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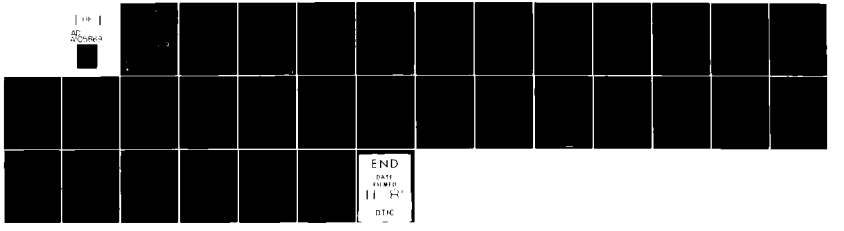

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Multiple Solvent Extraction System

With

Flow Injection Technology

by

Dennis C. Shelly, Thomas M. Rossi and Isiah M. Warner

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Department of Chemistry
Texas A&M University
College Station, Texas 77843

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Flow Injection Analysis (FIA) technology is used to automate a three stage extraction procedure permitting the rapid isolation of polynuclear aromatic hydrocarbons from a complex sample matrix. High performance liquid chromatographic and video fluorometric analyses of the automated and manually prepared extracts indicate nearly identical chromatographic and spectral profiles of the two extracts.

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Multiple Solvent Extraction System

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Brief

Flow Injection Analysis (FIA) technology is used to automate a three stage extraction procedure permitting the rapid isolation of polynuclear aromatic hydrocarbons from a complex sample matrix. High performance liquid chromatographic and video fluorometric analyses of the automated and manually prepared extracts indicate nearly identical chromatographic and spectral profiles of the two extracts.

Abstract

A three stage extraction procedure for the isolation of polycyclic aromatic compounds from complicated sample matrices has been automated using flow injection technology. Three single-step liquid-liquid extractions are linked together by multichannel pumping and resampling. In addition to the multiple extraction capability, the system demonstrates two other novel features. First, both Teflon and glass extraction coils are used to minimize sample carryover and memory effects. Second, microprocessor-controlled pneumatically-actuated valves control sample injection and effluent concentration. The performance of the system is evaluated by high performance liquid chromatography and video fluorometric analyses of both automated and manually performed extractions of a crude oil-ash residue sample. The three extraction system is rapid, reproducible and quantitative as compared to an identical manual procedure.

Introduction

There is an increasing need for specialized instrumentation for automated sample preparation. Three of the more important reasons for this realization are the inherent speed, precision and interfacing capabilities of automated procedures. For routine analyses, one frequently encounters (a) large sample loads, (b) introduction of bias or error associated with performing a given technique and (c) acquisition of a miriad of miscellaneous laboratory equipment. The virtues of automated sample preparation and automated analyses, in general, are discussed in a monograph by Foreman and Stockwell (1).

To date, the emphasis on laboratory automation has focused on the development of more efficient analysis systems. An example is the video fluorometer, which greatly speeds analysis time by implementation of a novel optical system, multichannel detection device and computerized data treatment (2,3). A result of these innovations is that sample preparation, particularly for a very complex matrix, is often the time-limiting step for the determination of one or more fluorophores. A very effective use of automated sample preparation would be either direct or indirect coupling to a sophisticated instrument such as the video fluorometer. In this way both routine and research oriented applications of multicomponent fluorescence determinations would be greatly expanded.

Both segmented flow analysis (SFA), also called continuous flow analysis (CFA), and flow injection analysis (FIA) have been employed for the automation of many types of chemistries. Several recent reviews enumerate the many applications for which SFA (4,5,6) and

FIA (7,8,9) methodologies were utilized. Both techniques, SFA and FIA, have been adopted in the automation of simple liquid-liquid extractions. Since the initial work of Karlberg (10), a great many applications of FIA automated solvent extraction have appeared in the literature (11-16).

The rapid development of automated solvent extraction is perhaps due to the frequency and importance of liquid-liquid extraction as a sample preparation technique. Several extraction procedures have been proposed for environmental analyses. Two schemes can be cited for the isolation of polynuclear aromatic compounds (PNAs) in fly ash extracts (17,18). Additionally, a method was reported for the extraction and determination of individual organic compounds, including polynuclear aromatics (PNAs), from shale oil (19). These procedures are characterized by incorporation of multiple extractions which, until now, have not been successfully automated by FIA methods.

