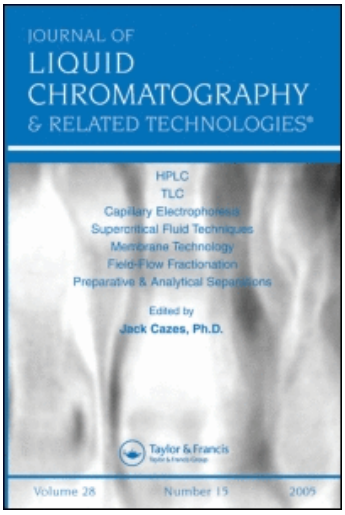

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Amberlite XAD-4 as a Stationary Phase for Preparative Liquid Chromatography in a Radially Compressed Column

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To cite this Article Pietrzyk, Donald J. and Cahill Jr., William J.(1982) 'Amberlite XAD-4 as a Stationary Phase for Preparative Liquid Chromatography in a Radially Compressed Column', Journal of Liquid Chromatography & Related Technologies, 5: 4, 781 — 795
To link to this Article: DOI: 10.1080/01483918208060586
URL: http://dx.doi.org/10.1080/01483918208060586

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(4), 781-795 (1982)

AMBERLITE XAD-4 AS A STATIONARY PHASE FOR PREPARATIVE LIQUID CHROMATOGRAPHY IN A RADIALLY COMPRESSED COLUMN

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ABSTRACT

Amberlite XAD-4, a nonpolar adsorbent with a particle size of 62 to 177 μm, was packed into 58 mm i.d. x 294 mm columns and radially compressed in the Waters Prep 500 preparative liquid chromatograph. Efficiency,loading capacity (multigram samples), resolution, recovery, and types of mobile phases were major parameters studied. Since XAD-4 is chemically inert mixed solvent and pH control from 1 to 13 can be successfully used in the mobile phase. Separations illustrating these advantages and the scope of the 58 mm i.d. column are described.

INTRODUCTION

Preparative high performance liquid chromatography (PLC) constitutes a wide range of column loadings extending from a few mgs to multigram samples. The scope of PLC applications extends to almost all scientific fields. Typical applications are purifications in synthetic chemistry, purification of standards, and characterization of trace compounds in physiological, environmental, plant, etc. type samples. Even commercial applications are

feasible. Details of these and other applications are reviewed elsewhere (1-3).

Commercial LC suppliers have responded to this interest and prepacked columns of 1/4 to 1 inch diameters containing either silica or alkyl-modified silica as the stationary phase are available. Larger diameter columns, which permit greater loading, are also available. Usually these are dedicated to a specific type of instrumentation. One such example is the Waters Prep 500, which employs silica or alkyl-modified silica packed in 58 mm i.d. x 294 mm plastic columns that are radially compressed.

Amberlite XAD-4 is a macroporous polystryene-divinylbenzene nonpolar adsorbent with a large surface area and porosity. It functions as a reversed stationary phase like the alkyl-modified silica and has been used in PLC 8 mm and 20.5 mm i.d. columns (4-6) and in analytical high performance liquid chromatographic (ALC) columns (7-8). Since XAD-4 is stable throughout the entire pH range of 1 to 13, strongly acidic and basic eluting agents, which cannot be used with the alkyl-modified silica, are readily used with XAD-4 columns without loss in efficiency or changes in chromatographic behavior. Other advantages and properties of XAD-4 including favorable mass loading capabilities are described elsewhere (4).

This report describes PLC experiments using XAD-4 in a 58 mm i.d. x 294 mm plastic column which is radially compressed in the Waters Prep 500. Mass and volume overload, efficiency, resolution, recovery, and mixed solvent, acidic, and basic mobile phases are evaluated.

MATERIALS

Reagents

The chemicals used were obtained from Curtin Matheson Scientific, Eastman Kodak, and Sigma Chemical Company and used as

received. Water was purified by passing distilled water through a mixed bed ion exchange column, a charcoal column and 0.2 μm Millipore filter disks. All solvent composition is expressed as percent by volume.

