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A NEW GRADIENT ELUTION CHROMATOGRAPH

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SUMMARY

The differential refractometer, a highly sensitive and virtually universal detector for liquid chromatography, has been considered inoperable with gradient elution chromatography. However, with proper attention to solvent selection and instrument design, the versatile refractometer can be used with solvent programming. Therefore, a new general purpose gradient elution liquid chromatograph has been developed using dual columns with both a differential refractometer and an ultraviolet photometer as detectors.

INTRODUCTION

Elution chromatography was introduced by REICHSTEIN and coworkers[1-3], who referred to this technique as liquid or flowing chromatogram[4]. As described and still widely practiced, a glass column 1–3 cm in diameter was packed with wide particle distribution active adsorbent such as silica, alumina or charcoal. The sample was loaded on the column and washed through with a series of solvents of increasing affinity for the adsorbent. This series, termed the elutropic series[5,6], lists solvents in order of increasing polarity, which for silica and most other adsorbents begins with branched hydrocarbons such as isooctane, followed by benzene, ether, acetone, ethyl acetate, alcohol, water, and acetic acid. This technique, as practiced, was subject to several limitations as follows: Adsorbents of excessive activity were often used. Wide diameter columns and large particle diameter adsorbents were used, resulting in low column efficiencies. Further, each elution step was carried out to completion (*i.e.*, until no significant amount of solute was detected in the latter volume of the solvent when evaporated to near dryness) before the next solvent was used. By this procedure, which generally did not employ a detector, each fraction frequently contained an overlapping mixture of components also present in both the preceding and following fractions. Thus, the procedure often provided a method of enriching, but not truly separating the mixture.

More recently ALM *et al.* developed gradient elution chromatography to overcome the problem of peak tailing by gradually increasing the eluting power of the flowing solvent[7].

Considerable attention has been given in recent work to high efficiency liquid–solid column chromatography by SNYDER *et al.*[8,9], who properly treat the theoretical conditions of high efficiency separations; however, a major limitation to the general usage of high speed–high resolution LSCC is the availability of suitable instrumentation to generate the necessary gradients and to detect the solute bands eluted from high efficiency columns.

While the ultraviolet detectors are readily applicable to gradient elution chromatography, they are of limited use, since they are not universal. The differential refractometer is virtually universal, but has been considered incapable of functioning with gradient elution chromatography.

It is the purpose of this work to report on the successful application of the differential refractometer to gradient elution chromatography.

A dual-column unitized high efficiency system was developed using a nine-chamber solvent programmer with both a UV and differential refractometer detector. Fractionation of UV absorbing materials was accomplished with the UV photometer

