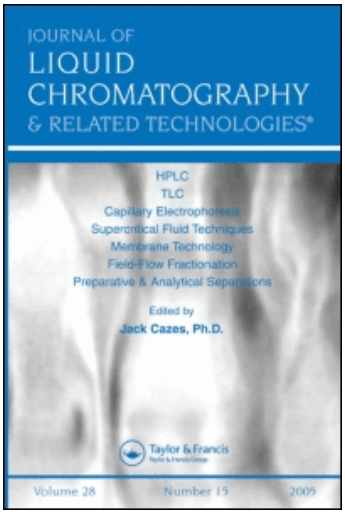

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Preparative Liquid Chromatographic Separation of Amino Acids and Peptides on Amberlite XAD-4

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PREPARATIVE LIQUID CHROMATOGRAPHIC SEPARATION OF AMINO ACIDS AND PEPTIDES ON AMBERLITE XAD-4

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ABSTRACT

Amberlite XAD-4, a polystyrene-divinylbenzene copolymic reversed phase adsorbent which has a 750 m^2/g surface area and 50Å porosity, was used as the stationary phase for the preparative liquid chromatographic separation of amino acids and peptides. Mixtures of > 40 mg and > 100 mg sample load were separated on 8.0 and 20.5 mm i.d. columns, respectively. Mixed solvent and acidic and basic solutions which cannot be used with silica and alkyl-modified silica, were evaluated as mobile phases. Mixtures of amino acids, diastereomeric di-and tri-peptides, diastereomeric dipeptides obtained from the reaction of tert-butyloxycarbonyl-L-amino acid-N-hydroxysuccinimide esters with D,L-amino acids, and enkephalin peptides were separated. Major and minor sample components were isolated.

INTRODUCTION

High performance liquid chromatography (LC) has become an increasingly powerful analytical cool for the separation and determination of amino acids (AA), AA derivatives, and peptides.

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Successful analytical liquid chromatography (ALC) of these compounds has been achieved on alkyl-modified silicas (1-4). Recently, it has been shown that many of these mixtures are separable on porous, high surface area polystyrene-divinylbenzene copolymers which are nonpolar adsorbents and are useful stationary phases for reversed phase chromatography. The most useful ones are Amberlite XAD-2 and -4 (5) and Hamilton PRP-1 (6); only the latter is commercially available as bulk form or prepacked columns. In general, the LC trends are similar for these stationary phases, however, the level of retention for a given mobile phase may differ due to difference in surface area. If XAD (7) and PRP-1 (6) microparticles are used efficiencies approaching that of alkyl-modified silicas of similar particle size are obtained.

A major advantage of the copolymers over silica or alkyl-modified silica, which are the two most used stationary phases in PLC, is that the copolymers are stable throughout the pH range of 1 to 13. Thus, strongly acidic and basic mobile phases, which often are the optimum eluting conditions for AA and peptide separations and cannot be used with the silica type stationary phases, are readily used on the copolymers. Other useful factors in PLC applications are: 1) the copolymers have large loading capacities, 2) eluting conditions on the copolymers have been well characterized (5,6), 3) prep columns are relatively inexpensive, and 4) efficiencies can be increased if required by reducing the copolymer particle size.

This report describes the use of XAD-4 for the PLC separation of AA and peptides. The XAD-4 surface area is the largest and thus it provides the highest loading capacity of the available XADs. Optimization of XAD-4 column variables such as flow rate, column diameter, particle size, packing density, volume and mass overload, capacity, recovery, and eluting conditions (8) is discussed elsewhere. Mixtures that have been

purified on prep XAD-4 columns include organic acids, bases, and polyaromatics (8), drug metabolites (9), and 2':3'-, 3':5'-cyclic nucleotides (10).

The separations described here were chosen to: 1) illustrate the range of useful mobile phase conditions with an emphasis on those that cannot be used with silica and alkyl-modified silica and 2) identify the ones which are especially useful in peptide chemistry. Often the success of a peptide synthesis is determined by development of separation procedures, removal of unwanted peptide chains, and/or purification of a single peptide from the synthetic process.