When utilizing such rapid techniques as video fluorometry (VF), high performance liquid chromatography (HPLC) and a combination of the two (HPLC-VF) for the determination of carcinogenic species in shale oil, it became evident to us that automated sample preparation would be beneficial for routine and research oriented investigations. This need in addition to our previous experience with the dimethyl-sulfoxide (DMSO)/pentane extraction of Natusch and Tomkins (18) lead us to automate this relatively useful multiple extraction procedure.

Theory

Principles of FIA Extraction

A fundamental principle, upon which FIA and SFA extractions are based, is selective wetting of component surfaces by both the organic and aqueous phases. In general, organic solvents wet Teflon surfaces preferentially to glass. Simiarly, aqueous solvents prefer glass to Teflon. The instrumentation and principles described by Karlberg (10) rely on this phenomenon for successful performance of solvent extraction. The three components necessary for both FIA and SFA based extractions are (a) a solvent segmenter, a device that produces alternating segments of two immiscible liquids; (b) an extraction coil, a length of small diameter tubing which carries the segmented solvents and promotes transfer of the extractant from one phase to the other; and (c) a phase separator, an apparatus which allows the phases to separate in such a manner that one or both phases may be recovered. Figure 1 diagramatically shows these three components, for which the designs of the solvent segmenter (Figure 1A) and phase separator (Figure 1C) are taken directly from the literature (10).

Since the operations of the solvent segmenter and phase separator are documented elsewhere and understood more completely than the actual mechanics of the extraction, it might be instructive to attempt a description of the extraction process. Figure 1B shows the two possibilities that can exist when employing either Teflon or glass coils for extraction. In both cases one solvent segment is excluded from the tubing surface while the other solvent freely wets the tubing inner wall. The excluded segment thus forms a plug

or bolus which is completely surrounded by the other solvent. Since both phases are being pumped continuously and the inter-plug distance remains relatively constant throughout the extraction coil, a question arises regarding the mechanism of the extraction process. In a static system, i.e. no phase mixing, the solute will migrate across the interface and at equilibrium

$$D_s = \frac{C_{org}}{C_{aq}}$$

where D_s is the distribution ratio for solute s, C_{org} is the equilibrium concentration of solute in the organic phase and C_{aq} is the equilibrium concentration of solute in the aqueous phase. The driving force for the system to reach equilibrium is the initial ratio of C_{org}/C_{aq} and C_{org}/C_{aq} at equilibrium or simply D_s. Obviously equilibrium will be achieved after a very long time in a static system where molecular diffusion is the primary transport process for bringing solute molecules from within the plug to the interfacial region. Alternatively, in a dynamic system, such as a two-phase flow in a coiled tube, secondary flow patterns can form, possibly similar to those described by Tijssen (20), which result in a much more vigorous transport of intra-plug solute molecules to the interface. This may also occur in the surrounding solvent resulting in a net flow of solute out of the plug and into the sheath solvent as the immediate environment of the interface is constantly changing. Similar and probably more vigorous conditions exist in SFA-based extraction manifolds due to air segmentation in addition to two-phase flow.

Most extraction coils in FIA manifolds are small diameter Teflon and most of the automated extractions are characterized by D_s much

greater than one. In situations of this type the solvent which wets the tubing contains a sufficiently higher concentration of extracted solute such that memory effects and sample carryover could be a problem because relatively high concentrations of solute would be present at the tubing inner surface. The obvious solutions to this problem is the glass coil since the organic phase is excluded in favor of the aqueous solvent. In multiple extraction schemes, one frequently encounters a back extraction step where the direction of the extraction is from organic to aqueous solvent. Thus it is advantageous to incorporate both Teflon and glass coils in the multiple extraction manifold.

DMSO/Pentane Extraction

The isolation of PNAs using the DMSO/pentane extraction is accomplished by performing three separate extractions. A flow diagram of the procedure is shown in Figure 2. The pentane or cyclohexane sample is first partitioned with DMSO to remove PNAs and other polar compounds. An amount of water is added to the DMSO which renders most of the polar, unionized species (including PNAs) somewhat insoluble in the very polar DMSO/water matrix. The second step is a back extraction with pentane to remove these relatively insoluble compounds. Finally, the pentane is washed with water to eliminate residual DMSO and other hydrophilic material. The remaining pentane fraction is reported to contain phthalates, aromatic bases and high molecular weight aliphatic acids in addition to PNAs when this procedure is performed on a soxhlet extract of fly ash particulate (18). Thus, the sample matrix complexity is markedly

reduced while ensuring analytically useful recoveries of the desired solutes.

