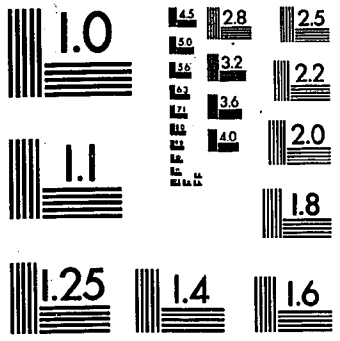

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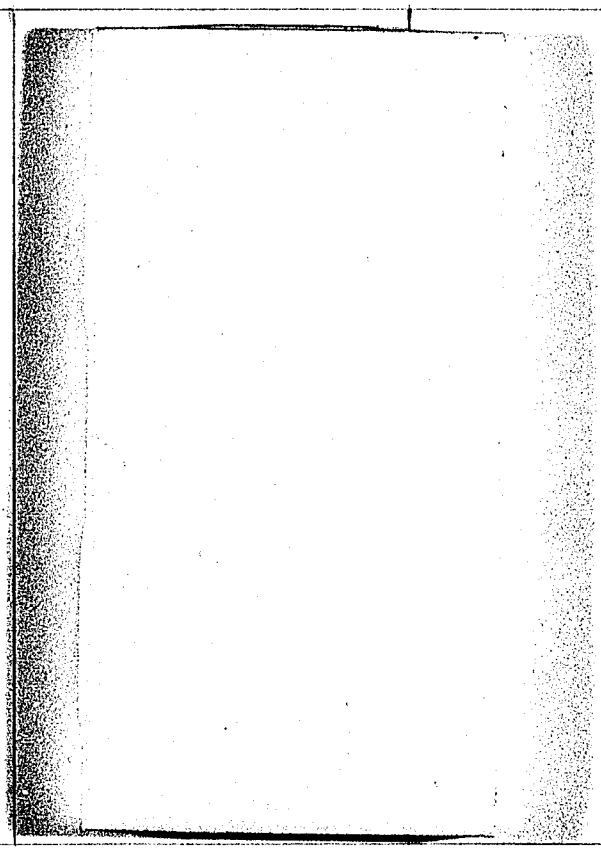

Chou, Chih-Kuang

PART I. HPLC DETERMINATIONS OF ORGANIC PEROXIDES. PART II. MULTI-MODE NORMAL BONDED-PHASE LIQUID CHROMATOGRAPHY OF POLYSTYRENES

City University of New York PH.D. 1985

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PART I. HPLC DETERMINATIONS OF ORGANIC PEROXIDES

PART II. MULTI-MODE NORMAL BONDED-PHASE LIQUID CHROMATOGRAPHY OF POLYSTYRENES

by

CHIH-KUANG CHOU

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1985

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David C. Lozke
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A. M.
Executive Officer

Ronald L Birke

Arthur G Baker
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The City University of New York

Abstract

PART I. HPLC DETERMINATIONS OF ORGANIC PEROXIDES

PART II. MULTI-MODE NORMAL BONDED-PHASE LIQUID CHROMATOGRAPHY OF POLYSTYRENES

by

Chih-Kuang Chou

Adviser: Professor David C. Locke

This dissertation is in two parts: (1) HPLC determinations of organic peroxides, (2) Multi-mode normal bonded-phase liquid chromatography of polystyrenes.

Part one is concerned with the determination of organic peroxides by using high performance liquid chromatography (HPLC) with an electrochemical detector. The electrochemically reducible organic peroxides are detected selectively by this detector in high sensitivity. Two types of organic peroxide samples are tested. The determination of hydroperoxides in air particulate samples is preliminary and needs further investigation; the determination of benzoyl peroxide in acne preparations is proved to be adquate and precise.

Part two demonstrates the study of possible interactions between normal bonded-phase PAC column packings and polystyrene molecules in a liquid chromatographic system with either a good or a poor solvent.

ACKNOWLEDGEMENTS

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CHAPTER I

I. INTRODUCTION:

Analysts today are called upon to determine organic peroxides in many and diverse analytical situations. For instance, commercial production of peroxides as industrial chemicals requires assays of purity. Process control needs rapid methods which can be used to determine not only pure peroxides but intermediates and peroxides at low concentrations as well, often in the presence of by-products which may interfere. Trace methods are needed in many situations, such as the detection of peroxidation in solvents, polymers (1), food fats and oils (2,3), or the monitoring of peroxide levels in industrial waste and air pollutants.

Instrumental techniques for peroxide determination include infrared (4,5) and near-infrared (6,7) absorption spectrophotometry, gas chromatography (8,9,10) and polarography (5,11-15). There are a number of colorimetric methods (16-22) for trace concentrations. Volumetric methods have been based on a variety of reducing reagents, including stannous chloride (23), arsenious oxide (24,25), titanous chloride (26) and ferrous ion (18).

More frequently used than any of these methods are the so-called iodine liberation methods (27-30), a family of volumetric procedures based on reduction of the

peroxide bond by iodide. Nearly all the peroxides of early analytical interest could be reduced in this way to liberate iodine quantitatively, or nearly so, even under conditions far from ideal. Thus these methods were popular for many years. However, the iodine liberation methods along with other volumetric and colorimetric methods can usually determine only the total content of peroxide; they can not differentiate among different peroxides. In other words, they are nonselective, and they are subject to interference by other oxidants.

With a few exceptions, the organic peroxides are thermally unstable and many are relatively nonvolatile, which properties lead to complications in gas chromatographic analyses. In principle, this problem can be overcome by using a complete PTFE-lined column at low temperature; the PTFE eliminates the decomposition of organic peroxides at high temperature catalysed by metal surfaces. However, because of its unreliability, this method is unsuitable for routine analysis.

