AN INVESTIGATION OF LIQUID–LIQUID CHROMATOGRAPHY WITH A RECORDING DETECTOR

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INTRODUCTION

The object of this work was to attempt to fit liquid–liquid chromatography to the gas chromatography model. The need for this seems evident. Even though liquid partition chromatography preceded gas chromatography by many years[1,2], the techniques of liquid chromatography, by and large, have remained what they were a decade ago. The equipment, method of elution and band detection have in no way paralleled the advance of gas chromatography. A relatively small number of workers have been using continuously recording U.V. or I.R. spectrometers, differential refractometers and interferometers. Some instruments were obtained commercially, others were designed and built in the respective laboratories using commercially obtained components. In spite of this advanced work by a few, it is reasonable to state that when a chromatographer shifted from gas chromatography to liquid chromatography, the changes in technique and equipment have been phenomenal: recording instruments have been replaced by fraction collectors, analysis time has increased 5 to 10 fold and separation efficiencies have decreased. The intent here is not to criticize liquid chromatography as a technique, but to look at both gas and liquid chromatography objectively in order to establish a correspondence between the two; then to attempt to fit liquid chromatography to a gas chromatography model to gain some of the benefits of gas chromatography in simplicity and speed of operation; perhaps in diversity of application.

The importance of liquid–liquid chromatography to analytical chemistry should not be under-rated. Even though high temperature gas chromatography, lightly loaded columns and glass bead supports have pushed the temperature limitations of gas chromatography to levels beyond expectation, the analytical chemist is still confronted with a multitude of materials which can not be volatilized, in mixtures which must be separated. For many of these problems, liquid–liquid chromatography affords the solution.

A comparison of the fundamental requirements of liquid–liquid chromatography and gas–liquid chromatography is contained in Table I. Discussion of this comparison is provided in this report along with the reported developmental results.

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TABLE I

COMPARISON OF REQUIREMENTS FOR GAS AND LIQUID SYSTEMS

	Gas chromatography	*Liquid–liquid chromatography*
Carrier	Gas	Liquid
Motion	Cylinder pressure	Gravity Pump Gas pressure
Sample inlet	Septum injection	Open column loading Septum injection
Column	Size and shape may be the same for either	
Solid support	Chromosorb	Chromosorb
Liquid phases	Low vapor pressure Suitable solvent characteristics	Immiscible with carrier Suitable solvent characteristics
Detector	Thermal detector, Ionization detector } universal	Visual, Chemical, U.V., I.R. } not universal Thermal, Refractometer, Interferometer, Mass } universal

EXPERIMENTAL

Detector

A universal detector was needed to carry out this investigation. A Waters Associates differential refractometer[3] was selected because:

1. It was reasonably universal.
2. It provided high sensitivity.
3. It provided small detector volume which is imperative to fast response.
4. It was moderately priced when compared to such devices as recording spectrometers or interferometers.

The schematic diagram in Fig. 1 shows its operating principle. The chief limitation of this detector as found in this work was that its full dynamic range is not as readily available as is customary with gas chromatography detectors.

The detector was built into a system as shown in the block diagram in Fig. 2.

Sample injection

The sample injection assemblies were prepared from Swagelok fittings. These were prepared either from Swagelok Tee's or from bulkhead fittings. The body of the bulkhead fitting was drilled with a No. 52 drill and a piece of $^1/_8$ in. O.D. tubing silver soldered into it. The sharp leading edge of the Swagelok fitting was ground flat to provide a seal for the septum. Injectors were used with either metal or glass columns ranging in size from $^1/_8$ in. to $^1/_2$ in. tubing.

