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CONDUCTIMETRIC DETECTION OF ORGANIC ACIDS IN REVERSED-PHASE CHROMATOGRAPHY

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SUMMARY

The response of various free acids, including monocarboxylic and dicarboxylic acids, phenol and substituted phenols, diketones, mercaptans, and aromatic acids in organic mobile phases was determined with conductimetric detection. Weak acids, such as phenols and mercaptans, have essentially no response. Substituted phenols with more acid strength respond slightly; carboxylic acids are most responsive, with detection limits in the range 1–10 mg/l. Carboxylic acids vary in response on an equivalent weight basis; response decreases with increasing size of the acid. Detection depends on the exchange of hydrogen for sodium on the silica matrix of the column, and the conjugate base of the acid is detected by conductivity.

INTRODUCTION

There is substantial interest in new and different detectors for liquid chromatography. Occasionally, a chromatographic system can be modified to take advantage of a specific type of detection. For example, the advent of ion chromatography has re-introduced the use of conductivity as a detector in liquid chromatography. Small *et al.*[1], in a novel use of ion-exchange chromatography and conductimetric detection, determined major inorganic cations and anions in diverse aqueous solutions. Also, organic acids and bases, such as formic acid, acetic acid, and methylated amines have been determined by ion chromatography with conductimetric detection[1,2]. In ion chromatography, the mobile phase is always water; interfering ions are removed by stripper columns consisting of ion-exchange resins.

To extend the technique of ion chromatography to more hydrophobic organic solutes, a technique has been developed which uses organic mobile phases of methanol, acetonitrile, and aqueous mixtures of these solvents. Interfering inorganic salts are not removed, but elute from the column prior to the organic compounds. For example, monocarboxylic acids from C_4 to C_{16} are detected in programmed runs of methanol–water and acetonitrile–water mobile phases. This technique appears to be a useful system of detection for aliphatic acids and other ionogenic, organic compounds which are insensitive to detection by ultraviolet spectroscopy.

This paper discusses the response of organic acids in methanol–water solvents and shows that the conductimetric detector may be used as a selective detector for organic acids in reversed-phase chromatography.

EXPERIMENTAL

Instrumentation
The liquid chromatograph consisted of (1) a set of Varian (Palo Alto, Calif., U.S.A.) Model 8500 pumps with programmer; (2) a Vari-Chrom UV–VIS spectrophotometer (Varian); (3) an air-actuated injection valve model CV-6-UHPa-C20 VALCO (Varian) with 200 μl sampling loop; (4) a model 701 ConductoMonitor conductivity detector from Laboratory Data Control (Riviera Beach, Fla., U.S.A.). The conductivity detector measures specific conductance with a common-electrode technique. The cell is a flow-through unit with a 2.5-μl volume.

Reagents
All test compounds were analytical-grade reagents from Chem Service, (West Chester, Pa., U.S.A.).

Standard solutions
All solutions of organic acids were prepared in methanol at 5.0 mequiv./l by equivalent weight. Non-ionic solutes were prepared in methanol at 5.0 mequiv./l per functional group. Because the response of the conductivity detector was related to the functional group on the organic molecule, it was important to compare all solutes at the same concentration of functional groups.

Chromatography
Analyses were performed on a 0.39 × 30 cm μBondapak C$_{18}$ column (Waters Assoc., Milford, Mass., U.S.A.). The column consisted of 10-μm particles of silica (SiOH) with a surface area of 300–350 m^2/g that has had a C$_{18}$ group chemically bonded to the SiOH at a 10% loading of C$_{18}$.

Mobile phases consisted of methanol and water gradients, acetonitrile and water gradients, and isocratic runs of methanol, acetonitrile, and water at flow-rates of 1 ml/min.

Procedure
The conductivity detector was tested by injecting 200 μl of the standard on to a reversed-phase column with either methanol or acetonitrile mobile phases, and by recording the response of the detector with a strip-chart recorder.