MATERIALS

The AA and peptides were obtained from Sigma Chemical Co. Several dipeptides were synthesized by the reaction of an amino acid or peptide with tert-butyloxycarbonyl-L-amino acid-N-hydroxy-succinimide ester (11). Ethanol (95%) and HPLC grade CH_3CN was obtained from Matheson, Coleman, and Bell. Distilled water was further treated by passage through a mixed bed ion exchanger, charcoal, and a 0.2 μm millipore filter disk. Solvent composition is expressed as per cent by volume. All inorganic reagents were analytical grade and used as received. Amberlite XAD-4 was obtained from Mallinckrodt Chemical Works. Procedures for cleaning, crushing, sizing, and column packing the 37-44 μm (325 to 400 mesh) and 75-105 μm (140 to 200 mesh) XAD-4 particles are described elsewhere (8). Columns used were 8.0 mm i.d. (3/8 in o.d.) x 25 cm and 20.5 mm i.d. (1 in o.d.) x 32 cm, respectively, equipped with 15 μm end fittings (Jones Chromatography, Inc.).

Instrumentation

Instrumentation used was described previously (8). ALC was done on an Altex Gradient LC Model 332 equipped with an Altex 210

injector using either a 8.0 mm i.d. x 25 cm, 37-44 μm XAD-4 column or a 4.1 mm i.d. x 15 cm, 10 μm Hamilton PRP-1 column.

Procedures

Mixtures were prepared from standards using water or mixed solvents (EtOH-H_2O) and acid and base only when necessary except for samples obtained from the Boc reaction (11). Literature LC data (5,6) and separations with analytical level (μg quantities) samples were used to establish the eluting conditions prior to scale up of the procedure to a preparative level. Conditions for the separations are listed in the Figures and/or discussion and represent an optimum eluting condition for the amount and type of mixture being separated.

RESULTS AND DISCUSSION

Mobile Phase Conditions

AA and peptides are ampholytes whose ionic form changes from cation, to zwitterion, to an anion as a function of pH. If the side chain groups contain ionization sites these also can contribute to the overall charge depending on their ionization constants and the pH.

Retention of AA and peptides on analytical XAD and PRP-1 columns is dependent on the ionic form of the sample (4-6). Thus, retention is high in acidic and basic solution where the sample is a cation and anion, respectively, and at a minimum at the isoelectric pH where it is the zwitterion form. If the side chain has an acidic or basic group the formation of an additional cationic (acid solution) or anionic (basic solution) site significantly reduces the retention. If all the ionization steps are accounted for, capacity factor, k', can be quantitatively related to mobile phase pH and ionization constants of the acid-base groups; this has been experimentally verified for AA and peptide

retention on the XAD and PRP-1 copolymers (5,6). Side chain polarity will have a significant effect on retention. Thus, retention changes in the order Leu > Val > Ala > Gly which correlates to the change in hydrophobicity for the side chain group; these effects are discussed in detail elsewhere (3-6). These same trends were observed when determining pH effects on retention of AA and peptides at large mass loadings on the prep XAD-4 column. Thus, the ALC data (5,6) are an excellent guide for predicting mobile phase conditions on the prep XAD-4 column.

Column selectivity (α) for a given pair of AA or peptides changes as a function of pH (5,6). In some cases the elution order can be reversed when switching from an acidic to basic mobile phase. The adjustment of mobile phase pH can then be used to make α as large as possible and thus permit a higher column loading. Often a strongly acidic or basic mobile phase, which are conditions that cannot be used with silica or alkyl-modified silica, rather than one with an intermediate pH provides the optimum α.

Addition of buffers, acids, or bases to the mobile phase might be undesirable for certain AA and peptide preparative separations. Resolution can often still be achieved by careful control of the type and amount of organic solvent in the organic solvent-water mobile phase. Even a completely aqueous mobile phase can be useful. In the absence of pH control, chromatographic bands tend to be broader and complete resolution for compounds with closely related k' values is often more difficult to obtain than in the presence of pH control. The addition of salts and/or counterions to the mobile phase sharpens chromatographic peaks on XAD-4 (12), but their presence also complicates recovery of the AA or peptide. Retention of AA and peptides on the prep XAD-4 column, like ALC on XAD-4 (4,5), increases as the percent organic solvent in the mobile phase decreases. The effect on eluting power follows the order CH_3CN > EtOH > MeOH. An elutropic order for other solvents has also been established (4-7, 13).

In general, k' on the prep XAD-4 column does not change appreciably if the AA or peptide mass load is well below the mass overload limit providing a volume overload is not present. When comparing different AA and peptides in a given mobile phase, the mass overload limit decreases as the k' for each increases. Furthermore, increasing the sample load above the limit decreases k'. These observations are consistent with those observed for the separation of other samples on the XAD-4 prep column (8). For AA and peptide separations at an acceptable resolution it is therefore important to consider the maximum amount to be loaded, the k' at this loading level, and the mobile phase conditions when attempting to establish the optimum separation conditions. The separations cited here focus on the advantages of the absence and presence of pH control in the mobile phase particularly at pHs where silica and alkyl-modified silica cannot be used, use of mixed solvents in the mobile phase, separation of closely related components, isolation of minor and major components, and separation of modest to gross mass overloads. Not all separations were done at or above the mass overload limit nor was it always determined. As is often the case in PLC the limiting factor is the solubility of the sample in the injection volume used and not the overload limit.