Carrier

The carrier or moving phase in liquid–liquid chromatography should be a comparatively poorer solvent than the stationary phase for the sample components. With the refractometer as a detector, the carrier liquid should have a refractive index different from the sample. The moving phase should also be less strongly retained by the solid

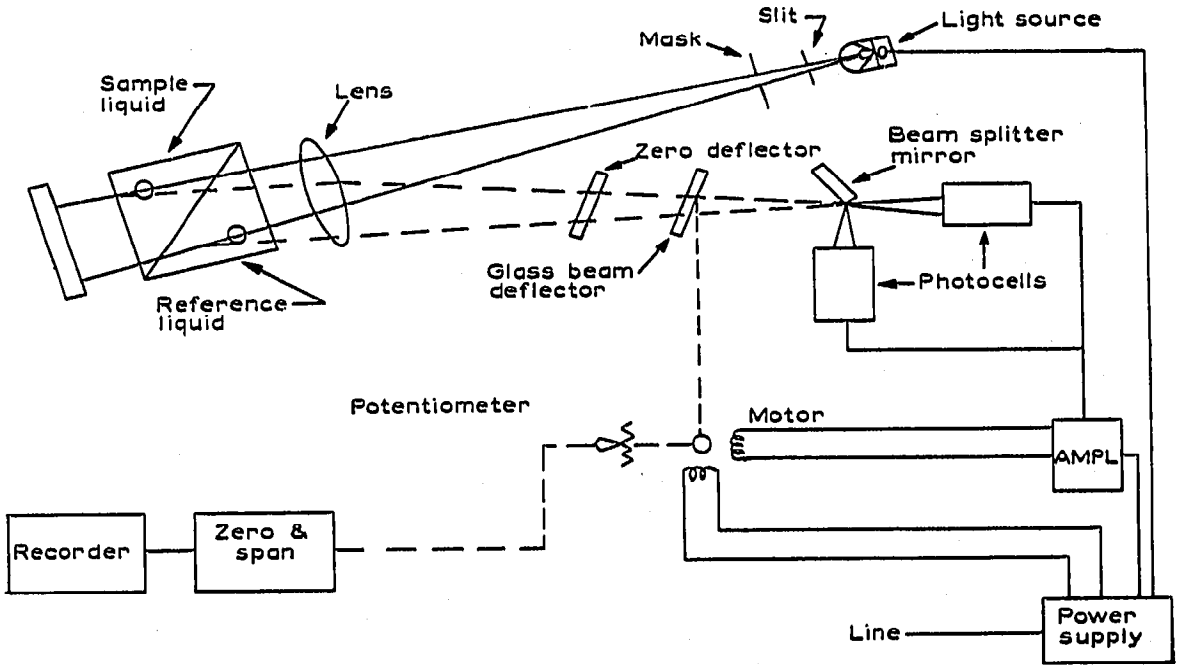

Fig. 1. Schematic diagram of Waters Associates liquid chromatography detector.