Gradient separation of butyric, octanoic, dodecanoic, and hexadecanoic acids was accomplished starting with a methanol–water (60:40) solution and programming to 100% methanol in 8 min at a flow-rate of 1.0 ml/min.

RESULTS AND DISCUSSION

Theory of acid response
Responses of the conductivity detector were determined for a variety of organic solutes including carboxylic acids, phenols, alcohols, aldehydes, esters, amides, ethers, and ketones in a mobile phase of methanol (Table I). As expected, organic solutes that do not ionize have no response because detection is based on conductivity of the solution.

TABLE I

RESPONSE OF COMPOUNDS BY CONDUCTIVITY DETECTOR IN A MOBILE PHASE OF
METHANOL AT SAME EQUIVALENT WEIGHT (5.0 mequiv./l)

Compound	Relative response*	Compound	Relative response*
Acetic acid	100	Phenol	2
Butyric acid	90	Picric acid	85
Octanoic acid	80	Pentane-2,4-dione	100
Dodecanoic	60	Dodecanol	No response
Myristic acid	60	Benzyl alcohol	No response
Hexadecanoic acid	35	Acetaldehyde	No response
Stearic acid	35	Benzaldehyde	No response
Succinic acid	80	Methyl formate	No response
Citric acid	50	Acetanilide	No response
Benzoic acid	100	Aniline	No response
Phthalic acid	65	Hexylamine	30
o-Nitrophenol	90	Tetrahydrofuran	No response
p-Chlorophenol	8	Acetone	No response
o-Cresol	0.1	2-Mercaptoethanol	1

* Acetic acid = 100.

The detector responded by approximately the same amount when either benzoic acid or sodium benzoate was injected onto the column. It was first thought that the solvated hydrogen ion and the sodium ion had the same equivalent conductance in methanol. This seems unlikely, because in water the hydrogen ion has about five times the equivalent conductance of the sodium ion. Another explanation is that an acid is only partially dissociated in methanol, and it is merely fortuitous that the responses are similar. However, when the conductivity of the standard benzoic acid solution was measured before injection onto the reversed phase column, it was very low. After benzoic acid had been passed through the C_{18} column, the conductivity was 50 times greater than that of the benzoic acid standard. The benzoic acid peak was collected, and sodium ion was measured by flame photometry. Sodium was 25 mg/l, as opposed to only 0.5 mg/l in the benzoic acid standard solution. This finding indicates that the reversed-phase C_{18} column is functioning as a weak cation-exchange resin, with the hydrogen ion of the acid being exchanged for sodium ions on the silica matrix. Apparently, the silica contains free $Si-O^-Na^+$ groups, which exchange for a H^+ ion. The sodium salt of the acid is then detected by conductivity, which explains the similar response of the conductivity detector for both free acid and sodium salt of the acid. Prior to the conductivity study, the C_{18} column had been used with sodium buffers, and active exchange sites contained sodium.

The cation-exchange capacity of the reversed phase column was measured by first saturating the sites with sodium, then exchanging sodium with hydrogen in a 10% acetic acid and methanol mobile phase. The exchange capacity of the column was 20 μequiv. for a 5-ml column. This exchange capacity is sufficient for 50 to 100 injections of standard solutions at 1.0 mequiv./l before regeneration is necessary. The detector responds identically within $\pm$ 2% for 50 injections.

Because organic acids are being measured as the sodium salt of the conjugate base, the organic anion is the most important variable that will affect the response of the conductivity detector for various acidic compounds. The molar conductance

is the sum of both the molar conductance of the cationic and anionic species. This is known as the *Law of the independent migration of ions*[3] and is expressed algebraically as:

$$\Lambda_0 = \Lambda_0^+ + \Lambda_0^-$$

where Λ_0 is the molar conductance of the solution at infinite dilution and Λ_0^+ and Λ_0^- are the molar ionic conductances at infinite dilution of the cationic and anionic species, respectively.