PLC of Amino Acids

Figure 1 illustrates PLC separation of two AA mixtures using an acidic mobile phase where the weight ratio of DL-Dopa, L-Tyr, and D-Phe (total weight of about 12 mg) is about 10:1:0.1 and 0.1:1:10, respectively. The k' values for the mobile phase using an analytical load are 3.6, 6.4, and >20, respectively. In the former mixture the last two AA are well below the mass overload limit while the first is at the mass overload limit. The opposite is the case for the latter mixture. The first two AA are the most difficult to separate (they differ by a -OH group in position 3 on the side chain) and the resolution of

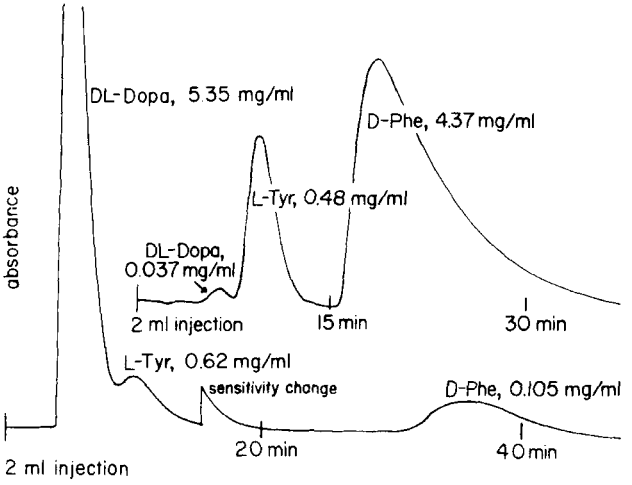


FIGURE 1

PLC of an Amino Acid Mixture

A 8.0 mm i.d. x 250 mm, 37-44 μm XAD-4 column was used with a 1.25% EtOH (95%)-98.75% H_2O, pH = 2 (H_3PO_4 buffer or 0.01M HCl), 0.1M ionic strength mobile phase at a flow rate of 5.6 ml/min. Sample injection was 2 ml.

this pair can be improved by decreasing the % EtOH in the 0.01M H_3PO_4 mobile phase; this also permits greater loading. If the mobile phase is at the zwitterion pH or in the absence of buffer or salt, resolution is significantly reduced. A basic pH is probably the optimum eluting pH (6, 7, 13) because of the acidic sites on the side chains of DL-Dopa and Tyr. This can't be used since DL-Dopa rapidly oxidizes in a strongly basic solution. A stepwise elution of increased % EtOH can be used to reduce the separation time for Phe removal.

DL-Dopa and Tyr are not very soluble. The sample load in Figure 1 was obtained by dissolving the mixture in a HCl solution. Since the sample solvent is a weaker eluent than the one used for the separation, it has little effect on peak shapes. The mixtures in Figure 1 also illustrate examples of the separa-

tion of a major and a minor sample component. In one case the DL-Dopa is about 88% of the sample while the Phe is at a 1.6% level. In the other the DL-Dopa is the minor constituent while the Phe is the major one. A separation of an Ala-Ile-Tyr mixture at a ratio of 21:7:0.1 using total loading levels of 7.1 and 14.2 mg was also carried out on the 8.0 mm i.d. XAD-4 column with an acidic mobile phase (8).

Figure 2 illustrates that PLC of more closely related AA is possible and that this can be done at high loads. At the conditions used and at an analytical load k' values in Figure 2A are ~0, 0.5, 2.1, and 16, respectively, while in Figures 2B and C they are ~0, 0.36, 1.2, and 1.9, respectively. Increasing the