Experimental

Extraction Apparatus

Figure 3 shows a schematic of the manifold, built to automate the preparation procedure outlined in Figure 2. Each extraction Section consists of three basic components (1) a solvent segmenter, (2) an extraction coil and (3) a phase separator. The specific designs of the solvent segmenters and phase separators are described in the literature (10). Some unique aspects of our apparatus are the use of both glass and Teflon extraction coils, incorporation of a cooling coil in extraction Section 2, microprocessor controlled valves, and a unique sample concentrator.

A Gilson eight channel peristaltic pump (Gilson Medical Electronics, Middleton, WI) was used for solvent delivery with different sizes and composition of pump tubing as indicated in Table I. Silicone and solvent flexible tubing (Fisher Scientific, Fairlawn, NJ) and black viton tubing (Gilson Medical Elec., Middleton, WI) were employed in the peristaltic pump.

The extraction coils for Sections 1 and 3 were glass lined stainless steel (Alltech Associates, Arlington Heights, IL) and prepared from a single section of 180 cm in length. The Section 2 mixing and extraction coils are 0.5 mm i.d. Teflon (Altex Scientific, Berkeley, CA), the former was wrapped around a small cold finger condenser and the latter around a 3 cm o.d. vial. Tap water was used for circulation through the condenser.

All fittings were .25 inch o.d. X 20 threads per inch polypropylene (Altex Scientific, Berkeley, CA) and 0.8 mm i.d. Teflon tubing (Altex Scientific, Berkeley, CA) was used throughout the mainfold.

The solvent segmenters and phase separators were modified A8 and A4 "T" connectors (Technicon Instruments, Tarrytown, NY), respectively.

The valves, pneumatic actuators and pneumatic interface were supplied by Altex Scientific, Berkeley, CA. An external air supply, maintained at 90 psi, was required for operation of the valves.

The sample concentrator consisted of a 30 mm X 2 mm i.d. glass tube packed with silica particles, obtained from a SEP-PAK cartridge (Waters Associates, Milford, MA).

All waste lines were joined together to form a common pathway to a single container.

Instrumentation

A Farrand Model 801 Spectrofluorometer (Farrand Optical, Valhalla, NY), fitted with a 10 µL flow cell, was used to monitor extraction and dispersion characteristics of fluorescent solutes.

All liquid chromatography was performed on an Altex Model 312 MP High Performance Liquid Chromatograph (Altex Scientific, Berkeley, CA) using a 25 cm Ultrasphere C_{18} column (Beckman Instruments, Irvine, CA). The HPLC microprocessor also controlled the valves with the pneumatic interface.

The video fluorometer has been previously described (3) except that a Model 1213 Cooled Detector Housing (Princeton Applied Research,

Princeton, NJ) has been added to the detector as well as a HR-320S, Model 1227, Spectrograph (Instruments SA, Metuchen, NJ). In addition, a HP9872A Digital Graphics Plotter (Hewlett-Packard, Ft. Collins, CO) was used for plotting of the fluorescence data.

Solvents and Reagents

"HPLC" grade pentane (J.T. Baker, Phillipsburg, NJ) was used after pumping through a silica cartridge (Waters Assoc., Milford, MA). Spectrophotometric grade DMSO (Aldrich, Milwaukee, WI) was used without purification as was perylene (Sigma, St. Louis, MO). Glass-distilled cyclohexane and acetonitrile (Burdick and Jackson Labs, Muskegon, MI) and Certified ACS grade benzene (Fisher, Fairlawn, NJ) was also used without purification. Type III reagent grade water was obtained by treatment using a Milli-Q water purification system (Millipore, Bedford, MA).

Sample Preparation Procedure

Approximately 5 g of burned oil residue was soxhlet extracted with benzene for 24 hours. The extract was concentrated to 0.035 gL^{-1} by evaporation to dryness and redisolving in glass distilled cyclohexane. To remove particulates the extract was filtered through a 0.5 μm fluorocarbon membrane filter, and the final filtered extract was partitioned into 500 μL aliquots for treatment by both the manual and automated DMSO/pentane extractions.

Results and Discussion

System Design and Construction

The design of the FIA system was accomplished by considering each of the three extractions separately and sequentially. By

resampling (repumping) the proper extraction phase, the three steps were linked together with reagents and solvents added in confluence at the appropriate points. The final configuration of the system is shown in Figure 3. The sample is injected into a pentane stream. The DMSO and pentane solvents are segmented, extracted in a glass coil and separated in Section 1. The heavier phase (DMSO) is resampled through pump channel 3 and mixed with water. The DMSO/water mixture is segmented with pentane, extracted in a Teflon coil and separated in extraction Section 2. Pentane, the lighter phase, is resampled in pump channel 6 and sent to Section 3. Water is added at this point and by segmentation, extraction in a glass coil and separation, the third extraction is completed. The pentane layer (lighter phase) is resampled in channel eight and pumped through silica adsorbent. After the entire sample has adsorbed to the silica an aliquot of acetonitrile is injected, by syringe, onto the column thus eluting the adsorbed sample constituents. In this way the final output can be analyzed directly by reversed phase HPLC.

