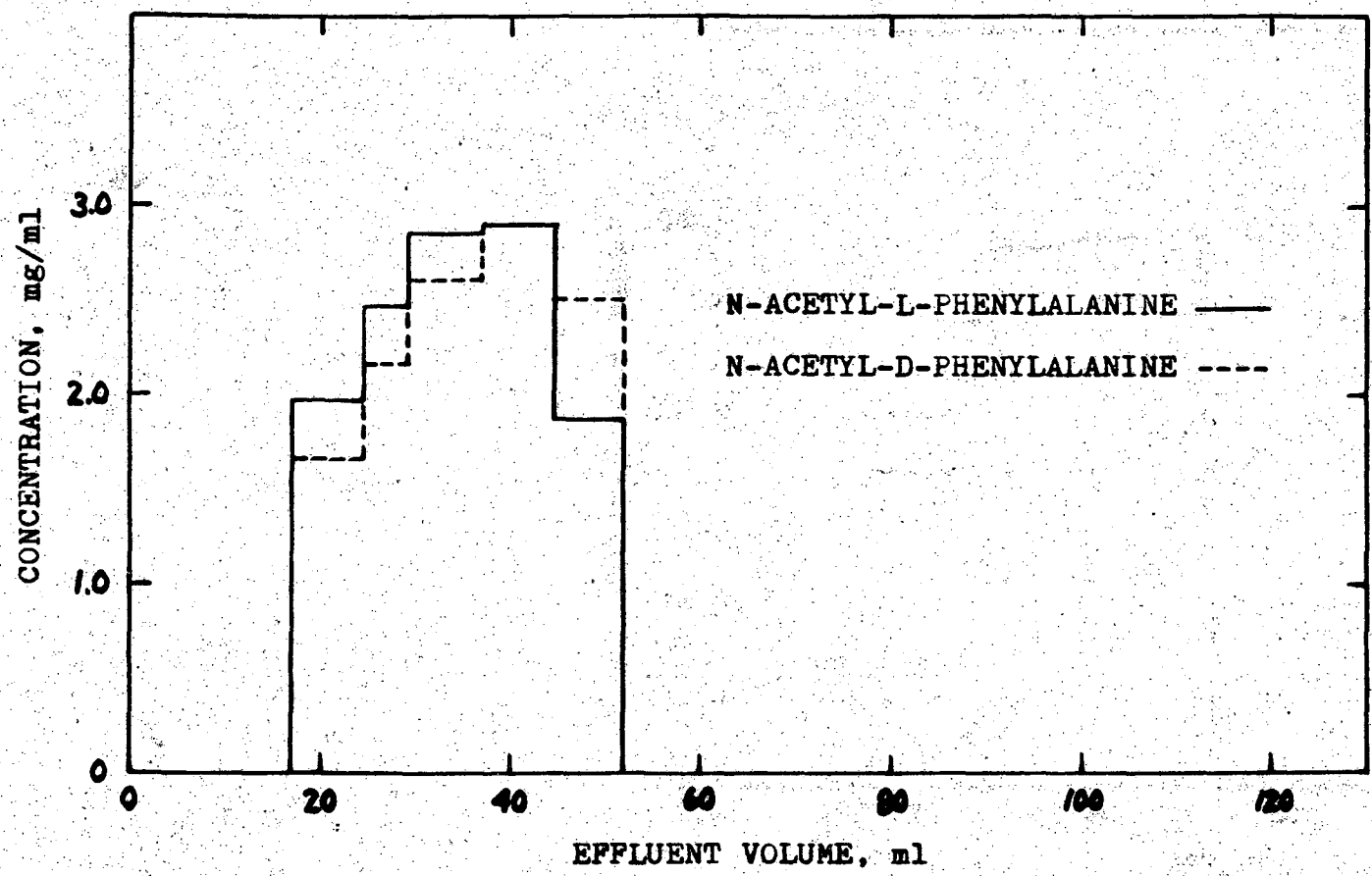


Figure 6. Resolution of N-Acetyl-DL-Phenylalanine in Methanol with 0.10M Chloroacetic Acid Elution.