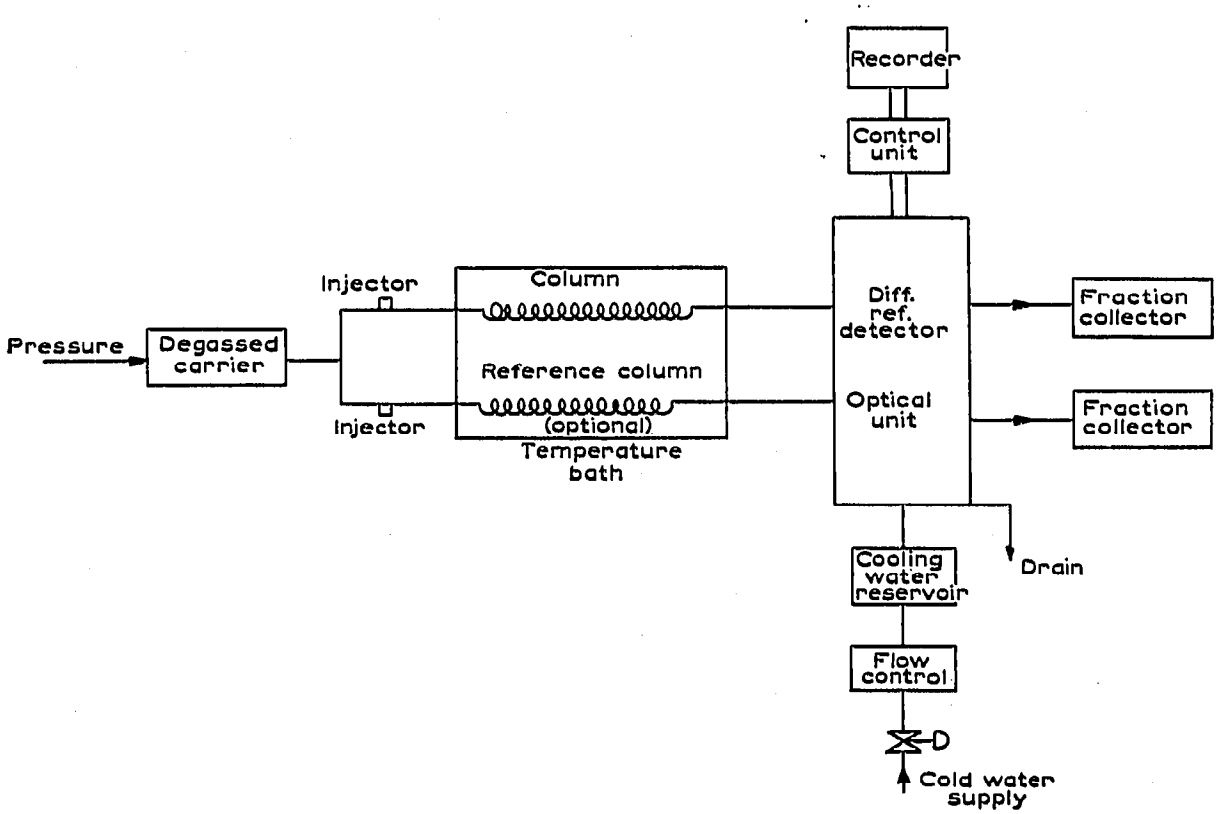

Fig. 2. Block diagram of liquid chromatography system.

support than the stationary phase. Immiscibility between the stationary and moving phases is desirable but not essential. Immiscibility implies a difference in polarity between the two phases. Materials ranging in polarity from water to isooctane may be used. Carrier liquids used in this investigation were *n*-heptane, ligroin, iso-octane, methanol, ethanol, and water.

Carrier motion

Moving the carrier is a problem of choice in liquid–liquid chromatography. Many types of pumps and constant head devices have been reported. In this work, three methods of carrier motion were used:

1. Gravity plus a hydrostatic head using a dip leg.
2. A microbellows pump supplied by Research Appliances Co., Allison Park, Pa.
3. Helium head pressure from a cylinder, controlled by a 2-stage regulator.

Much of this work was done with the microbellows pump arranged into an assembly with an auxiliary bellows used to provide the analog of a resistance-capacitance filter to smooth flow[4]. The method was only partially successful because smoothing was not complete and an occasionally encountered high pressure drop column caused rupturing of bellows. The preferred operation was obtained with method 3.

Degassing the carrier liquid

Degassing carrier liquids prior to use was found to be imperative for smooth operation of pressurized systems. Fig. 3 shows the difference between chromatograms produced with air-saturated carrier solvent and degassed carrier solvent. In the investigation a carrier was degassed by refluxing and allowing it to cool in a closed vessel. Under gravity flow with low head pressure, degassing the carrier was not necessary. However, when pressure was applied to the carrier liquid, the pressure drop across the column permitted outgassing. The gas bubbles in the line produced spiking shown in Fig. 3.