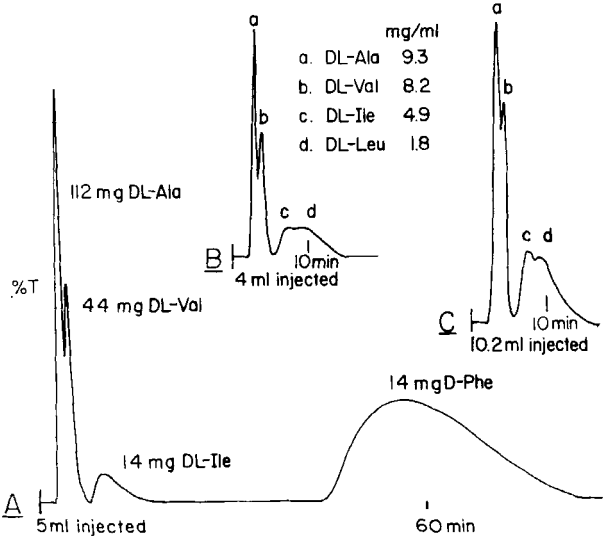


FIGURE 2

PLC of Amino Acid Mixtures on a Large Diameter Column

A 20.5 mm i.d. x 320 mm, 75-105 μm XAD-4 column was used for A, B, and C. An aqueous 0.02M H_3PO_4 mobile phase was used for A while a 0.02M Na_2HPO_4 mobile phase was used for B and C. Flow rates were 22 ml/min.

column diameter to 20.5 mm permits larger loading. The total load is 184, 96.8, and 248 mg in Figures 2A, 2B, and 2C.

In Figure 2A only the highly retained Phe is above the mass overload limit. This accounts for its elongated peak shape. The Phe elution time can be reduced and its peak shape improved by increasing the pH or adding more EtOH to the mobile phase stepwise after the appearance of Ile, or initially, if resolution of the first three AA is not desired. It should be noted that Phe, which is about 7.7% of the sample, can be easily separated from the mixture in a single pass at even much lower levels.

In Figures 2B and C all AA are below the mass overload limit even at the higher sample loading. The inability to obtain a better resolution is due to their similarity in k' values. It should be noted that Ile and Leu are isomeric and differ from Val and Ale by only one and two side chain carbons, respectively. Using an acidic eluent did not improve resolution while a more basic eluent than used in Figures 2B and C gave a slight improvement.

PLC of Peptide Diastereomers

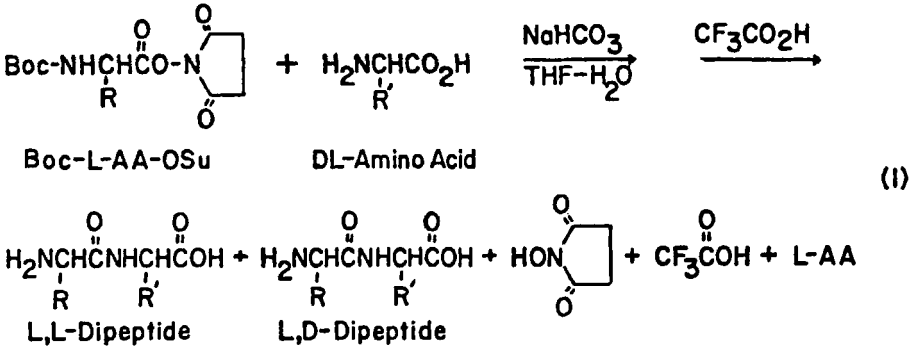
The ALC data for dipeptide diastereomers is readily available (4-6, 11). For all examples studied the L-L and D-D dipeptides always coelute first and are followed by the coelution of the L-D and D-L dipeptides. This order has been explained by considering the spatial arrangement around the chiral centers.

A mixture of dipeptide diastereomers, made by mixing pure L-Leu-L-Tyr (10 mg) and D-Leu-L-Tyr (4 mg), was separated on a 8.0 mm i.d. XAD-4 column. A 5:95 95% EtOH-H_2O mixture without pH control was used as the mobile phase. This sample loading is well above the mass overload limit. Since retention ($k'_{LL} \cong 7$ and $k'_{LD} \cong 13$) is high for this eluent the mass overload limit for the column is reduced and the peaks tend to

broaden. If the sample load is reduced resolution and peak shape are improved. Decreasing the % EtOH also increases resolution. However, this also increases the separation time and lowers the mass overload limit particularly for the second component.

A commercially available DL-Ala-DL-Val sample was also separated on the 8.0 mm i.d. XAD-4 column (9). An acidic aqueous mobile phase was used and quantities as high as 48 mg total dipeptide were separated into D-D, L-L and D-L, L-D components in a single pass through the column.

The reaction between an AA or peptide and the appropriate tert-butyloxy-carbonyl-L-amino acid-N-hydroxysuccinimide ester is often used in peptide synthesis to increase the chain length by one unit (11). This is illustrated in reaction 1 where a DL-amino acid is the starting material. Since the Boc reagent



introduces a L-amino acid, the final mixture should contain an equivalent amount of L-L and L-D dipeptide. Reaction 1 is useful in peptide synthesis because it goes to completion rapidly without loss of optical activity and many Boc reagents are commercially available. The reaction has even been used for the determination of optical purity of D,L-amino acids and diastereomeric dipeptides (11).