The amounts of charge carried by Λ_0^+ or Λ_0^- may not be equal, and the transport number or transference number (t_0) equates the fraction or percentage of charge on each species. Thus, a cation transport number, t_0^+ (ref. 3), is equal to:

$$t_0^+ = \frac{\Lambda_0^+}{\Lambda_0}$$

The transport number, $t_0^{Na^+}$, for sodium is constant for acids listed in Table I. However, transport numbers for organic anions are not constant; as the size of the acid increases, transport numbers for anions should decrease, because the mass of the anionic solute is increasing. This result can be shown for simple fatty acids. The response of acetic, butyric, octanoic, dodecanoic, and hexadecanoic acids decrease gradually with relative response factors of 100, 90, 80, 60, and 35, respectively (Table I). Hexadecanate has four times the mass of acetate.

Another effect which may decrease conductivity response of dicarboxylic and tricarboxylic acids is solvation of the anion. It is known that the molar conductance of inorganic cations decreases with decreasing atomic radii, because of the hydration shell that forms around the solvated ion[3]. Ions with a smaller atomic radius have larger hydration shells, and thus they migrate more slowly. Similarily, dicarboxylic and tricarboxylic acids have larger solvation shells than monocarboxylic acids which act to decrease their rate of migration. This effect of solvation can be seen in the relative responses of phthalic acid (65), succinic acid (80), and citric acid (50) compared with those of acetic acid (100) and benzoic acid (100) (Table I). The responses of the dicarboxylic and tricarboxylic acids on an equivalent-weight basis are less than those of the monocarboxylic acids.

Selective detection by conductivity

Simple monocarboxylic acids gave the highest conductimetric response. Dicarboxylic acids respond less than monocarboxylic acids, and phenols vary from no response to a response identical with that of acetic acid.

An interesting class of compounds which can be detected by conductivity are the β-diketones. The proton on the carbon between the two carbonyl groups is acidic, and pentane-2,4-dione gave a response identical to acetic acid on an equivalent-weight basis (Table I).

The detection limit for acetic acid is 13 μequiv./l (0.8 mg/l); the detection limit increases to *ca.* 40 μequiv./l (10 mg/l) for palmitic acid, based on a peak to noise ratio of two. The detection limits for the other acids listed in Table I vary between these two extremes. This limit is determined with the detector in the absolute mode,

which must be used for gradient programming from water to methanol solvents. If the detector is used in the differential mode (that is, comparing conductivity of the sample to the blank cell that contains the mobile phase), sensitivity is enhanced *ca.* 5 to 10 times. If chromatography is done isocratically, the differential mode may be used.

The response of the conductivity detector is linear for a 10–15-fold concentration range of dilute organic acids. For example, the standard curve for benzoic acid (Fig. 1) is linear from 0.1–2.5 mequiv./l (20–300 mg/l); similar relationships are noted for other acids. The expected conductivity response would not be linear at higher concentrations (greater than 1000 mg/l), because the response is based on the concentration of charge-carrying species, which is a function of the activity of the solute. Detector response does vary from acid to acid, and is related to the variation in equivalent conductance of the acid being measured.
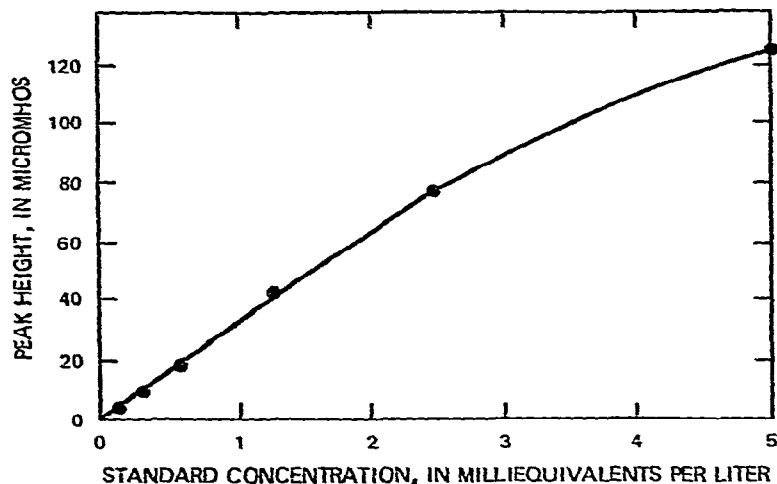

Fig. 1. Benzoic acid standard curve.