System Performance

Overall system performance was evaluated using two approaches. First, a 1.0×10^{-4} M pentane solution of perylene was extracted and the final output (without concentration on silica) was examined by the Farrand fluorometer. The results of this experiment are shown in Figure 4. Curve a shows the extractor output while curve b shows the theoretical output of the same sample without dispersion. If we were able to measure the theoretical output it would have the same area as curve a, since at each phase separator the proper phase was

resampled with nearly 100% recovery of the phases. In this way we get an idea of the dispersion characteristics, residence time and dilution effects of the system. A closer examination of curve a also shows the effect of sample size. Recall from Figure 3 that a 500 µL loop was used for sample injection. This sample size is very much larger than the actual segment size produced by the solvent segmenters. The conventional sample size for FIA methods is usually less than 50 µL or roughly comparable to segment size. This very large sample leads to the rounded broad peak in Figure 3 because most of the 500 µL is divided into segments of approximately equal solute concentration. Possibly by decreasing sample size, minimizing dead volume and increasing flow rate more efficient performance can be achieved. After such measures have been exercised the output profiles may more closely resemble those of single extraction manifolds.

We evaluated the performance of the system, on a chemical basis, by comparing the automated extraction output with an equivalent manually prepared sample. HPLC chromatograms of the extracts are shown in Figure 5. There is remarkable similarity between the two traces with the exception that fewer polar components are present in the FIA extract than in the manually prepared extract. The low extracted amounts of polar species is not due to non-elution from the silica concentrator since this device was not used for preparation of the FIA sample. A more likely explanation is that extraction conditions are less vigorous in the FIA procedure compared to the manual method and since these species probably have low extraction efficiencies between DMSO/water and pentane they are extracted to a lesser extent than in the manual technique. We also examined the

fluorescence properties of the two extracts by video fluorometry. The data for this study are shown in Figure 6. Again, there is good agreement between the procedures as evidenced by the similarity of the two spectra. The sample concentrator was used for this experiment.

The results of this work indicate the feasibility of automating multiple extraction procedures with FIA technology. This aspect was thought to be not possible due to the "limited" control of sample dispersion and extent of extraction using FIA principles. Our data show this to be a somewhat foredrawn conclusion since we have adequately demonstrated the capabilities of FIA solvent extraction for multiple extraction schemes.

A more thorough study of the performance and optimization of the basic design are currently under investigation.

Acknowledgement

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Credit

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Table I

Tubing Selection and Corresponding Flow Rates of Individual Channels of the Manifold Pumping System

Channel #	Solvent	Tubing Type and Internal Diameter (mm)	Flow Rate (mL/min)
1	pentane	solvent flexible, 1.4	1.13
2	DMSO	Viton, 0.76	0.47
3	DMSO	Viton, 0.76	0.47
4	water	silicone, 1.3	0.90
5	pentane	solvent flexible, 1.4	1.13
6	pentane	solvent flexible, 1.4	1.13
7	water	silicone, 1.3	0.90
8	pentane	solvent flexible, 1.4	1.13

Figure Captions

Figure 1. Basic Components of FIA Liquid-Liquid Extraction. A. Solvent Segmenter: (1) Platinum capillary; (2) Glass capillary; (3) Inner Teflon tube; (4) Outer Teflon sheath; (a) Organic solvent input; (b) Aqueous solvent input; (c) Segmented stream output. B. Extraction Coils: (5) Teflon extraction coil; (d) Aqueous plug; (e) Organic stream; (6) Glass extraction coil; (f) Organic plug; (g) Aqueous stream. C. Phase Separator: (7) Teflon insert; (8) Glass inlet branch; (h) Segmented stream inlet; (i) Organic stream outlet; (j) Aqueous stream outlet.

Figure 2. Flow Diagram of DMSO/Pentane Extraction Scheme.