Several Boc reactions where carried out and the products separated on the prep XAD-4 column to illustrate the column's usefulness in peptide synthesis. To obtain the dipeptides from reaction 1 it is necessary to separate them from each other, from the blocking groups and other reagents used in the synthesis, from the L-amino acid introduced from the Boc reagent if used in excess or if the reaction is incomplete, and from the amino acid or peptide reactant if it is used in excess or if the reaction is incomplete. Figure 3 illustrates the PLC for three

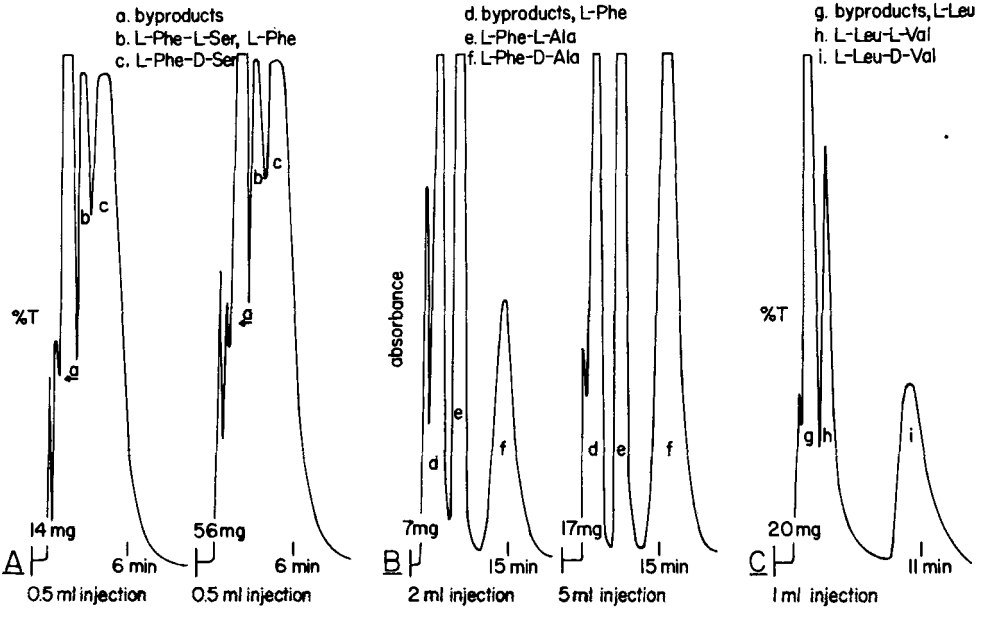


FIGURE 3

PLC of Three Different Boc Reaction Mixtures

The column in Figure 1 was used for all three separations. The mobile phases were 100% H_2O for A (k' = 1.3 for L-Phe-L-Ser and 2.3 for L-Phe-D-Ser), 5:95 95% EtOH:H_2O for B (k' = 1.7 for L-Phe-L-Ala and 3.8 for L-Phe-D-Ser), and 1:9 95% EtOH:H_2O for C (k' = 1.4 for L-Leu-L-Val and 4.2 for L-Leu-D-Val) at a flow rate of 5.6 ml/min.

different mixtures based on reaction 1. ALC separations with standards verified the peak designation.

In Figure 3A the reactants were DL-Ser and Boc-L-Phe-OSu and after workup a 0.5 ml sample containing 14 or 56 mg of the dipeptides (assuming 100% conversion) was injected and separated. The L-Phe is due to the excess Boc-L-Phe-OSu used in the reaction and appears in the L-Phe-L-Ser peak. No attempt was made to separate the L-Phe-L-Ser from the L-Phe, however, this mixture has been separated at an analytical level using pH control (5,6). A lower % EtOH will improve the separation of the dipeptides from the by-products but since the bands tend to broaden due to overloading resolution of the two dipeptides is not improved. At lower loading the resolution can be improved. In Figure 3B DL-Ala and Boc-L-Phe-OSu was used in reaction 1. After workup a 2 and 5 ml aliquot containing about 7 and 17 mg of dipeptides, respectively, (assuming 100% conversion) was injected and separated. Even at the larger mass overload a baseline separation of the dipeptides was obtained. Consequently, it should be possible to separate the mixture on the 80 mm i.d. column at a gross overload. In Figure 3C the sample was obtained from the reaction of DL-Val and Boc-L-Leu-OSu. After workup a 1 ml aliquot containing about 20 mg of dipeptides (assuming 100% conversion) was injected and separated. Resolution of L-Leu-L-Val from the by-product peaks can be increased by reducing the EtOH in the mobile phase, however, the retention time and peak broadening of the L-Leu-D-Val is significantly increased.