The conductivity detector responds to ionogenic solutes, a characteristic which may be used for selective detection. For example, the chromatograms for impure acetaldehyde (Fig. 2) and benzaldehyde (Fig. 3) show that only the acidic oxidation product of the aldehydes responds to conductivity. Acetic acid, an oxidation product of acetaldehyde, gives a large response by conductivity; however, the aldehyde does not respond. Likewise, benzoic acid responds by conductivity, but benzaldehyde does not. The acetic acid/acetaldehyde (Fig. 2) chromatogram illustrates an advantage of the conductivity detector for compounds insensitive to UV detection. Acetic acid has a good response by conductivity, but is almost undetectable by UV. Although acids may be detected at low wavelengths (206 nm) gradient programming at this wavelength causes large shifts in baseline, which makes sensitive detection impossible. Also, many extraneous compounds absorb at low wavelengths, and cause interference. Therefore, a combination of conductivity and UV detection is useful for determination of aliphatic acids.
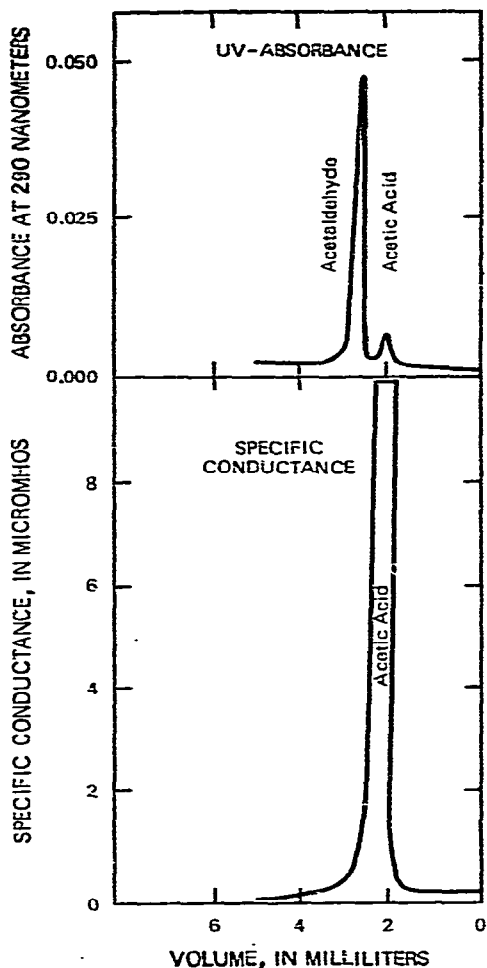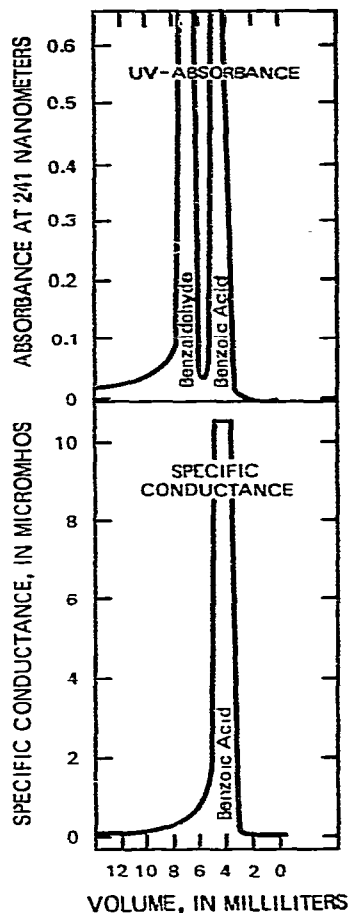
Fig. 2. Chromatogram of impure acetaldehyde.