Amberlite XAD-4 (20 to 50 mesh) was purchased from Mallinckrodt Chemical Works. Cleaning, crushing and sizing procedures are described elsewhere (4). Irregular shaped particles (62 to 177 μm) were used to pack plastic columns of 58 mm i.d. x 294 mm (Waters Associates) equipped with either 15 μm porous stainless steel or polyethylene column end fitting disks (Waters Associates).

Instrumentation

A Waters Prep 500 liquid chromatograph (Waters Associates) was used and the column effluent was monitored by a refractive index detector (Prep 500) and an Altex Model 153 fixed wavelength (254 nm) detector equipped with a 2 μl, 0.5 mm cell. Sample was introduced with 10 or 30 ml syringes designed to fit the Prep 500 injector. ALC separations were performed on an Altex Model 332 LC using a 4.1 mm i.d. x 150 mm, 10 μm, Hamilton PRP-1 column.

Procedures

The exit frit was inserted into the plastic column and the column was wrapped with cloth duct tape up to but not under the sealing rings. Approximately 450 g of dry XAD-4 with a size distribution of 430 g of 74 to 149 μm, 10 g of 62 to 74 μm, and 10 g of 149 to 177 μm was carefully poured into the column in small amounts (about 30 ml) and pressed into a tight bed with a plexiglass rod. This was continued until the column was full. The last two or three inches of the column which could not be pressed with the rod was tightly packed by hammering a frit (this served as the column outlet) into place using a rubber stopper to protect the frit. If the lower part of the column was not packed tight enough

a second frit was hammered in (served as column inlet). The packed column was then inserted in the Prep 500, radially compressed, and 1:5 95% EtOH:H_2O pumped through the column at 50 ml/min for approximately 1 to 2 hrs. Since the XAD-4 particles will swell as the % EtOH increases a change in % EtOH in either direction must be gradual to prevent channeling or bursting of the column. At 50 ml/min and 1:5 95% EtOH:H_2O inlet column pressures were about 15 atm. The radial compression was about 34 to 40 atm.

After the LC system stabilized in the appropriate mobile phase the sample, which was in water, ethanol, their mixture, in acidic, or basic solution, was introduced by syringe. In general, sample solvents were weaker eluting agents than that used for the separation. Effluent fractions, when desired, were collected manually.

RESULTS AND DISCUSSION

Column Properties

The LC properties of the 58 mm i.d. XAD-4 column which varied from column to column was largely due to the inability to pack the columns in an uniform manner. However, with packing practice the differences were small. The properties also change with usage; this is the largest when the column is exposed to widely different mobile phases. The properties cited here are average ones observed prior to exposing the column to widely different mobile phases.

The 58 mm i.d. x 294 mm, 62 to 177 μm XAD-4 column had an average void volume of about 260 ml determined for a mobile phase that was in the range of 5 to 10% ethanol. This corresponds to approximately 55-60% stationary phase.

The column permeability changes with solvent composition. As the organic solvent concentration in the mobile phase increases, the XAD-4 particles swell, and the column inlet pressure required to maintain the flow rate increases. For a 5 to 10% ethanol mobile

phase at a flow rate of 50 ml/min inlet pressure was about 20 atm. Inlet pressures did not change appreciably when dilute EtOH mobile phases contained 0.1M HCl or 0.1M NaOH. Because of pressure limits separations were done at either 50 or 100 ml/min.

The plastic columns were taped to increase their wall strength and to prevent the stationary phase particles from entering the instrument compression chamber if the column splits. With use wrinkles appear in the column; the more tightly the column is packed the smaller the wrinkles are. Since the column inlet end is the most difficult to pack tightly, wrinkling was the largest at this end and column splitting, if it occurred, was usually at the inlet end. In general, longer column life and reproducibility was favored by tightly packed columns and by minimal changes in the EtOH:H_2O ratio of the mobile phase. However, column and retention properties were reasonably reproducible even when the column was subjected to modest change in the solvent composition with and without acid (up to 0.1M HCl) or base (up to 0.1M NaOH) providing the transition from one mobile phase to another was a gradual one.