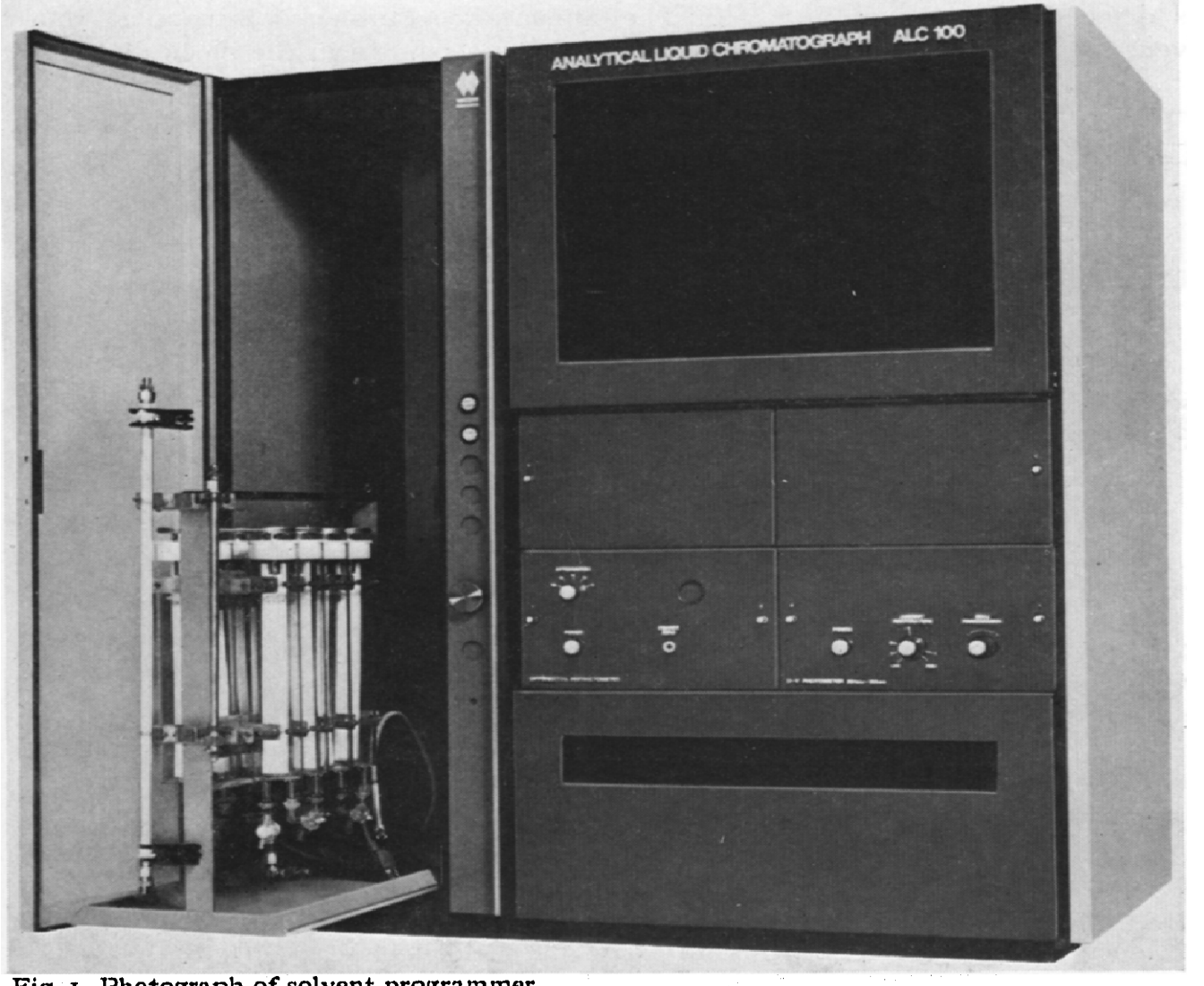

Fig. 1. Photograph of solvent programmer.

and the differential refractometer in series. With the non-UV absorbing materials, the refractometer was used alone.

EXPERIMENTAL

Solvent programmer

A nine-chamber variable gradient device with five chambers in use, pictured in Fig. 1, was employed to provide a solvent gradient. Solvent programs were calculated as described by PETERSON AND SOBER[10]. The programmer consists of up to nine solvent chambers, interconnected at the bottom and stirred by a common drive. The programmer was used both to elute the sample and to regenerate the columns after elution prior to re-use.

Chromatographic system

A Waters Associates ALC 100 (ref. 11) was used as the chromatographic system. The solvent programmer was installed in the column cabinet in place of the column rack, since the large number of columns commonly used in gel separations was deemed unnecessary in LSCC. The solvent programmer was coupled to the solvent pump in place of the regular solvent tank as shown in the block diagram in

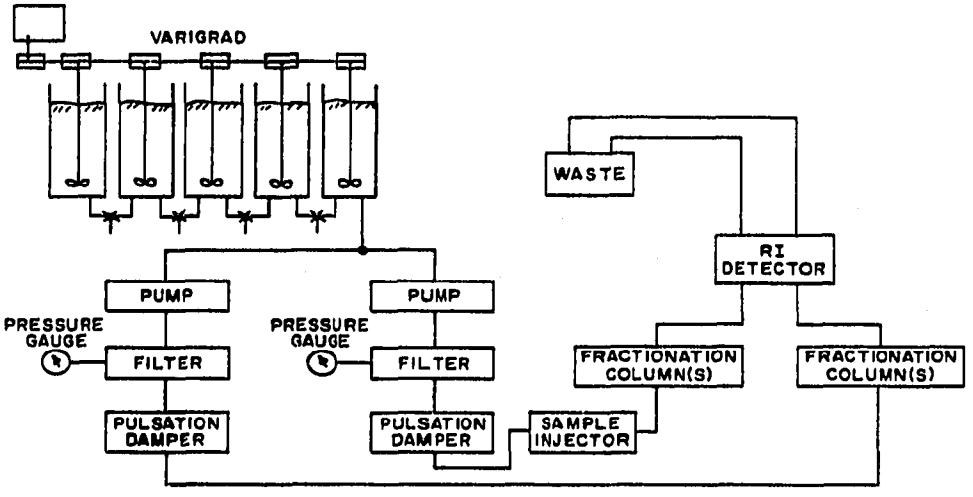

Fig. 2. Block diagram of dual column–split flow system.