Fig. 3. Chromatogram of impure benzaldehyde.

Effect of solvent on conductivity detection

The two most common solvents for reversed phase chromatography, methanol and acetonitrile, were compared to determine their effect on the sensitivity of the detection system. The measured conductance of organic acids was considerably greater in methanol than in acetonitrile. It varied directly as acid strength, or inversely to pK_a (Table II). Acids with a pK_a less than 7.2 respond in a methanol solvent, and additionally, acids having a pK_a less than 5.0 respond in acetonitrile. Very strong organic acids, such as picric acid, respond more in acetonitrile than in methanol. The response of the conductivity detector for the majority of acids is greater in methanol; therefore, methanol was chosen for reversed-phase separation of organic acids.

The data in Table II can be explained by the ion-exchange phenomenon. In

TABLE II

RESPONSE OF CONDUCTIVITY DETECTOR IN ORGANIC SOLVENTS RELATED TO pK_a

Compound	pK_a	Response* (methanol)	Response* (acetonitrile)
o-Cresol	10.20	0.1	No response
Phenol	9.89	2.0	No response
p-Chlorophenol	9.18	8.0	No response
o-Nitrophenol	7.17	90.0	No response
Acetic acid	4.75	100.0	40
Benzoic acid	4.19	100.0	40
Picric acid	0.38	85.0	200

* Acetic acid = 100.

methanol, a hydrogen ion of a free acid with pK_a less than 7.2 exchanges with a sodium ion on the silica matrix. A strong acid, such as a carboxylic acid, donates its proton to the weaker silica acid. Because phenol is a weaker acid than silica and does not donate a proton, free phenol is not detected by the conductivity system. Apparently, the solvent has an effect on this exchange reaction. Carboxylic acids do not respond as much in acetonitrile as in methanol, which may be due to increased acid strength of the silica surface in acetonitrile. This is suggested by the pK_a and response data for acetonitrile (Table II), where there is a reversal in conductivity response for picric acid. That is, the response is greater in acetonitrile than in methanol.

The effect of water in the mobile phase was examined. The organic acids tested would not elute in a water mobile phase, and so methanol–water mixtures were tested. Water did not significantly alter the response of the conductivity detector for hydrophobic organic acids, when tested from methanol–water (25:25) mixture to 100% methanol. The baseline shift from water to methanol was small, ca. 2 μmho, and aliphatic acids were detected at 13–40 μequiv./l (1–10 mg/l).

The response of hexylamine is affected by the amount of water in the mobile phase. In methanol, the response of hexylamine is 30 (Table I). But if water constitutes more than 50% of the mobile phase, the response of hexylamine increases dramatically. This is probably due to the extraction of a proton from water by hexylamine, with the detector responding to the protonated amine and OH^- group.

Applications of the conductivity detector

The conductivity detector is especially suited for organic acid detection and for those ionic solutes which absorb weakly in the UV. A separation of butanoic, octanoic, dodecanoic, and hexadecanoic acids was performed as a test of the conductivity detector (Fig. 4). The acids were injected at 250 mg/l, about 20 times above maximum sensitivity. A μBondapak C_{18} column was programmed from 60% methanol and 40% water to 100% methanol in 8 min. Owing to ion exchange, the acids were chromatographed as their sodium salts. The inorganic salts were not retained and eluted at the void volume. Therefore, they did not interfere with conductimetric detection of organic acids.