Figure 3. Schematic Diagram of FIA Manifold for Automated DMSO/Pentane Extraction: (V1) Sample injection valve, 500 µL loop; (a) Solvent segmenter; (b) Glass extraction coil, 100 cm X 0.7 mm i.d.; (c) Phase separator; (d) Teflon cooling coil; 50 cm X 0.5 mm i.d.; (e) Teflon extraction coil, 75 cm X 0.5 mm i.d.; (f) Glass extraction coil, 80 cm X 0.7 mm i.d.; (g) Silica concentrating column; (h) Syringe to inject eluting solvent; (w) waste; (V2) Switching valve.

Figure 4. Comparison of Experimental and Theoretical System Response (a) Experimental response showing influence of dispersion, (b) Theoretical response due to the absence of dispersion.

Figure 5. Reversed-Phase HPLC Chromatograms of Manual and Automated Extractions. Gradient profile is shown at top. (——) Manual extraction and (----) Automated extraction. Flow rate is 1.0 mL min^{-1}.

Figure 6. *Rapid Scanning Fluorescence* Spectra of the Automated and Manually Performed Extraction Extracts. (a) Manual extraction and (b) Automated extraction.

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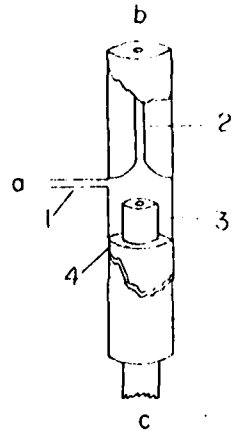

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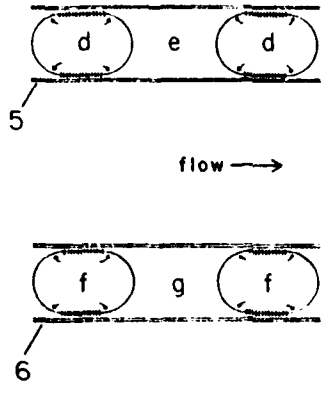

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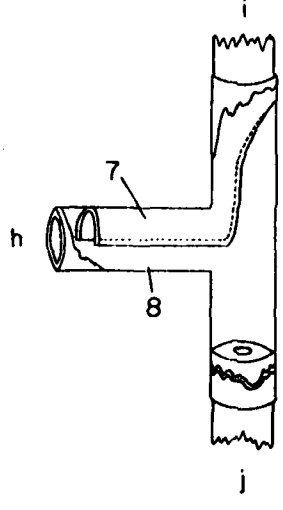