Column Performance

Figure 1 shows how plate number, N (number of plates for the 294 mm column), for the radially compressed XAD-4 column changes with sample loading. The arylsulfonic acid and tetraalkylammonium salt samples (k' values of 2.47 and 1.88, respectively) were used because of their large solubility in water. Thus, they could be introduced as small volumes of concentrated solution (20 and 17 ml in Figure 1, respectively, or about 5 to 7% of the void volume) without exceeding the volume overload. Greater loads were not examined because these could only be attained by a large increase in sample volume. Although not shown, k' decreased as sample loading increases which is consistent with previous results (4).

The mass overload limit, which by definition is the load that produces a 10% change in efficiency (4), occurs at about 2 to 2.5 g.

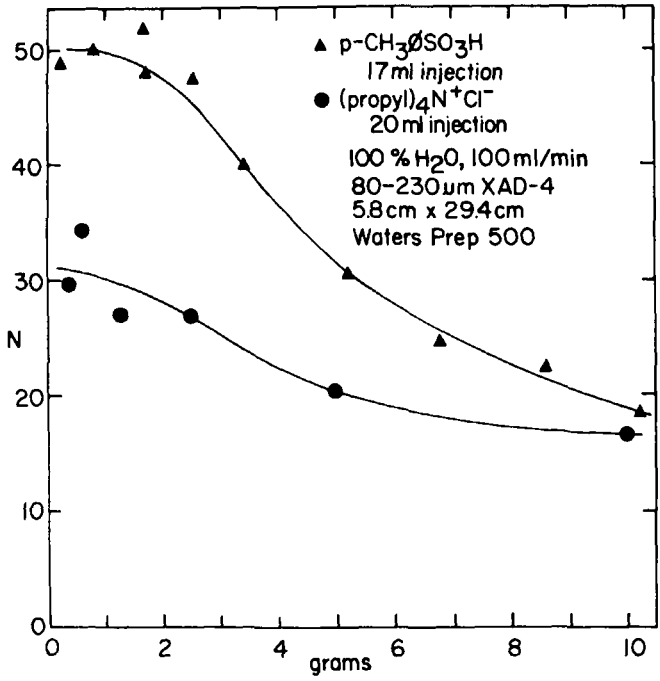

FIGURE 1

The Effect of Mass Loading on the XAD-4 Column

A 58 mm x 294 mm, 62 to 177 µm XAD-4 column was used with 100% H_2O at a flow rate of 100 ml/min.

However, even at a column load in excess of the mass overload limit many plates are still available. For example, at 4 to 5 times the mass overload limit (a 10 g load) approximately 20 plates are still obtained. Furthermore, a symmetrical peak shape was retained throughout the major part of this load range and only at higher loads does tailing begin to occur. Improvement in column efficiency would be realized by reducing both the XAD-4 particle size and its range. This has been shown in both XAD analytical and smaller diameter prep columns (4,9).

Similar loading experiments were done with pyridine and picoline samples using an aqueous 0.1M HCl mobile phase and with o-chlorophenol using a 1:5 95% EtOH:H_2O, 0.1M NaOH mobile phase. The mass overload limit in the acidic and basic mobile phases were consistent with the results found in Figure 1.

On the average for the separations reported here, the number of plates developed on the radially compressed XAD-4 column when operated below the mass overload limit was about 40 to 60 plates. In comparison, the number of plates typically found for the commercially available silica and C_{18}-modified silica columns that can be radially compressed are in the order of 100 to 130 plates (10, 11). Contributing to this difference is the fact that the commercially available silica and C_{18}-modified silica are smaller particles than the XAD-4 and that the samples and eluting conditions used in the latter studies are not the same as that used on the XAD-4.

The injection system on the Prep-500 requires the injected sample to replace liquid through and against the pressure of the column-detector. This prevents an accurate determination of the volume overload. Within experimental error no significant effect on efficiency was found up to a 50 ml injection (20% of the column void volume). This was shown by determining plate number for the elution of 1.88 g of p-methylbenzene sulfonic acid as a function of injection volume at a flow rate of 100 ml/min. Even at a 100 ml injection (40% of the column void volume) a volume overload effect was small.