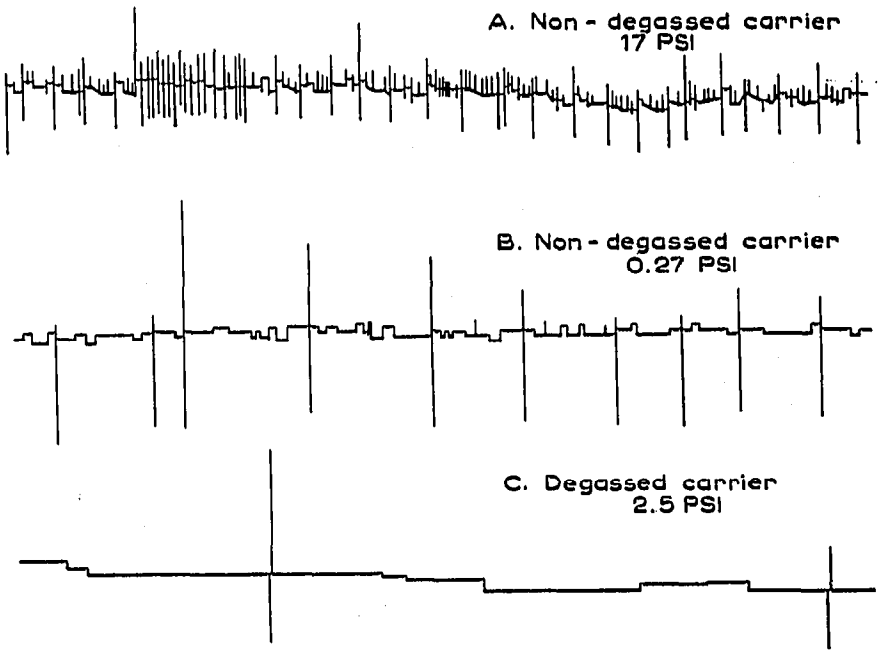

Fig. 3. Effect of gas in moving phase.

The stationary phase

Ideally the stationary phase should be completely insoluble in the carrier liquid. In practice, this is virtually unattainable. It has been the practice, therefore, to saturate the moving phase with the stationary phase to prevent depletion of the stationary phase during use. This technique has found limited application in gas chromatography also[5]. Although the saturated carrier solves the problem of stationary phase "permanence" it introduces a new problem in that the stationary phase contaminates the sample with materials that may be difficult to remove. The stationary phase usually must be similar in functionality to the sample component in order to provide the desired solubility and therefore desired retention of the sample components.

As a result the stationary phase may be as difficult to separate from the components of interest as the original contaminants in the sample. To avoid this problem, the stationary phase must be chosen with discretion. This in turn limits the choice of stationary phases available to a need. An approach to this problem is to use high polymers as stationary phases. A copolymer stationary phase for example can provide multiple functionality and be very sparsely soluble in a moving phase. Of necessity, the solubility of the sample components in the copolymer substrate will be very small and will require the use of very small sample loads monitored by a high sensitivity detector. We tried this approach with moderate success with a separation of nonane and tetradecane on a column of ethylene–methyl acrylate copolymer using methanol as a carrier. Many problems remain to be solved, but the results are sufficiently promising to warrant continued investigation.

Columns

A number of column systems were investigated. These are shown in Table II. The ethylene glycol–heptane system is a well known system. It was used in this work to evaluate the equipment and to develop a working knowledge of the technique. The polyglycols, copolymer and Sephadex systems were extensions into the realm of high polymers.

TABLE II

COLUMN SYSTEMS USED IN THIS INVESTIGATION

No.	*Moving phase*	*Stationary phase*	*Solid support*	*Length in meters*
1	*n*-Heptane*	Ethylene glycol	Chromosorb P	1.3
2	*n*-Heptane*	PEG 400	Celite	1.3
3	*n*-Heptane*	PEG 400	Celite	5.0
4	Methanol	Polyethylene	Chromosorb P	4.0
5	Methanol	Ethylene–methyl acrylate copolymer	Chromosorb P	5.0
6	*n*-Heptane	Ethylene–methyl acrylate copolymer	Chromosorb P	5.0
7	0.5% and 0.2% butanol in *n*-heptane	Butanol	Silica Gel	5.0
8	*n*-Heptane*	Ethylene glycol	Chromosorb	1.7
9	Ligroin*	Ethylene glycol	Chromosorb	1.7
10	Ligroin*	Carbitol	Chromosorb	1.5
11	*n*-Heptane*	Water	Chromosorb	5.0
12	*n*-Heptane*	Water	Silica Gel	4.5
13	0.4% and 10% butanol in *n*-heptane*	Water	Silica Gel	4.5

* These materials were saturated with stationary phase for use.