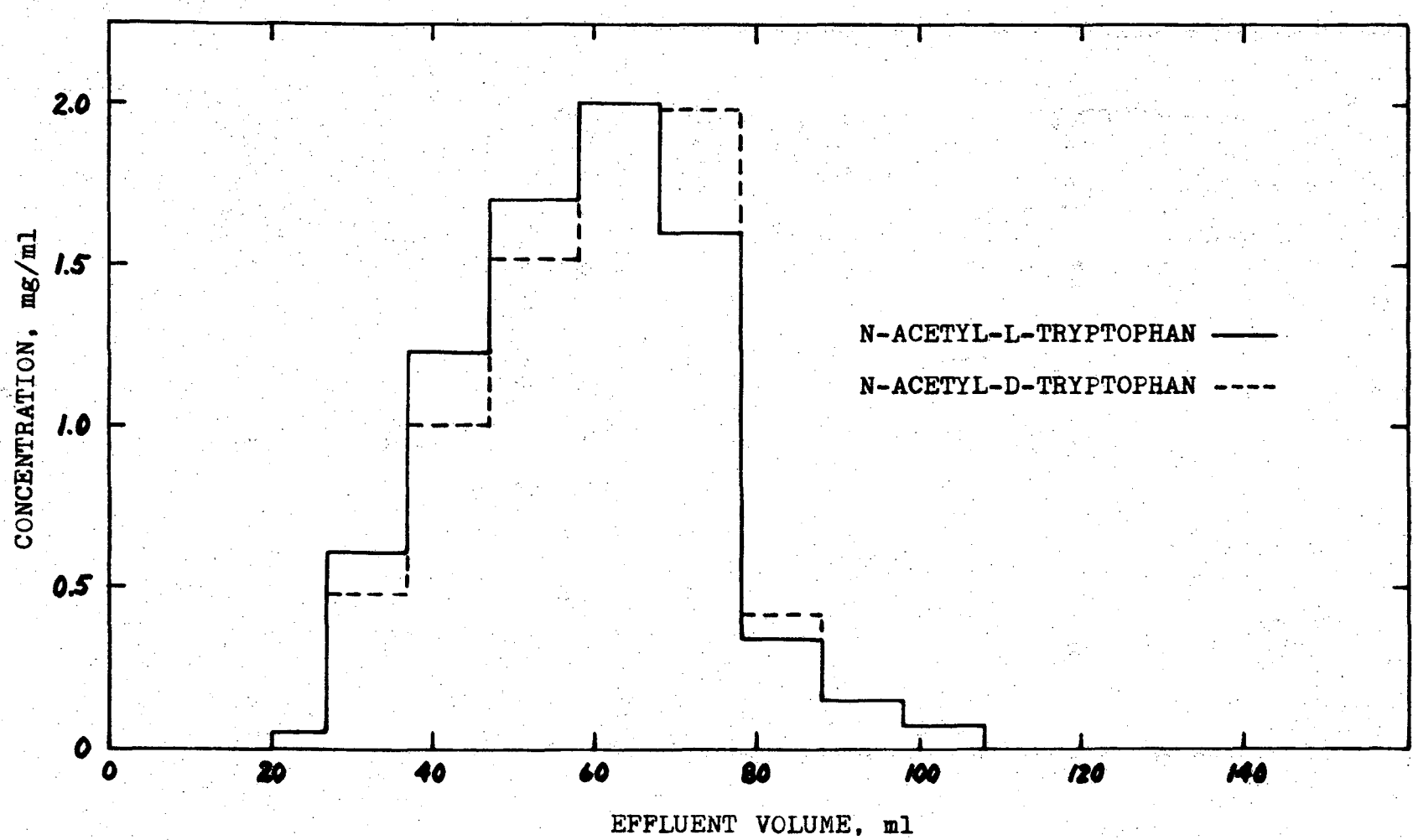


Figure 7. Resolution of N-Acetyl-DL-Tryptophan in Methanol with 0.10M Chloroacetic Acid Elution.

isomer ratios from 1.10 to 1.25 resulted.