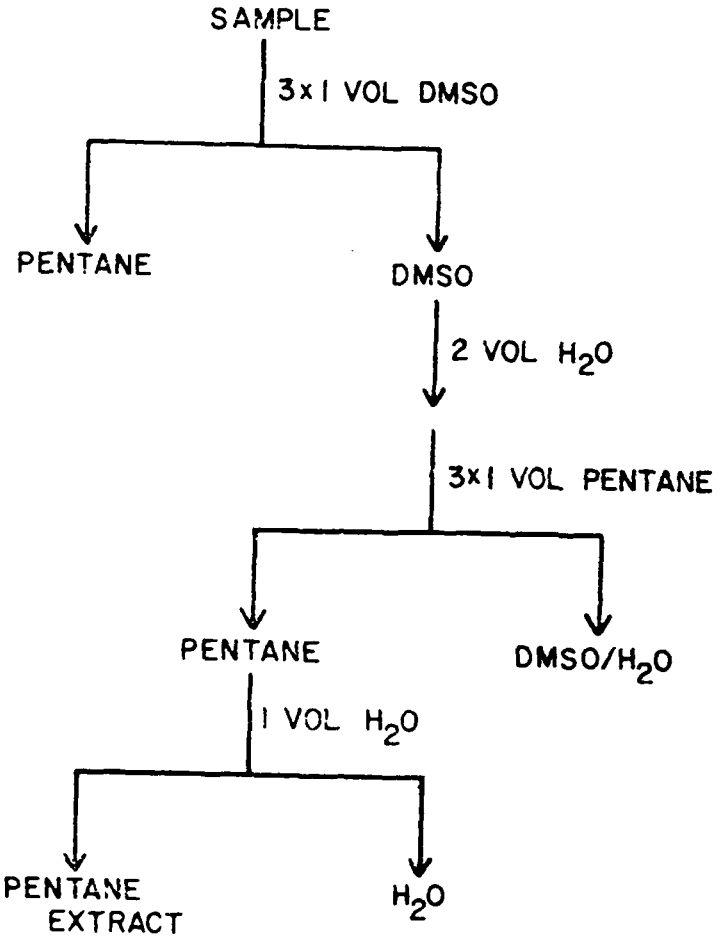
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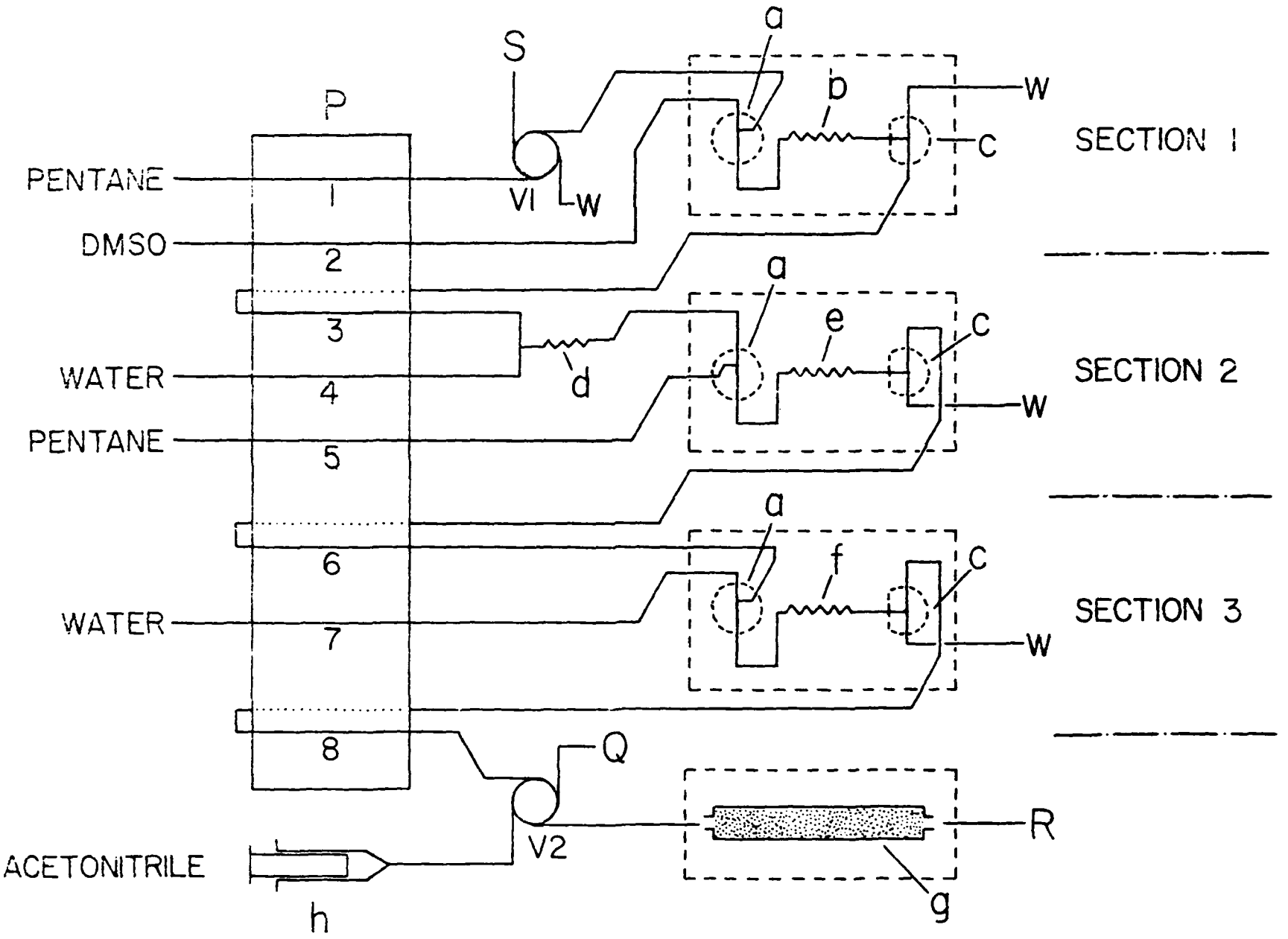
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