Resolution

Figure 2 illustrates the preparative separation of two benzenesulfonic acids at loading levels above the mass overload limit relative to each component. At low loading the k' values of the p-methyl- and 2,5-dichloro- derivatives are 1.3 and 2.7, respectively. For a single pass a resolution of 0.93 is obtained

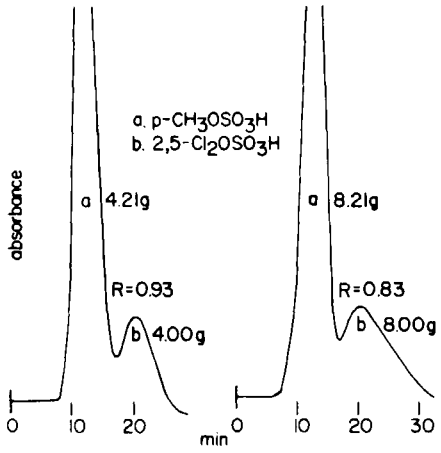

FIGURE 2

Separation of a Mixture of Benzenesulfonic Acids at a Mass Overload

A 58 mm x 294 mm, 62 to 177 μm XAD-4 column was used with 1:4 95% EtOH:H_2O at 43 ml/min. Injection volume was 9 ml.

at a total load of 8 g (see Figure 2). At lower loadings resolution is more favorable. For example, at 1.6 g total load, a resolution of 1.0 was found. As loading increases, efficiency and subsequently resolution decreases. Thus, at 16 g total load (Figure 2) resolution drops to 0.83. Two major factors which contribute to this are an increase in peak broadening and a decrease in retention with increased loading. However, even with these effects enough plates are still available to permit isolation of a significant amount of each component free of the other in a single column pass even though the column is grossly overloaded.

Mobile Phase Versatility and Sample Recovery

Retention order is the same as found on analytical XAD-4 columns and these data can be used to predict elution order and

optimum mobile phase (7-9). Thus, retention is reduced when the analyte is in a charged form, retention increases as the percent organic solvent in the mobile phase is reduced, and the eluting power changes in the order CH_3CN > EtOH > MeOH. Because of XAD-4 swelling and the flexibility of the plastic columns the better column performance is achieved when the mobile phase composition is only modestly changed during packing, conditioning and elution and when H_2O is the major mobile phase component.

A mixture of p-methyl-and 2,5-dichlorobenzenesulfonic acids were separated at a total load of about 3.7 g where the two acids are in the weight ratio of 40/1, 1/1, and 1/40. The 1/1 mixture represents the isolation of two major components while the other two are examples of separation of a minor (2.5% of the sample) and a major (97.5% of the sample) component. These chromatograms are shown in Figure 3. Verification of the separation was done by

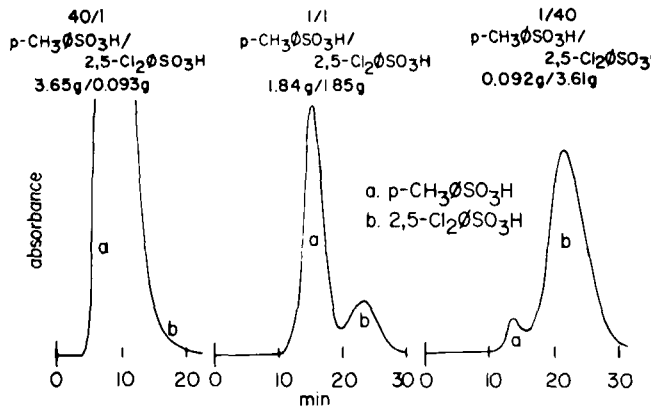

FIGURE 3

Separation of a Mixture of Benzenesulfonic Acids

The conditions and column described in Figure 2 were used. Injection volume was 5 ml.

collecting 2 min (86 ml) fractions and analyzing each fraction by ALC. The fraction analysis for each chromatogram is shown in Figure 4.