Fig. 2. An additional pump and column were installed to provide dual column split flow to the refractometer detector. When the UV detector is used, single column operation is satisfactory.

A 1 ft. × 3/8 in. stainless steel column packed with 30–100 μ Porasil 60 was used for the separations. Isopropanol and *n*-hexane were used as polar–non-polar solvent pair.

Applications

Samples of polypropylene glycol (UCON 50HB55) and Triton X45 (ethylene oxide derivative of octylphenol) were fractionated using linear elution and solvent programmed elution chromatography. The differential refractometer was used with

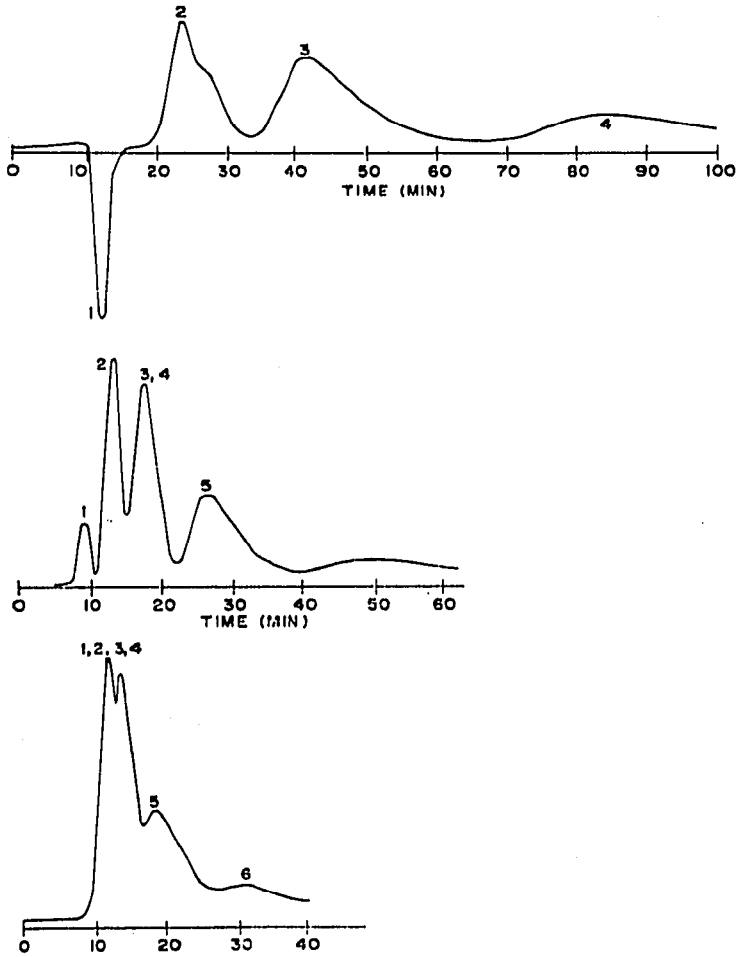

Fig. 3. Effect of solvent composition on elution of UCON 50HB55. Column, 1 ft. × $^3/_8$ in. Porasil 60; flow (column), 1.06 ml/min; flow (ref.), 1.01 ml/min. Solvent: top, 8% isopropanol in *n*-hexane; center ,30% isopropanol in *n*-hexane; Bottom, 70% isopropanol in *n*-hexane.

