dehyde derivatives at pH 4. In none of these studies were enough data obtained to permit the calculation of k_d or k_f values.

Sørensen and Andersen report a pK_a of 11.7 for the bisulfite adduct of formaldehyde at 25°. We have calculated their data, allowing for the acidity of formaldehyde hydrate,[21] which had been neglected, and get a pK_a of 11.8 ± 0.5, $K_{S^{2-}}$ of (2.8 ± 2) × 10^5 M^{-1}, and K_{SH^-} of 10^{10} M^{-1}. The uncertainty in the pK_a and in both constants, $K_{S^{2-}}$ and K_{SH^-}, is attributable to the uncertainty in the intercept (which is related to the pK_a and very near to zero) in the plot correlating $1/K_{obsd}$ with $1/[OH^-]$. However, the magnitude of K_{SH^-} agrees well with the value of 1.6 × 10^{10} M^{-1} that can be obtained by combining the results of Skrabal and Skrabal[22] with the equilibrium constant for hydration of formaldehyde. Stewart and Donnally report a pK_a of 9.16 for the bisulfite adduct of benzaldehyde at 21° and ionic strength 0.10. This corresponds to a value of about 9.6 at zero ionic strength. Thus the thermodynamic pK_a values of compounds of the type $RCH(OH)SO_3^-$ are about 11.8, 11.3, and 9.6 when R is hydrogen, isopropyl, and phenyl, respectively. Acids of the type $RCH_2NH_3^+$ in which R is separated from the acidic proton by the same number of atoms, have pK_a values of 10.7, 10.4, and 9.3, at 26°, when R is hydrogen, isopropyl, and phenyl, resepctively.[23] Thus the effect of changing R from hydrogen to isopropyl in one series is the same, within the experimental uncertainty, as in the other. Phenyl, however, is an anomalously effective acid strengthening substituent in the α-hydroxy sulfonate series, not only by comparison to the ammonium ions but also by comparison to simple alcohols. Benzyl alcohol is about eight times as strong an acid as isobutyl alcohol and is only slightly stronger than methanol in isopropyl alcohol solution.[24] It may be relevant that if the titrimetric K_{app} values obtained above pH 10 by Stewart and Donnally were too large because of imperfect quenching, as ours were, too small a pK_a value for the bisulfite addition compound would result. We feel that their quenching method, in which acid but no cooling was employed, is probably not as effective as ours. However, if k_d is as much smaller for the benzaldehyde adduct as they report, perhaps a less effective quenching method would still be effective enough.

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Registry No.—Sodium 1-hydroxy-2-methylpropanesulfonate, 13023-74-0; sodium bisulfite, 7631-90-5; isobutyraldehyde, 78-84-2.

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An Automated Preparative Liquid Chromatography System

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The design, construction, and operation of an automated preparative liquid chromatography system capable of separating multigram quantities of materials is presented. The system repetitively injects the sample, monitors the effluent, detects and separately collects, as programmed, entire chromatographic bands, then distills and reuses the eluting solvent.

The general utility of liquid chromatography systems is now widely appreciated, several commercial units being available. Because these commercial systems are basically analytical units which operate at high pressures and employ small columns packed with expensive adsorbents, they are not particularly well suited for the routine separation of multigram quantities of materials. Recognizing a need among organic chemists for instrumentation capable of such separations, we herein describe an automated low-pressure preparative liquid chromatography system which repetitively injects the sample, monitors the effluent, detects and separately collects, as programmed, entire chromatographic bands, then distills and reuses the solvent. Apart from its ability to separate multigram quantities through unattended repetitive operation, the system obviates the use of large quantities of solvent, substantially