Changing the N-substituent of DL-phenylalanine from an acetyl group to a benzoyl group, which is considerably larger, produced no detectable resolution. ($[\alpha]_D$ for N-benzoyl-L-phenylalanine was found to be -35°).

Switching the elutant from 0.01M chloroacetic acid to 0.10M acetic acid in methanol (pH = 2.88) dramatically increases retention and resolution of N-acetyl -DL-phenylalanine. Isomer ratios up to 2.3 resulted, when the same conditions as for Figure 5 were used, but with a shift to acetic acid elution; this result is shown in Figure 8.

Shifting the elutant to a 0.01M ammonium acetate-acetic acid methanolic buffer of pH 3.75 gave improved retention and isomer ratios of up to 2.9, as shown in Figure 9. (Over the range of concentrations of N-acetyl-phenylalanine encountered in the column effluent, the L-isomer was found to have a $[\alpha]_D$ of 55° in the pH 3.75 buffer solution).

Conclusions and Discussion.

Chitosan has been used successfully in the chromatographic resolution of racemic forms of mandelic acid and of several N-acetyl amino acids. It should therefore be applicable to the separation of a wide range of similar compounds. Unfortunately chitosan was not successful in separating optical isomers of the amino acids themselves.

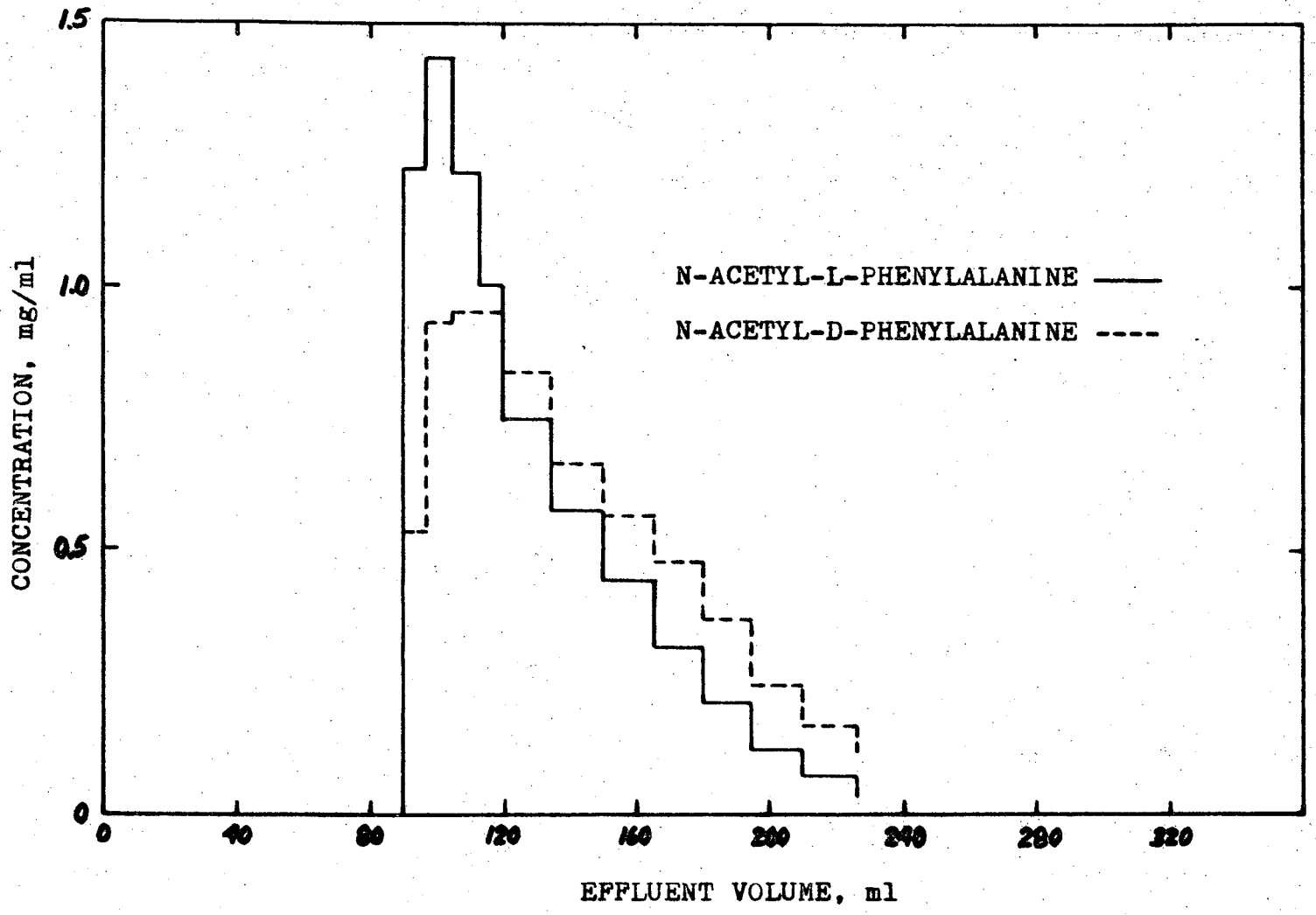


Figure 8. Resolution of N-Acetyl-DL-Phenylalanine in Methanol with 0.10M Acetic Acid Elution.