The conductivity detector lends itself to preparatory reversed-phase chromatography owing to its wide dynamic range of seven orders of magnitude. In this labora-
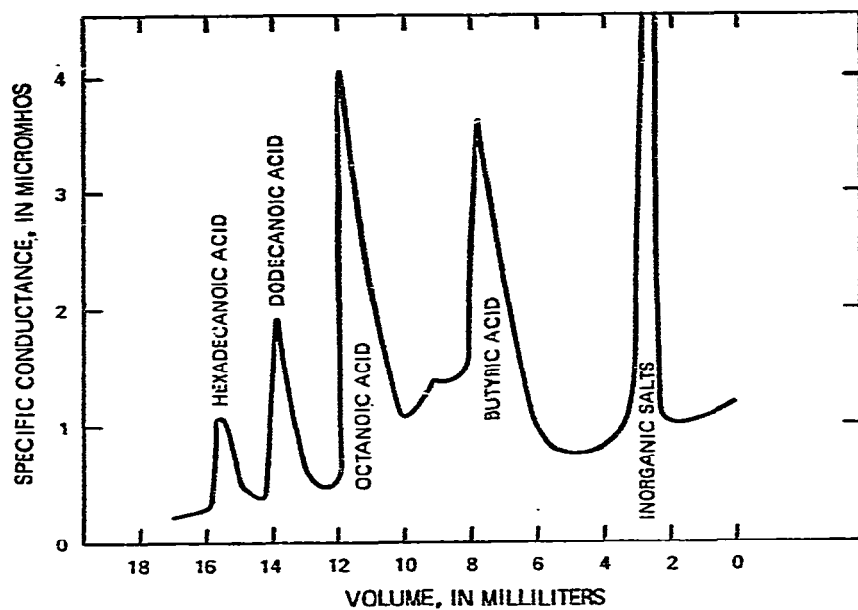
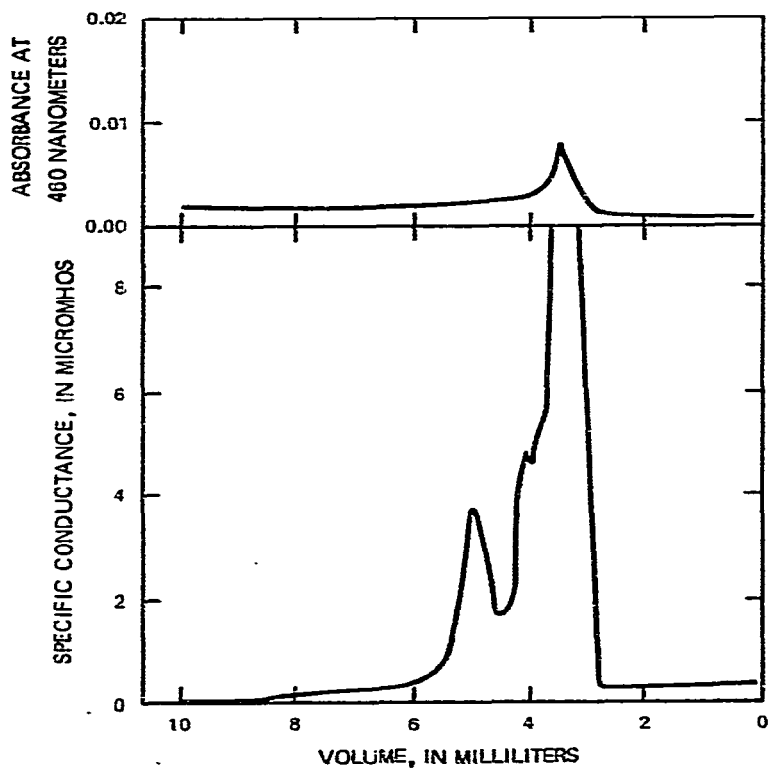Fig. 4. Chromatogram of aliphatic acid separation.