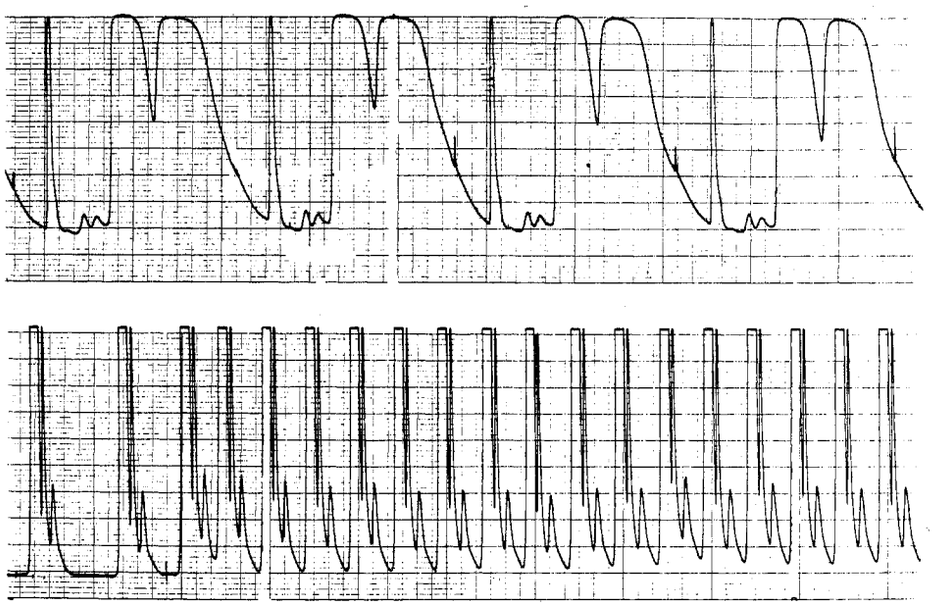

Figure 1. Two representative series of automated repetitive chromatography runs. Absorption at 280 nm is plotted against time. Repetition rate for the upper chromatogram was once per 5 hr. For the lower chromatogram, the final repetition rate was once per hour. Owing to saturation of the ultraviolet monitor, the extent of separation is greater than the recorder trace suggests.

reduces the number of fractions with which one must deal, and minimizes the problem of waste solvent disposal. This extensively tested system has been found to be flexible in application, reliable, and simple to use; consequently, it should prove valuable for the isolation of natural products, the separation of reaction mixtures, and the routine purification of organic compounds.

Although we do not claim that this system provides resolution equal to that of commercial analytical units, it does, in our hands, afford separations equal or superior to those attained by tlc but on a considerably larger scale. One demonstration of the utility of this system is that it has, in our laboratories, made possible the preparative-scale resolution of a variety of chiral alcohols[1] which had resisted resolution through the more usual (and tedious) methods of fractional crystallization of diastereomeric derivatives.

While this system routinely affects the chromatographic separation of 6–10 g of diastereomers/24 hr, in some cases, samples of up to 50 g have been chromatographed in a single pass. Two examples of the repetitive separations provided by the system are shown in Figure 1.

Limitations of the present system when operating in the automatic mode are (a) solvent gradients cannot be employed (although mixed solvents can be used),[2] (b) the compounds being collected must be stable[3] and of low volatility at 100°, and (c) nonvolatile reagents (salts, buffers) cannot be employed in the solvent system. These limitations are a consequence of the reclamation of solvent, and can be avoided if solvent reclamation is foregone.

Figure 2 is a block diagram depicting component lay-out. After initial application of the sample to the column, the sample pump stops and the main pump commences operation. The main pump feeds from a reservoir which is continually refilled with reclaimed solvent. From the pump, the eluting solvent flows through a pressure gauge, a one-way check valve (a part of the sample injection system), the column, a flow cell of short path length, through whichever of the four solenoid selector valves has been selected, and into the corresponding still. The function of the still(s) is to recover solvent from the eluent and to return the solvent to the reservoir. Nonvolatile materials eluted from the column remain in the boiling kettles of the stills. The absorbance of the column eluent is continuously determined, displayed

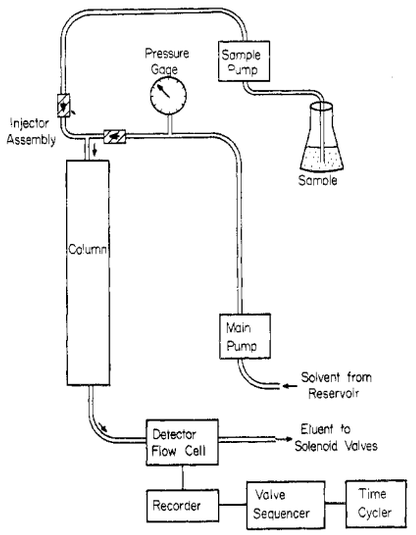

Figure 2. Block diagram of the automated preparative liquid chromatography system.

on the recorder, and monitored at regular short intervals by the valve sequencer unit. This unit, through appropriate circuitry, notes and counts bands of absorbing[4] material as they are eluted, and activates appropriate solenoid selector valves to divert any given band of material into the boiling kettle of the still specified by the settings of the programming switches. The entire band is collected in one kettle since that valve arrangement is maintained until the next band begins to emerge, and is only then changed if the switches are so programmed. With four stills, three chromatographic bands can be separately collected, additional bands being collected in the fourth kettle for discard or rechromatography. Although it is clearly possible to increase the number of stills, situations necessitating the separate collection of more than three fractions have seldom been encountered. When all chromatographic bands have been eluted, the cycle timer stops the main pump and starts the sample pump which introduces a fresh sample onto the column through a one-way valve. Simultaneously, the valve sequencer and the solenoid valve arrangements return to the start position. The main pump resumes action upon

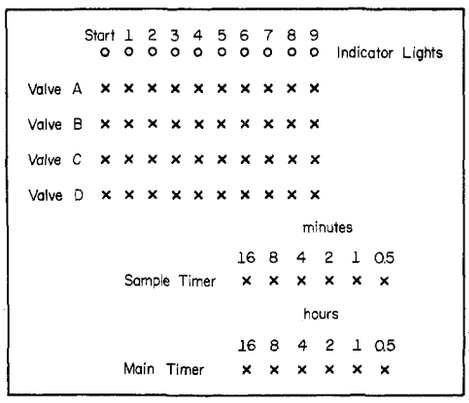

Figure 3. Control panel for the valve sequencer and time cycler units. Each x represents an on–off toggle switch.