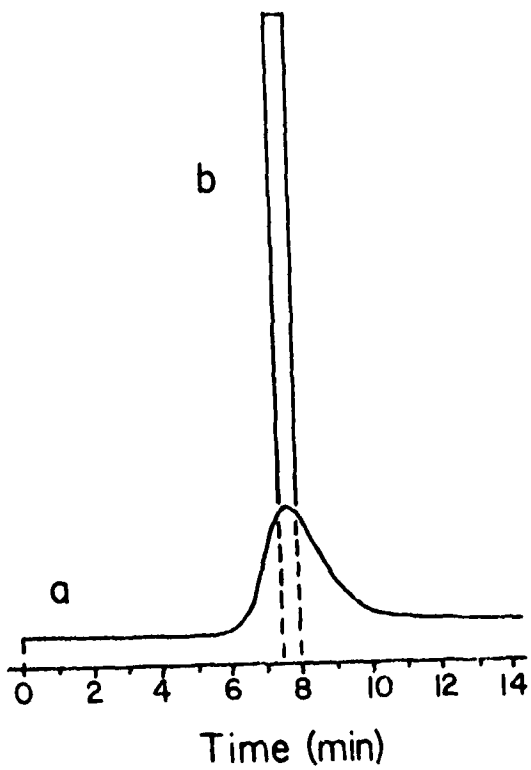
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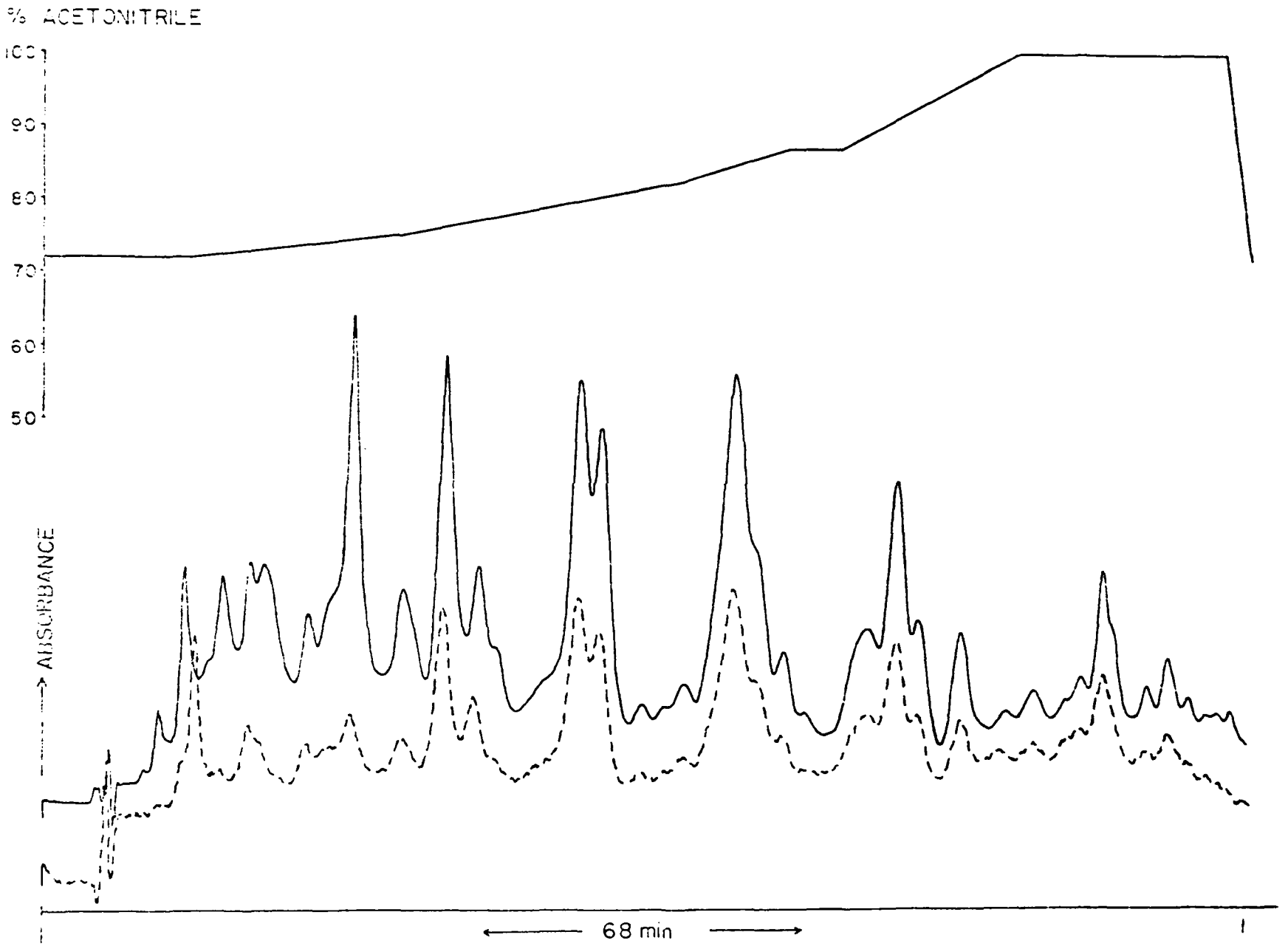
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