The prep separations in Figure 4 were carried out with an aqueous-EtOH mobile phase. The inorganic compounds are from the reaction mixture and appear in the by-product peak. Thus, recovery of the peptides from appropriately collected fractions is simplified. If pH control is used, resolution in most cases can be markedly improved (5,6), however, this requires an additional step to remove the buffer salts from the peptide.

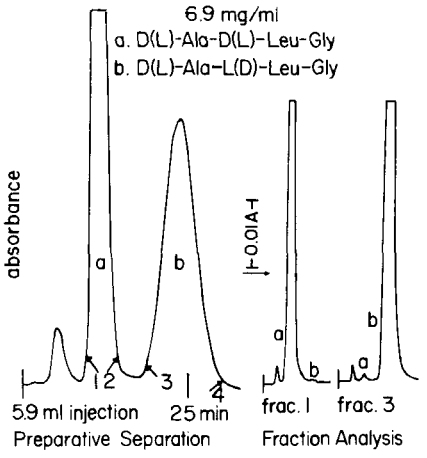


FIGURE 4

PLC of a Diastereomeric Tripeptide

The preparative separation was done on the column used in Figure 1 using a 5:95 CH_3CN:H_2O mobile phase at a flow rate of 5.6 ml/min. The fraction analysis was done on a 4.1 mm i.d. x 150 mm, 10 µm PRP-1 column using a 3:97 CH_3CN:H_2O, pH = 6 (phosphate buffer), 0.1M ionic strength mobile phase at a flow rate of 1.4 ml/min.

Often this can be achieved on the same XAD-4 column using a salt free mobile phase.

Figure 4 illustrates the PLC separation of a commercially available sample of the tripeptide, DL-Ala-DL-Leu-Gly, on a 8.0 mm i.d. XAD-4 column. The L-L and D-D tripeptides (k' = 6.3) coelute as the first peak while the L-D and D-L tripeptides (k' = 15.7) coelute as the second peak. The sample was injected as 5.9 ml of 6.93 mg DL-Ala-DL-Leu-Gly/ml. At this 40 mg loading level, which is a gross mass overload, a baseline separation is obtained, because of the large difference in k' values. (At lower loadings resolution and peak shapes are even better.) Fractions were collected and these are indicated by number in the prep chromatogram in Figure 4. Purity of these fractions

was established by ALC on a PRP-1 column (6). Only the analytical chromatograms for fractions 1 and 3 are shown in Figure 4. The major peak is off scale and the chromatogram focuses on the trace quantity. By comparing peak heights or peak weights, the purity of the major diastereomer is estimated to be greater than 99% after a single pass through the prep column. No attempt was made to recycle the peak to improve purity. Since resolution is so favorable even a greater mass overload than shown in Figure 4 would be tolerable. Also, the option of improving peak shape and resolution through pH control of the mobile phase is still available.

PLC of Peptides

The enkephalins are peptides that have been isolated from brain extracts and are reported to be natural opiate receptor agonists. Figure 5 contains an analytical and preparative level chromatogram, where the total load is 27 mg (9 mg of each peptide), for the separation of three enkephalin peptides. The mixture was prepared from commercially available enkephalins. Since retention for the three enkephalins is high, k' values are 4.2, 7.6, and 12.2, respectively, the extent of mass overload increases as the peptide retention increases. This accounts for the increased broadening of the peaks as the retention times for the three enkephalins increases. To demonstrate that the three enkephalins can be isolated in high purity and good yield, fractions of the prep-chromatogram were collected; these fractions are identified by number in Figure 5. Analysis of each fraction was done by ALC. Only the chromatograms for the key fractions, numbers 1, 3, and 6, which correspond to each of three enkephalins, are shown in Figure 5. Clearly, the separation in terms of yield and purity is favorable in a single pass through the prep column. To improve enkephalin yield, the cross contaminated fractions (fraction 2 for the first pair of enkephalins and fractions 4 and 5 for the second pair of enkephalins) could be recycled.