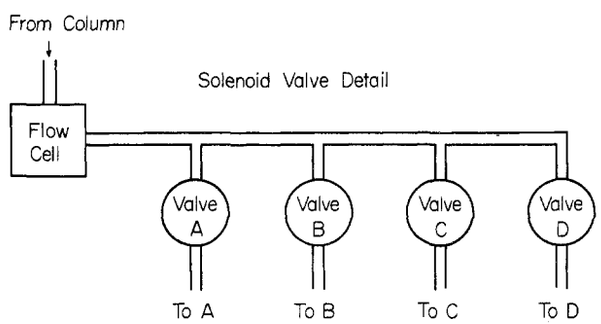

Figure 4. Solenoid valve arrangement for the automated preparative liquid chromatography system. Each valve conducts eluent into the corresponding still.

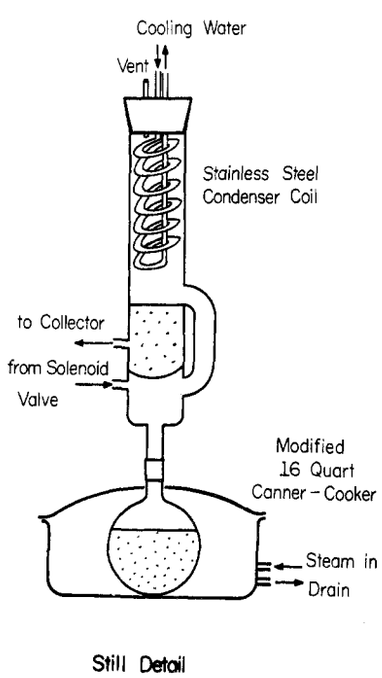

Figure 5. Details of the solvent still and steam heating baths. Four such stills (A–D) are employed.

shutdown of the sample pump. The lengths of the two pump cycles are programmable by switch settings on the timer unit. Figure 3 illustrates the control panels of the valve sequencer and the time cycler units, whereas Figures 4 and 5 illustrate the solenoid valve layout and the solvent still–heating bath arrangement, respectively.

This system employs large commercial or home-built glass chromatography columns holding from 1 to 7 kg of 0.05–0.2 mm silica gel or alumina. These comparatively inexpensive adsorbents are easily packed and allow pressures of less than 30 psig at flow rates of 2.5 l./hr. The low pressures simplify design, operation, and component requirements. Larger columns (12.5 cm diameter, 125 cm length, *ca.* 20 kg of adsorbent) have been fabricated and successfully employed. While larger samples can be accommodated and resolution has been satisfactory, per diem capacity has not been increased by the use of very large columns owing to the pumping rate limitation (2.5 l./hr) of the present main pump. Assuming satisfactory resolution, the principle factor influencing per diem capacity of the system is simply the rate at which solvent can be cycled through the system. Clearly, use of larger columns and greater pumping rates would increase the per diem capacity of the system, although drastic increases in pumping rates will necessitate redesign of the solvent stills.

To assist those who wish to construct similar systems,[5] additional description, circuit diagrams, and dimensioned drawings for chromatography columns have been made available separately as supplemenary material.

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Supplementary Material Available. To assist those who wish to construct similar systems,[5] additional description, circuit diagrams, and dimensioned drawings for chromatography columns will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-3901.

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(1) For a detailed account of this broad-spectrum resolution method, see W. H. Pirkle and M. S. Hoekstra, *J. Org. Chem.*, **39**, 3904 (1974).
(2) Since solvent reclamation is essentially a flash distillation, it is not necessary to use constant boiling mixtures of solvents to avoid changing the solvent composition through fractional distillation from the stills. However, solvent composition might well fluctuate enough to adversely affect detector systems sensing changes in index of refraction.
(3) It should be possible to use a continuous low temperature vacuum evaporation technique to remove solvent from thermally sensitive materials.
(4) While it is not essential that an absorbance detector be employed in the system, the presence of upright and inverted peaks, as might sometimes be afforded by index of refraction or Christenson effect detectors, would constitute a minor problem since the latter would not be counted by the valve sequencer unit. In this event, it would be necessary to include circuitry to automatically reverse the roles of the valve sequencers' positive and negative slope detectors by sensing whether the signal level from the elution detector is greater than or less than that of the base line level.
(5) Almost predictably, the control panel for the sequencer and cycler units arrived from the shop in which it was fabricated bearing the title "Pirkleator No. 1," a name which seems to have taken hold among the users of this system.