In the 40/1 mixture the p-methyl derivative swamps the detector cell because of its large concentration and masks the 2,5-dichloro peak. Fraction analysis (Figure 4) verifies the separation and shows the minor component at the tail of the peak. For a single pass and with careful fraction collecting 80 to 90% of the major component can be isolated at high purity level. The minor component could be isolated in mg quantities at a high purity level by collecting the peak tail, or a large amount of it could be collected where its enriched and purified via a second pass through the column. For the 1/1 mixture the chromatogram (Figure 3) and fraction analysis (Figure 4) indicate that a significant amount (up to 70 to 80%) of each compo nent can be isolated at a high purity level by just a single column pass. In the 1/40 mixture, Figures 3 and 4, about 80 to 90% of the second or major component can be isolated in mg quantities of high purity by collecting the beginning of the first peak. Or, larger quantities of an enriched mixture can be isolated and purified by a second pass through the column.

Silica and C-18 modified silica are the only stationary phases that have been used in radially compressed columns in the Waters Prep-500. Although they are readily used in mixed solvents, their applications are limited to a pH range of 2 to 8. This is not the case for XAD-4 which is chemically stable throughout the pH range of 1 to 13.

The application of an acidic and basic mobile phase in PLC offers several advantages. 1. Organic acids, bases, and ampholytes can be separated as charged species rather than neutral ones. 2. Retention of charged species is greatly reduced which broadens the useful range of eluting conditions. 3. The aqueous solubility of the ionized sample is increased which allows smaller volumes of concentrated sample solution to be

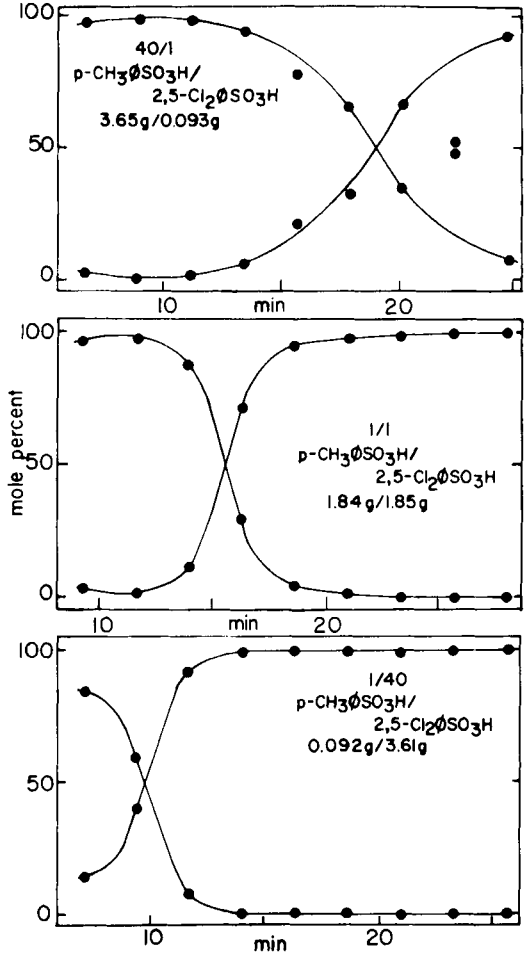

FIGURE 4

Fraction Analysis for the
Chromatogram in Figure 3

ALC was done on a 4.1 mm x 150 mm, 10 µm, Hamilton PRP-1 column using a 8.5:91.5 $CH_3CN:H_2O$ pH=6.0 (phosphate) mobile phase at a flow rate of 1 ml/min.

injected. 4. Adjustment of pH relative to the K_a values for the analytes can often improve the selectivity. 5. Addition of electrolyte to the mobile phase often reduces band width and improves selectivity when using XAD-4. Perhaps the major disadvantage of using acids, bases, or buffer salts in PLC is that the separated components contain these electrolytes. By adjustment of mobile phase conditions so that the sample is converted back to the neutral form the same or a second XAD-4 column can be used to separate the sample from the salts.