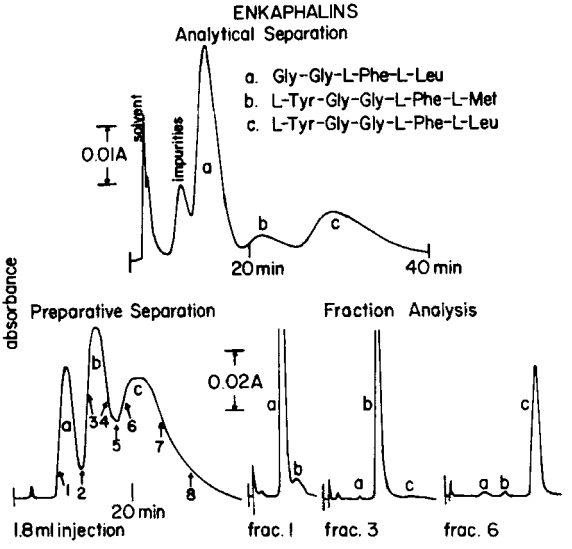


FIGURE 5

PLC of a Mixture of Enkaphalin Peptides

The preparative and analytical separation was done on the column used in Figure 1 using a 17:83 CH_3CN:H_2O, pH = 2.2 (H_3PO_4 buffer), 0.1M ionic strength mobile phase at a flow rate of 5.6 ml/min. The fraction analysis were done on a PRP-1 column (see Figure 7) using the above mobile phase at a flow rate of 1.4 ml/min.

Using a mobile phase without pH control would be preferred if the enkephalin is to be isolated. However, suitable resolution at a reasonable loading was not obtained in the absence of pH control. A basic pH will not only decrease the level of retention but also it will change the elution order to b < c < a. This is due to the influence of the additional charge site on the Tyr that forms in basic solution. Depending on which peptide is being sought the basic mobile phase might be preferred.

Separation of the three enkephalins is rapidly achieved at an analytical level even though the peptides are very similar.

For example, the Leu enkephalin and Met enkephalin (b and c in Figure 5) are 5-unit peptides that differ only in the fifth unit, the former contains L-Leu at unit-5 while the latter contains L-Met at unit-5. The difference in polarity between these unit-5 side chains accounts for the elution of the methionine enkephalin prior to the Leu enkephalin. The [des Tyr^1]-Leu enkephalin (a in Figure 5) is a 4-unit peptide and is similar to unit-2 to unit-5 in the Leu enkephalin. The additional side chain provided by the Tyr at unit-1 in the Leu and Met-enkephalins accounts for their greater retention over the [des Tyr^1]-Leu enkephalin. The correlation of a significant change in chromatographic retention with a modest change in peptide structure has been observed before (3-6).

The dipeptide separations shown in Figure 6 clearly illustrate a key advantage of a prep XAD-4 column in peptide separations, namely its utility with either a strongly acidic or basic mobile phase. Neither of these can be used with silica or alkyl-modified silica columns. Also changing the mobile phase pH from an acidic to basic one often leads to an elution order reversal.

In the 0.01M HCl mobile phase the k' values for L-Phe-L-Ser and L-Ser-L-Phe at an analytical load are 1.0 and 3.5, respectively, while in the 0.01M NaOH mobile phase retention is reversed so that their k' values are 3.1 and 1.4, respectively. The prep chromatograms for the separation of these two dipeptides for the two mobile phases are shown in Figure 6.

In Figure 6A, where the acidic mobile phase is used, L-Phe-L-Ser is separated from L-Ser-L-Phe at ratios of 400:1 and 1:100. The same separations, but with a reversed elution order due to the basic mobile phase, are shown in Figure 6B. It is readily seen that if recovery of L-Phe-L-Ser as the minor component is desired the basic mobile phase is best, while for recovery of L-Ser-L-Phe as the minor component the acidic mobile

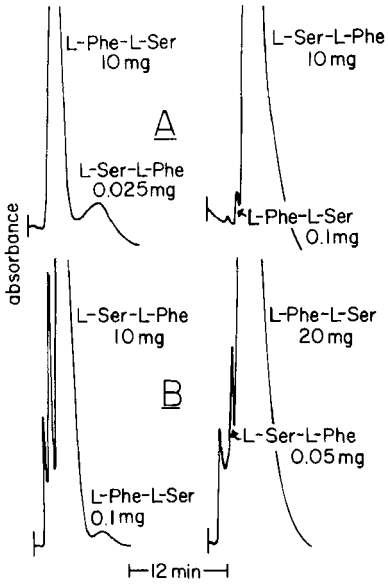


FIGURE 6

PLC of a Dipeptide Mixture by a Strongly Acidic and Basic Mobile Phase

The column in Figure 1 was used with a 0.01M HCl 5:9 95% EtOH:H_2O mobile phase in A and a 0.01M NaOH 5:95 95% EtOH:H_2O mobile phase in B. Flow rate was 5.0 ml/min.

phase is best. For major component recovery the reverse is best, that is, an acidic mobile phase for L-Phe-L-Ser and a basic one for L-Ser-L-Phe. The chromatograms in Figure 6 suggest that larger total mass loadings could still be separated. However, the limitation is sample solubility in the injection volume used rather than effects due to excessive mass overloading. Although not shown here reversals in elution order for other peptide mixtures are possible on the prep XAD-4 column.