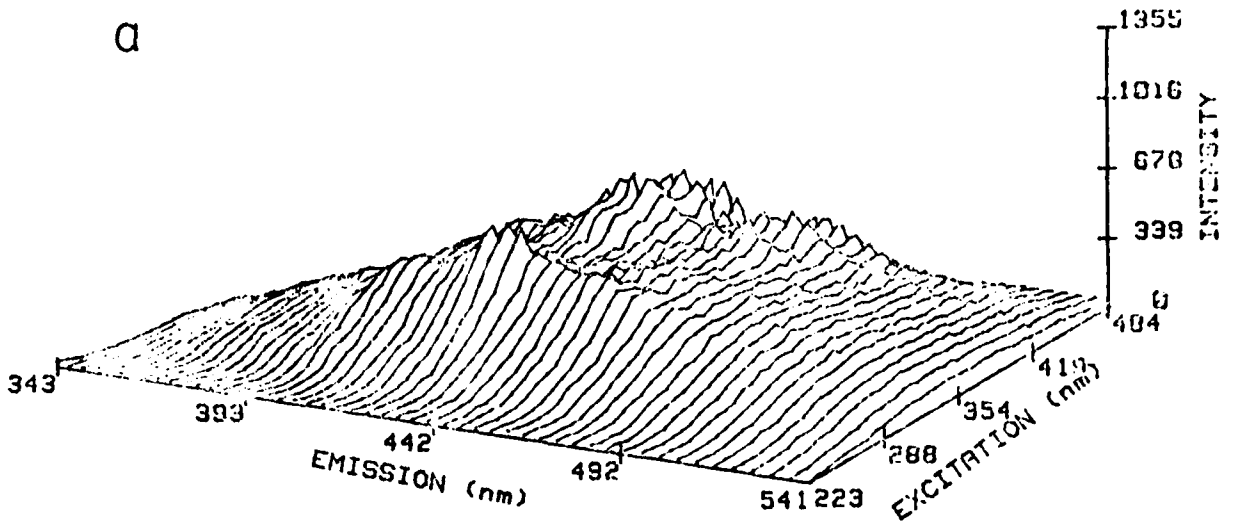
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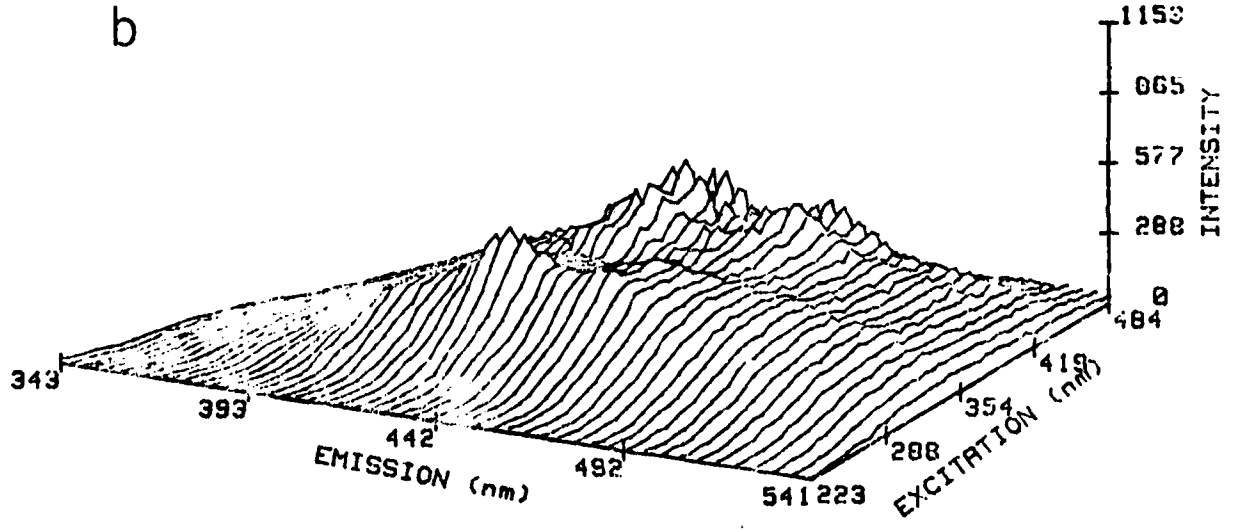
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