Fig. 5. Chromatogram of brown-colored substances isolated from water.

tory, the conductivity detector has been used for preparatory chromatography of natural organic acids concentrated from surface waters. Chromatograms of colored organic substances concentrated from the South Platte River near Denver, Colorado, on a XAD-8 (non-ionic polyacrylic ester) preconcentration column, show that the colored material eluting in methanol is predominantly ionic (Fig. 5), owing to the large conductivity response. The small visible-absorbance peak in Fig. 5 (a brown-colored fraction eluted with methanol from XAD-8) has a corresponding large conductivity trace. This indicates that the brown component contains numerous ionic functional groups. These groups are probably acidic, because previous studies[4] have shown that the majority of ionic organic solutes in water are acidic, and comprise 30–50% of the dissolved organic carbon. It is not likely that either phosphate or amine functional groups are responsible for the conductivity trace, because they are labile in natural waters. Therefore, the conductivity detector is selective for organic acid functional groups in the methanol fraction of organic concentrates from natural waters.

Selectivity for the functional group of the organic acid can be seen in Fig. 6 (a green-colored fraction eluted with methanol from XAD-8). The first visible-absorbance peak was determined to be a chlorophyll c degradation product by visible scan and retention time. This finding is consistent with the conductivity trace in that chlorophyll c contains several carboxylic functional groups, which leads to a high conductivity trace. The examples above demonstrate that the combination of con-
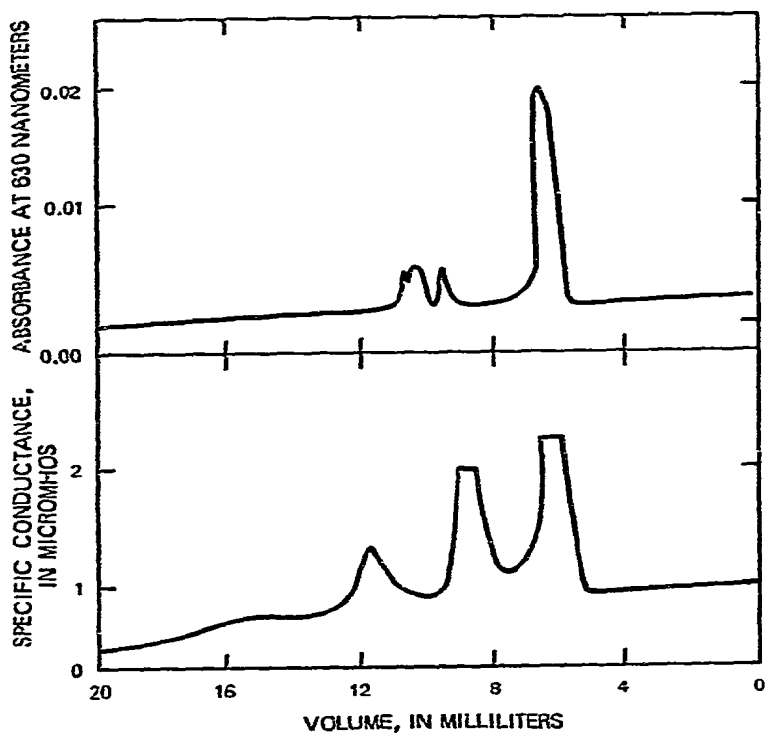

Fig. 6. Chromatogram of green-colored substances isolated from water.

ductivity and UV–VIS detection is very useful and can give both quantitative and qualitative information about unknown organic solutes in concentrates from natural waters.

CONCLUSIONS

Conductivity detection of organic acids is an extention of ion chromatography to hydrophobic, organic, ionic solutes in organic mobile phases. The mechanism for detection is weak ion exchange of hydrogen for sodium on the silica matrix of the reversed phase column. The combination of conductivity and UV–VIS detection may give both quantitative and structural information about unknown solutes. The wide dynamic range of the conductivity detector makes it appropriate for reversed-phase preparatory liquid chromatography of ionogenic solutes. More work is needed to establish conductivity detection as a routine analytical method for the analysis of aliphatic organic acids in water. Two areas of needed research are (1) lowering of the detection limit and (2) removal of interference from natural high-molecular-weight organic acids, such as humic acids.

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