Sephadex G-25–water–ethanol

A relatively ideal column for use with polar solvents was prepared from the polydextran Sephadex G-25 (Pharmacia, Uppsala, Sweden[6]). Normally, this material is used in a fully swelled state for gel filtration by molecular exclusion. Its use is usually limited to large molecules with molecular weights above 2000. Its range was extended to smaller molecules by using it in a partially swelled state. Fig. 4 shows a separation

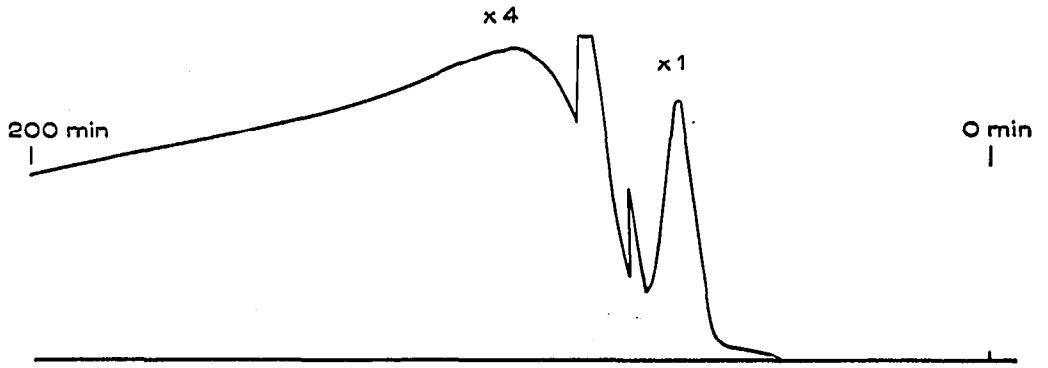

Fig. 4. Chromatogram showing separation of methyl naphthalene sodium sulfonate from ammonium nitrate. Column: 120 cm × 1.3 cm, Sephadex G-25. Carrier: 95% ethanol–5% water. Flow rate: 1.22 ml/min.

of methylnaphthalene sodium sulfonate from ammonium nitrate on a 120 cm × 13 cm O.D. column of partially swelled Sephadex using water as the stationary phase and ethanol–water (95:5) as the moving phase.

This separation was made in 2 h with an efficiency of 312 plates[7]. More complete

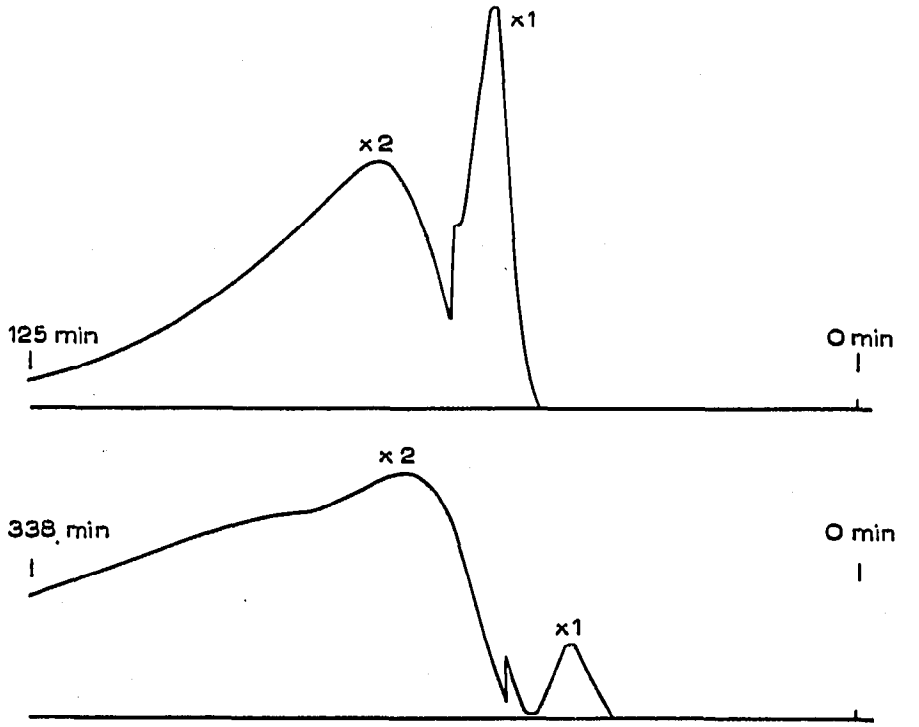

Fig. 5. Effect of flow rate on separation. Column and carrier same as Fig. 4. Flow rate: top 1.7 ml/min; bottom, 0.5 ml/min.

resolution was obtained at a slower flow rate as shown in Fig. 5, but analysis time was much longer. Carrier flow rate was maintained by a head pressure of helium using a 2-stage regulator. The top chromatogram in Fig. 5 was prepared at a head pressure of 5 p.s.i. which produced a flow rate of 1.7 ml/min. The bottom was prepared at 0.27 p.s.i. with a flow rate of 0.5 ml/min. Separation times were 125 and 338 min, resp.