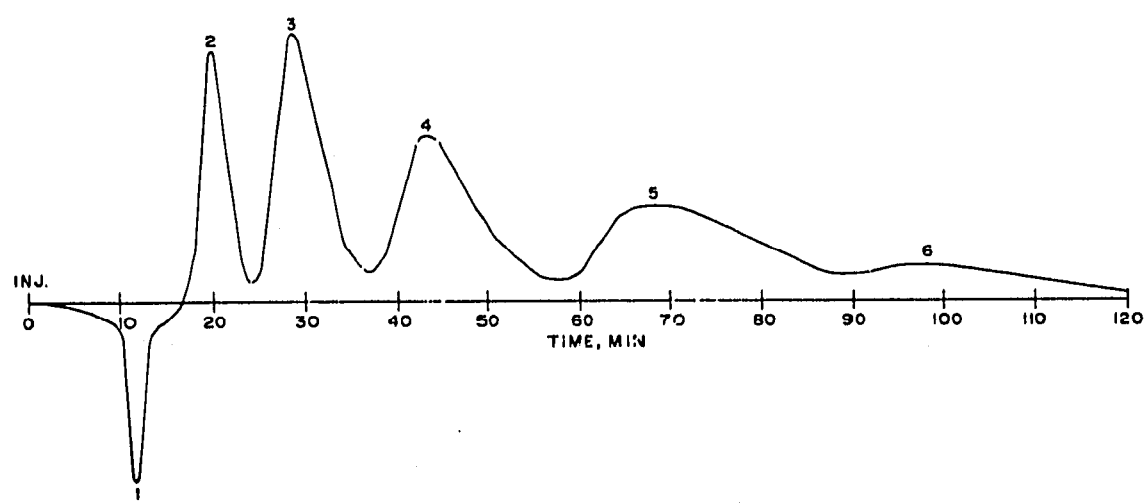

Fig. 4. Solvent programmed separation of UCON 50HB55, using a differential refractometer detector. Column: 1 ft. × $^3/_8$ in. Porasil 60. Solvent program: 8–30% isopropanol in *n*-hexane. flow rate of sample and ref., 1.05 ml/min.

TABLE I

REFRACTIVE INDICES OF POLAR–NON-POLAR SOLVENT PAIRS

Non-polar solvent	*RI*	*RI*	*Polar solvent*
n-Heptane	1.3855	1.3854	*n*-Propanol
n-Hexane	1.3754	1.3776	Isopropanol
n-Propyl ether	1.3807	1.3807	Methyl ethyl ketone

both materials. The UV detector was usable only with the Triton, since UCON is transparent to UV.

RESULTS AND DISCUSSION

Chromatograms of UCON 50HB55, fractionated by linear elution chromatography at each of three solvent compositions, are shown in Fig. 3. It is evident that the non-polar solvent affords the best resolution to the early peaks, but requires excessive time to elute the more polar peaks. Solvent conditions which elute the more polar compounds in reasonable time fail to resolve the early peaks. The chromatograms in Fig. 4 show complete resolution of the six peaks by a solvent program of 8% isopropanol in *n*-hexane to 30% isopropanol using a differential refractometer as a detector.

The differential refractometer had been considered unsuitable for use with solvent programming; however, by choosing two solvents which differ widely in polarity but which are similar in refractive index (RI), solvent programming with a refractive index detector is practicable. Several solvent pairs are shown in Table I.