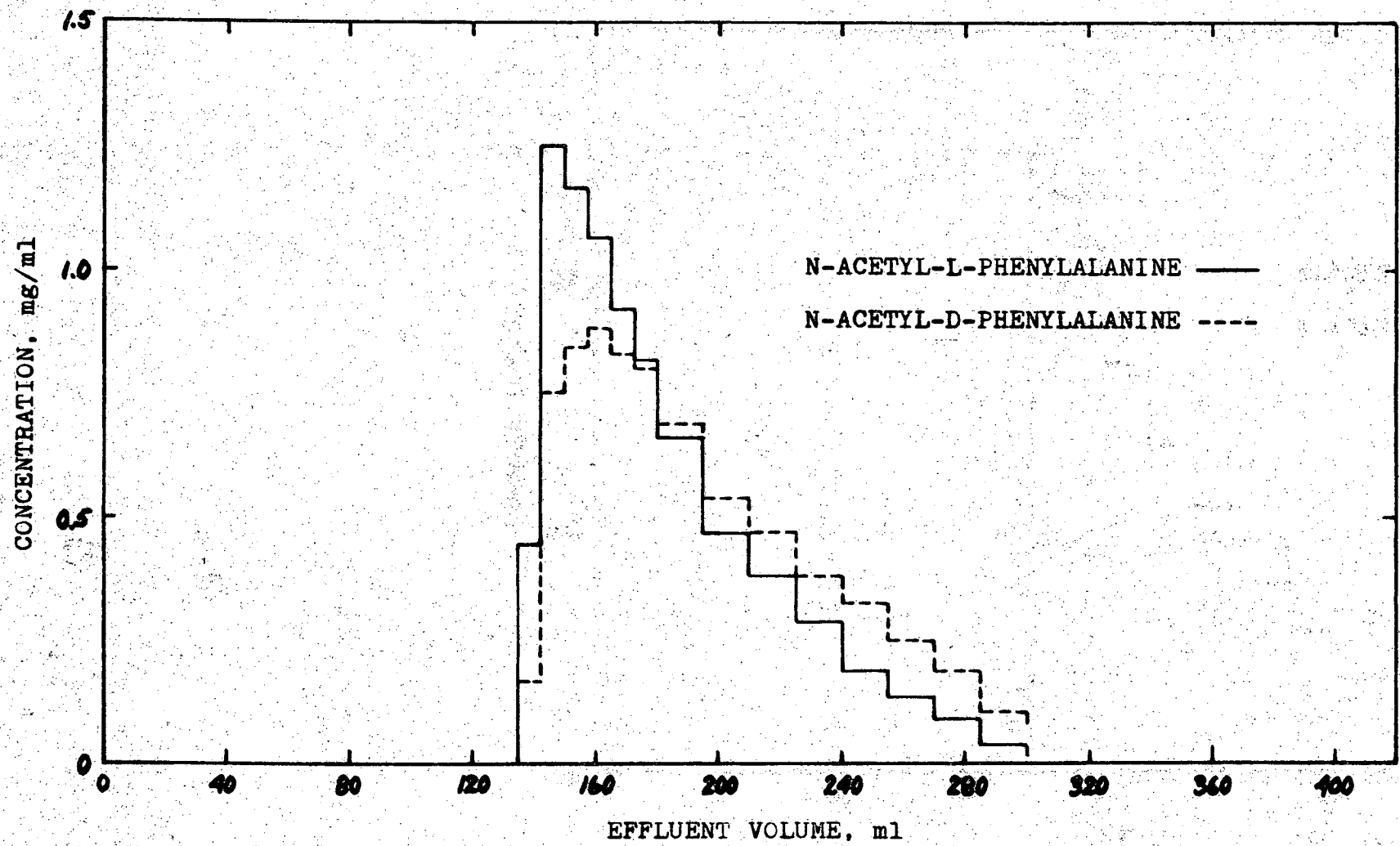


Figure 9. Resolution of N-Acetyl-DL-Phenylalanine in Methanol with 0.10M Ammonium Acetate-Acetic Acid Buffer Elution (pH = 3.75).

However, N-substitution, which can be readily undone by refluxing with dilute HCl, converts DL-amino acids to true acids and allows them to be resolved on chitosan. Furthermore, it appears that at least in some cases N-substituted amino acids can be as useful nutritionally as the amino acids themselves (2).

It is possible to predict the conditions under which a separation such as the one shown in Figure 9 might be made complete. Although the chromatograms of Figure 9 are not symmetrical, the Gaussian approximation and Figure 16-9 of Vermeulen *et al.* (14) are used for this purpose. The average band widths indicate a number of transfer units less than that predicted for mass transfer outside the particles; transport within the particles controls the rate. As a result, the number of transfer units is inversely proportional to the flow rate through the column and directly proportional to the amount of sorbent used. With this information, the Gaussian relation can be used to scale up the separation. Figure 10 shows the complete separation which can be attained by decreasing the flow rate to one-fourth the original (to 1.5 ml/hr) and increasing the amount of sorbent by a factor of 36 (to 210 gm). To obtain the chromatograms of Figure 10, six times as much N-acetyl-DL-phenylalanine (30 ml 0.15*M*) should be introduced to