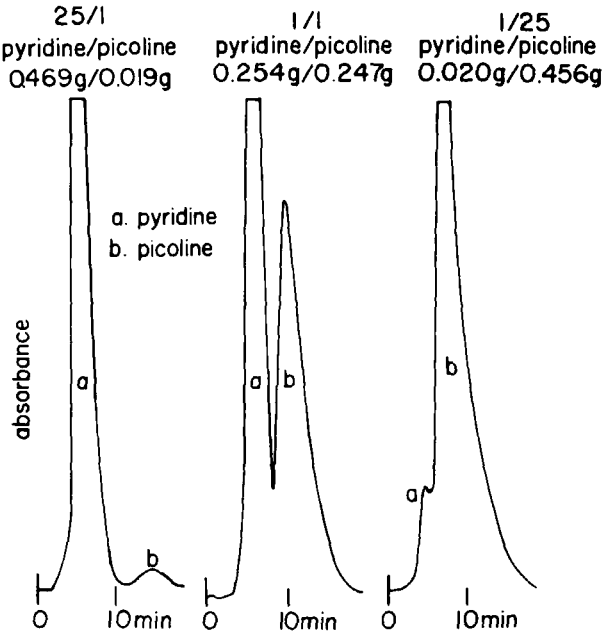

FIGURE 5

Separation of a Pyridine/Picoline Mixture with a Strongly Acidic Mobile Phase

A 58 mm x 294 mm, 62 to 177 μm XAD-4 column was used with 0.1M HCl at 102 ml/min. Injection volume was 5 ml.

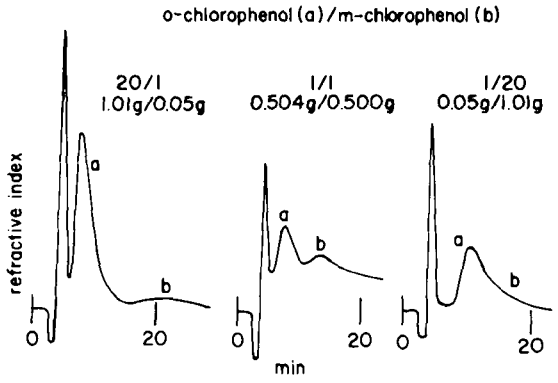

FIGURE 6

Separation of a o-Chlorophenol/m-Chlorophenol Mixture with a Strongly Basic Mobile Phase

A 58 mm x 294 mm, 62 to 177 μm XAD-4 column was used with 3:7 95% EtOH:H_2O, 0.1M NaOH at 100 ml/min. Injection volume was 10 ml.

Two separations were carried out on the XAD-4 column to demonstrate the feasibility of using an acidic and basic mobile phase. Figure 5 illustrates the separation of a pyridine/picoline mixture at a 25/1, 1/1, and 1/25 ratio using an aqueous 0.1M HCl mobile phase while Figure 6 shows the separation of a o-chloro/m-chlorophenol mixture at a 20/1, 1/1, and 1/20 ratio using 1:5 95% EtOH:H_2O, 0.1M NaOH mobile phase. No undesirable chromatographic properties are introduced into the separation as a result of using the strongly acidic or basic eluent other than the presence of HCl or NaOH in the collected fractions.

For the pyridine/picoline mixture, where the k' values are 1.6 and 4.5, respectively, chromatographic peaks are found for both components in all three mixtures. This was verified by fraction analysis. Only for the 1/25 mixture is the resolution low and this occurs because the picoline k' decreases with the increased picoline loading. Thus, the minor component in the

25/1 and 1/25 mixture can be isolated in significant amounts at a high purity level. Similarly, the major component in all three mixtures can be isolated in large quantities at a high purity level. The pyridine/picoline mixture could be separated in mixed solvent in the absence of acid, however, the EtOH concentration in the mobile phase must be significantly increased since the pyridine/picoline are now retained as neutral species. Also, in the absence of the acid the peaks become much broader. The conclusions regarding the o-chloro/m-chlorophenol separation are similar. Since the k' values in a basic mobile phase (retention as anions) are favorable a baseline separation is obtained except for the 1/20 mixture. In the absence of HCl (retention as neutral species) the EtOH would have to be about 40 to 50% to affect the separation; also, peaks would be broader.

ACKNOWLEDGEMENTS

We thank J. D. Stodola and D. L. Bakken for their help in column packing and Waters Associates for their advice and for supplying the empty column cartridges. This investigation was partly supported by Grant CA 18555 awarded by The National Cancer Institute, DHEW, and partly by Grant CHE 79-13203 awarded by the National Science Foundation. Initial experiments were reported at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Atlantic City, N.J., March, 1981 as part of paper Number 582.

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