Balancing the flow while solvent programming with a dual column single pump

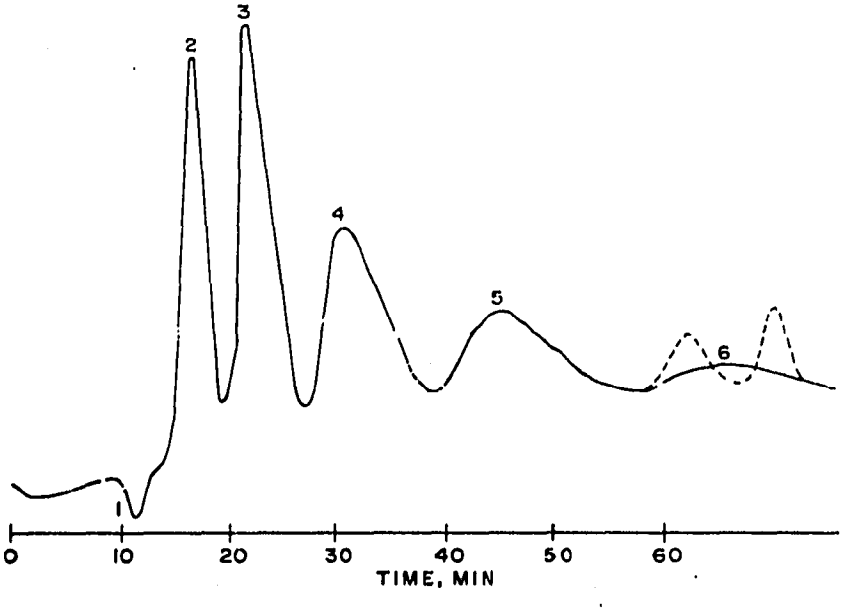

Fig. 5. UCON fractionation with increased gradient, using a differential refractometer detector. Conditions: same as Fig. 4 except solvent program: 10–70% isopropanol in *n*-hexane.

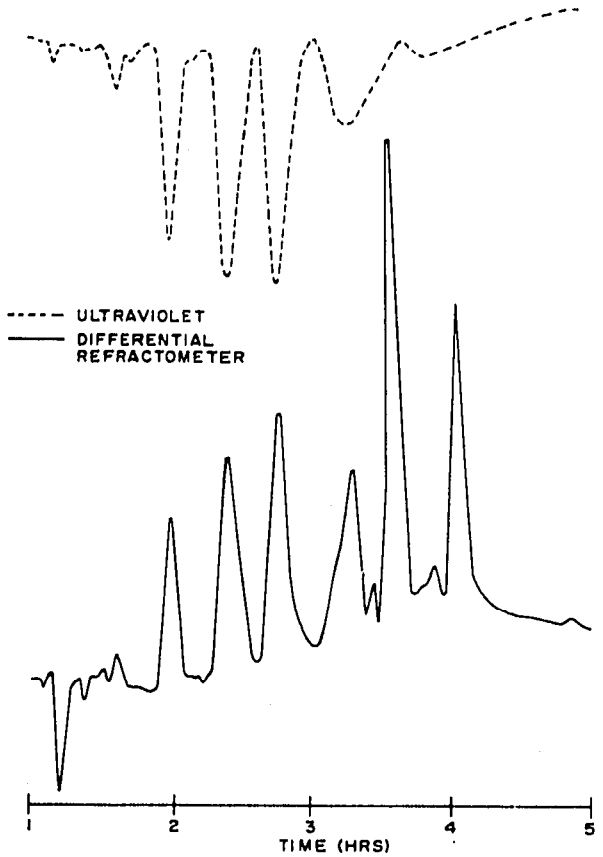

Fig. 6. Fractionation of Triton X45 ,using a differential refractometer and UV photometer in series. Conditions: same as Fig. 5 except step gradient at peak 6.

split flow system was difficult, particularly when refractive index differences were increased. Therefore, a dual pump system was employed. The chromatogram shown in Fig. 5 was produced with 10–70% isopropanol in *n*-hexane, using the dual pump system. The increased program rate produced sharper peaks and effected the separation in 60 min rather than 100 min with the 8–30% isopropanol program.

The Triton X45 chromatograms shown in Fig. 6 were prepared using the differential refractometer and UV detector in series. A 5–80% isopropanol program was used until peak 6 was eluted, after which pure isopropanol was step programmed through the system. The pure isopropanol produced no upset in the UV detector, but produced two large spikes on the RI chromatogram. From this it is evident that the RI detector may be used with programmed solvent chromatography provided that parallel columns are used, solvent flow is properly matched, and composition changes are not abrupt. Materials which differ in polarity as much as isopropanol and *n*-hexane, and yet differ in refractive index by only 0.002, provide ideal systems for program solvent analytical liquid chromatography.

Column regeneration

It has been common practice in gradient elution chromatography to start with a freshly packed column containing active adsorbent and to discard the column after a single use. The procedure, though acceptable with inexpensive adsorbents, becomes

unreasonable when costly, narrow mesh, spherical packings are used to pack high efficiency columns. Regeneration and re-use of such columns is desirable. It has been shown in this work that Porasil columns can be regenerated by reverse programming, simply by flushing the columns with 1.5 volumes of pure *n*-hexane after each use. In cases where the solvent program involves immiscible materials (*n*-hexane–isopropanol–water) it is necessary to regenerate with isopropanol to remove water and *n*-hexane to remove isopropanol.

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