Chromosorb–water

Columns containing water on red chromosorb were shown to be useful in liquid–liquid chromatography in the separation of surfactants. Many of these high molecular weight polyfunctional molecules are not sufficiently volatile to permit their separation by gas chromatography. They are not readily separated by chemical methods or by simple extraction techniques. They may be separated by liquid–liquid chromatography using water as the stationary phase and *n*-heptane as the moving phase. Table III shows the relative retention of a number of surfactants with a number of different chemical compositions. They include anionic, nonionic and cationic materials.

TABLE III

RETENTION OF SURFACTANTS (RELATIVE TO BUTANOL) ON COLUMN II

Surfactant	*No. of ethylene oxide units*	*HLB index*	*Relative retention*
Triton X-15	1	—	0.00
Span 85	—	1.8	0.03
Span 80	—	4.3	0.05
Ethofat 60/15	5	—	0.35
Span 60	—	4.7	0.41
Triton X-35	3	—	0.46
Triton X-45	5	—	0.46
Duponol C	—	—	0.67
Span 40	20	6.7	0.93
Ethofat 60/25	15	—	1.04
Tween 65	20	10.5	2.37
Myrj 45	—	11.1	2.37
Siponate DS-11	—	—	2.37
Morpholinium Oleate	—	—	2.37
Triton X-102	12	—	2.76
Tween 20	—	16.7	> 3.00

They are separated by their solubilities in heptane relative to water. Elution is in order of their HLB (hydrophylic–lipophylic balance) index[8]. More hydrophylic materials which exhibit larger HLB indices are retained longer than the lipophylic materials. Within any class of polyoxyethylene type material, retention is a direct function of the number of ethylene oxide units.

With a reversed phase system, elution order should be reversed.

Celite–PEG 400

Alcohols and acids are eluted from a PEG column in order of increasing ionization constant.

Although this separation was poor by gas chromatography standards, the information is included to illustrate the reverse order separation. Carboxylic acids and their salts may be eluted in order of carbon number or ionization constant in liquid–

liquid chromatography by choosing the proper moving and stationary phases. In gas chromatography elution order is predominantly a function of boiling point or carbon number in any series.

Gradient elution in liquid–liquid chromatography

Gradient elution is generally used in liquid absorption chromatography where progressively more polar solvents are used to elute solutes from the column in order of increasing polarity. Gradient elution may be used to great advantage in liquid–liquid chromatography, but it has no parallel in conventional gas–liquid chromatography.

A separation of butyne-1,4-diol from 4-hydroxy-2-butynyl N-(3-chlorophenyl)-carbamate was required. Using dichloroethane as the moving phase and water as the stationary phase, the diol was permanently retained on the column. It could be eluted from the column readily with water. By a two-solvent gradient elution technique the separation was complete on a very short column.

The separation could have been made with a single solvent system. For example, by using an appropriate mixture of benzene, ethanol and water the familiar differential chromatogram could have been obtained. However, a longer column and greater attention to conditions would have been required to effect a complete separation.

SUMMARY

A liquid–liquid chromatography system patterned after the gas chromatography model was built and investigated. Liquid–liquid chromatography and gas–liquid chromatography were found to have many principles in common. Much of the gas chromatography instrument technology was applicable to liquid–liquid chromatography. The system built for this investigation afforded simplified operation and provided rapid separations which were not readily attainable by other methods. Although problems remain to be solved, the results warrant continued investigation. The subtle differences which separate the techniques were found to result from basic differences between gas–liquid equilibrium and liquid–liquid equilibrium. When properly treated, however, these differences may be used to advantage.

In the unitized system, water was shown to be a desirable stationary phase, particularly when working with surfactants. Both chromosorb and the polydextran Sephadex G-25 were found to be desirable solid supports. Multifunctional copolymers showed promise as stationary phases and warrant further investigation.

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