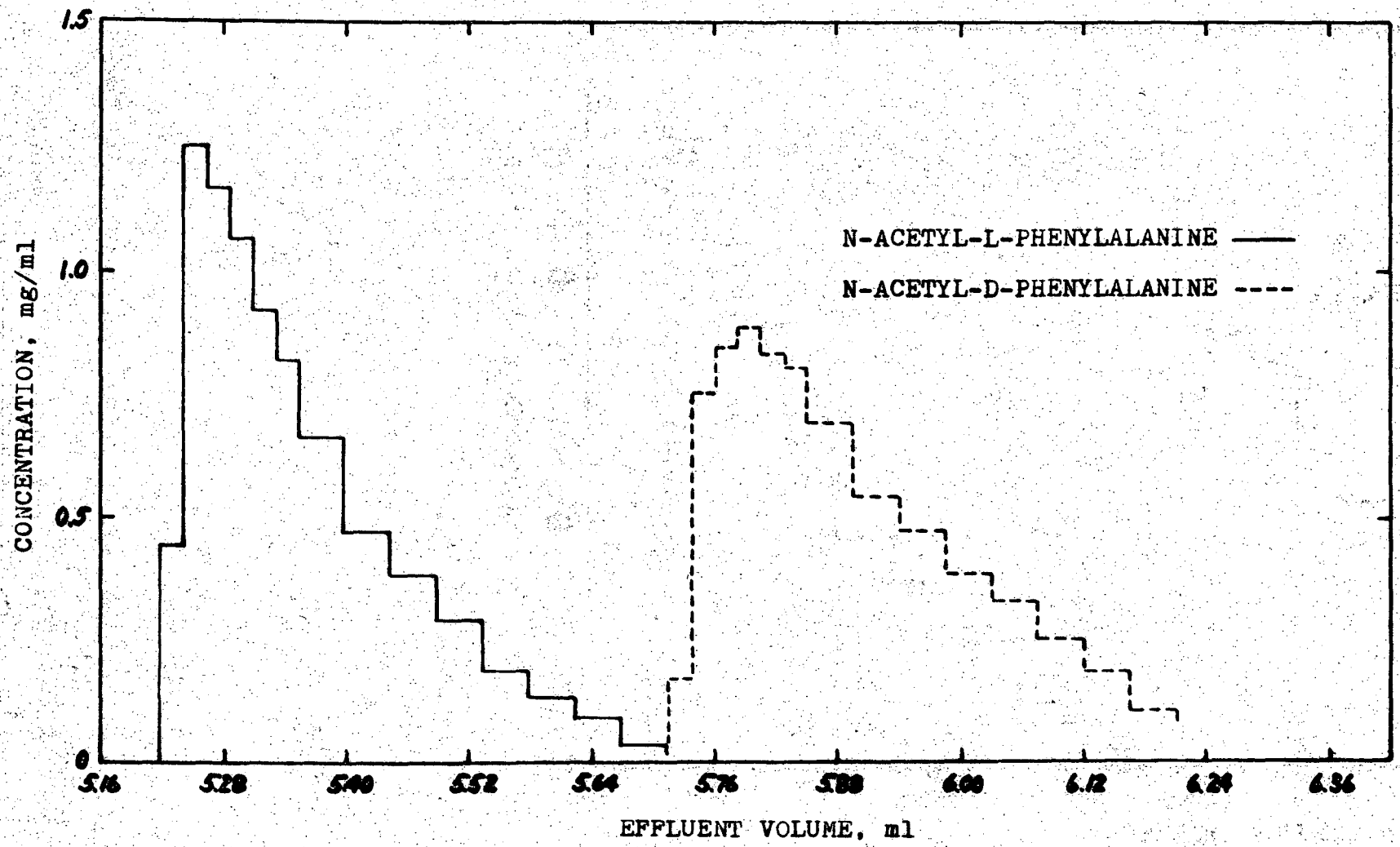


Figure 10. Calculated Scale-Up for Resolution of N-Acetyl-DL-Phenylalanine.

the column.

Somewhat less demanding conditions can allow good partial resolution. Using a column flow rate of 1.5 ml/hr and 23 gm of chitosan, 56% of the N-acetyl-L-phenylalanine (the useful amino acid) should be recovered in pure form. Lower-purity mixed fractions could be recycled, increasing the recovery of pure N-acetyl -L-phenylalanine to 72%.

In either scaled-up scheme, only 20 to 30% of the total column volume would be in use at any given instant. In such cases the sorbent utilization can be improved by multiple cycling--that is, by absorbing additional feed charge before elution of the first charge has been completed (13,14). In this way,,the effluent can become an almost continuous succession of chromatographic zones.

Noguchi et al. (11) also report the hydrolysis and crosslinking of chitin, with the objective of producing an asymmetric anion exchanger capable of resolving acidic optical isomers. In their work, deacetylation was carried out with 55% NaOH at 150°C for 1 hour and resulted in 86% conversion with a product capacity of 5.2 meq/gm. After dissolution in 1.5% aqueous acetic acid and reprecipitation from 5% NaOH solution, the chitosan was pretreated with benzyl chloride or styrene oxide and crosslinked with epichlorohydrin to the extent of one epichlorohydrin molecule per glucose unit. Attempts at chromato-

graphic resolution of racemic mandelic acid by this material were unsuccessful.

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ACKNOWLEDGMENTS

This work was done under the auspices of the U. S. Atomic Energy Commission. A Regents' Fellowship (JMK), NDEA Title IV Fellowship (JMK) and National Institute of General Medical Sciences Grant (DRB) are also acknowledged with thanks.

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