Summary

Preparative LC columns packed with 37-44 μm XAD-4 particles can be used to separate a variety of AA and peptide mixtures at

large mass loadings for purposes of purification, and are viable alternates to silica and alkyl-modified silica columns. Of particular significance is that only the XAD-4 can be used with strongly acidic and basic mobile phases which often are the ones that provide the best resolution of AA and peptide mixtures. The acid, base, or buffer can then be usually removed from the isolated AA or peptide by passing the collected fraction through a XAD-4 column using a mixed solvent mobile phase where only the AA or peptide is retained.

In general mass overloading occurs at about 0.23% and 0.25% wt/wt for the 8.0 and 20.5 mm i.d. XAD-4 column, respectively, at k' values of about 2. Useful column efficiencies are still obtained even when k' > 10 and often at modest to gross overloads particularly when k' for the sample components differ appreciably. Depending on the mobile phase the 37-44 μm XAD-4 columns at preparative mass loadings develop efficiencies of about 500 to 1500 plates/meter. Increased efficiencies can be obtained by using smaller XAD-4 particles. Not all separations can be done at or above a mass overload because, as is often the case in PLC, sample solubility in the injection volume is not high enough to permit the large overload.

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REFERENCES

1. Walton, H. F., Ion Exchange and Liquid Column Chromatography, Anal. Chem. 52, 15R-27R (1980).

2. Krummen, K. and Frei, R. W., Quantitative Analysis of Nonpeptides in Pharmaceutical Dosage Forms by HPLC, J. Chromatogr. 132, 429-436 (1977).

3. Molnar, I. and Horvath, C., Separation of Amino Acids and Peptides on Non-Polar Stationary, Phases by HPLC, J. Chromatogr. 142, 623-640 (1977).

4. Kroeff, E. P. and Pietrzyk, D. J., HPLC Study of the Retention and Separation of Short Chain Peptide Diastereomers on a C-8 Bonded Phase, Anal. Chem. 50, 1353-1358 (1978).

5. Kroeff, E. P. and Pietrzyk, D. J., Investigation of the Retention and Separation of Amino Acids, Peptides, and Derivatives on Porous Copolymers by HPLC, Anal. Chem. 50, 502-511 (1978).

6. Iskandarani, Z. and Pietrzyk, D. J., Liquid Chromatographic Separation of Amino Acids, Peptides, and Derivatives on a Porous Polystyrene-Divinylbenzene Copolymer, Anal. Chem. 53, 489-495 (1981).

7. Baum, R. G., Saetre, R., and Cantwell, F. F., Liquid Chromatography Columns of Microparticle Amberlite XAD-2, Anal. Chem. 52, 15-19 (1980).

8. Pietrzyk, D. J. and Stodola, J. D., Characterization and Applications of Amberlite XAD-4 in Preparative Liquid Chromatography, Anal. Chem. 53, 1822-1828 (1981).

9. Dieterle, W., Faigle, J.W., and Mory, H., Preparative Reversed Phase Chromatography of Polar and Nonpolar Metabolites on Columns Packed with Micronized XAD-2 Resin, J. Chromatogr. 168, 27-34 (1979).

10. Uematsu, T. and Sasaki, T., Separation of 3':5'-and 2':3'-Cyclic Ribonucleotides from Their Related Mononucletoides by Amberlite XAD-4 Column Chromatography, J. Chromatogr. 179, 229 (1979).

11. Cahill, W. R., Kroeff, E. P., and Pietrzyk, D. J., Applications of tert-Butyloxycarbonyl-L-Amino Acid-N-Hydroxysuccinimide Esters in Chromatographic Separation and Determination of D,L-Amino Acids and Diastereomeric Dipeptides, J. Liq. Chromatogr. 3, 1319-1334 (1980).

12. Rotsch, T. D., Cahill, W. R., Pietrzyk, D. J., and Cantwell, F. F., Ionic Strength Effect on Liquid Chromatographic Retention of Benzenesulfonates on Nonpolar Adsorbents, Can. J. Chem. 59, 2179-2183 (1981).

13. Robinson, J. L., Robinson, W. J., Marshall, M. A., Barnes, A. D., Johnson, K. J., and Salas, D. S., Liquid Solid Chromatography on Amberlite XAD-2 and Other Styrene-Divinylbenzene Adsorbents I. Development of a Solvent Eluotropic Scale, J. Chromatogr. 189, 145-167 (1980).