The polarographic behavior of organic peroxides in aqueous and nonaqueous solvents has been investigated intensively. Most of the organic peroxides are electrochemically reducible at the dropping mercury electrode. One of the advantages of the polarographic technique is that different groups of organic peroxides have markedly different half-wave potentials, so that in

many cases a polarographic method can be devised which will distinguish between classes of peroxides. However, the half-wave potentials of organic peroxides of the same class are close together, since the polarographic reduction of peroxides is generally irreversible, the waves are somewhat drawn out; so that individual members of a class of peroxides cannot be distinguished. Thus polarography is unsuitable for trace analysis of mixtures of organic peroxides.

Spectroscopic studies of organic peroxides have been carried out using ultraviolet (31,32), infrared, Raman (33) and mass spectroscopy (34). Most of this work has been concerned with structure determination and these methods are of limited value for the quantitative analysis of peroxides. In absorption spectrophotometry, the influence of the peroxy bond on the spectra of other functional groups present in the molecule is more significant than the absorption of radiation by the peroxy group itself. Among these techniques, only infrared methods are potentially useful for the determination of organic peroxides.

Chromatographic methods other than gas chromatography have also been used. Separations were performed by using column(35), paper(36,37) or thin-layer(38,39) chromatography. By carefully choosing stationary and mobile phases, different members of homologous series of

peroxides can be separated. Since the separation process is fast, and relatively inert materials used, highly reactive peroxides can also be determined.

Recently high performance liquid chromatography (HPLC) has been used for the qualitative and quantitative determination of selected, mostly aromatic, organic peroxides (40,41). These studies were done with UV detectors using either normal or reversed phase eluents. The high resolving power makes HPLC the most promising technique for organic peroxides determination. However, there is still a major problem in trace analysis of peroxides with HPLC, since the peroxy bond itself shows no absorption in either the visible or UV region. Thus an accurate and highly sensitive detection system for alkyl and other UV nonadsorbing peroxides is required.

In the last few years, electrochemical detection systems became popular for using with liquid chromatography. The main advantages of electrochemical detectors over the UV detector are selectivity and sensitivity. With electrochemical detectors, only those compounds which are electrochemically active in the LC mobile phase can be detected, and the selectivity can be enhanced further by varying the potential on working electrode. In favorable case, the detection limit can be as low as pico-moles per injection.

The electrochemical detector consists of at least a

pair of electrodes separated by a solution in a microcell. This solution is flowing, usually but certainly not necessarily, out of a liquid chromatographic column. The solution is part of an electric circuit through which current flows. There is, then, a requirement for charge carriers in the solution. This is why most applications are found in chromatography with polar eluents which may be made conductive by adding salts, acids or bases. Less polar eluents may be used but only if they can be rendered conductive. The general feature of electrochemical detectors is that the solution containing the analyte flows through the cell containing a working electrode. Cells are conveniently classified into two categories, those which electrolyze all of the analyte flowing through the cell and those which do not. The former are known as coulometric detectors (42) and the latter as amperometric detectors (43,44). Diagrams of several amperometric detectors may be seen in figure 1-1. Coulometric detectors are shown in figure 1-2.

In figure 1-1, there are three different types of amperometric detectors cells. The channel design microcell is the most popular one. The original publication was that of Kissinger et. al. (45). In this type of cell, the solution is forced through a rectangular thin layer channel, one wall of which contains the working electrode, the cell volume can be made less than 1 ul, and the

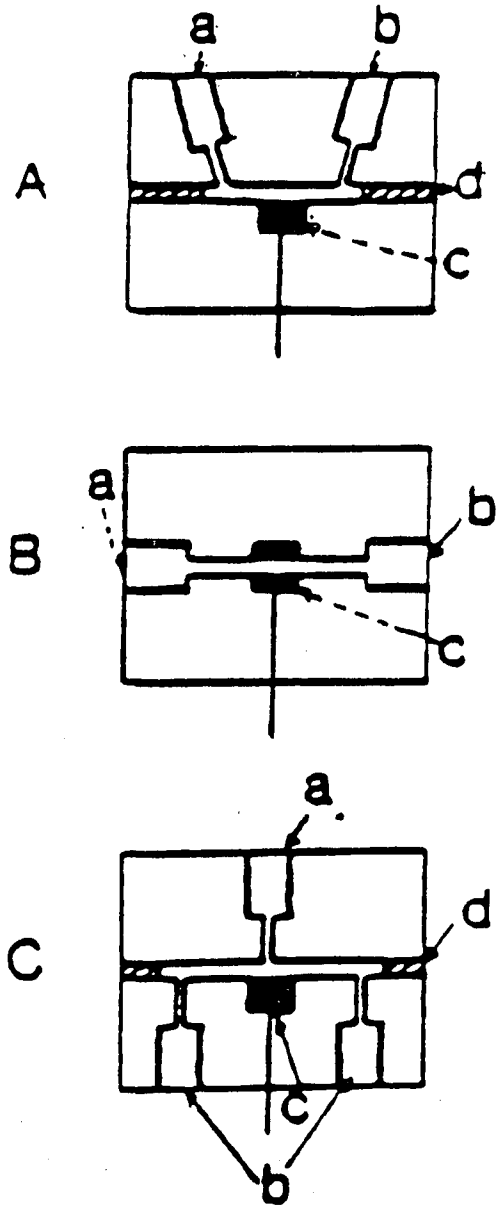

Figure 1-1 Amperometric detector schematics
A, channel; B, tubular; C, "wall-jet";
a, entrance; b, exit; c, working electrode;
d, spacer.

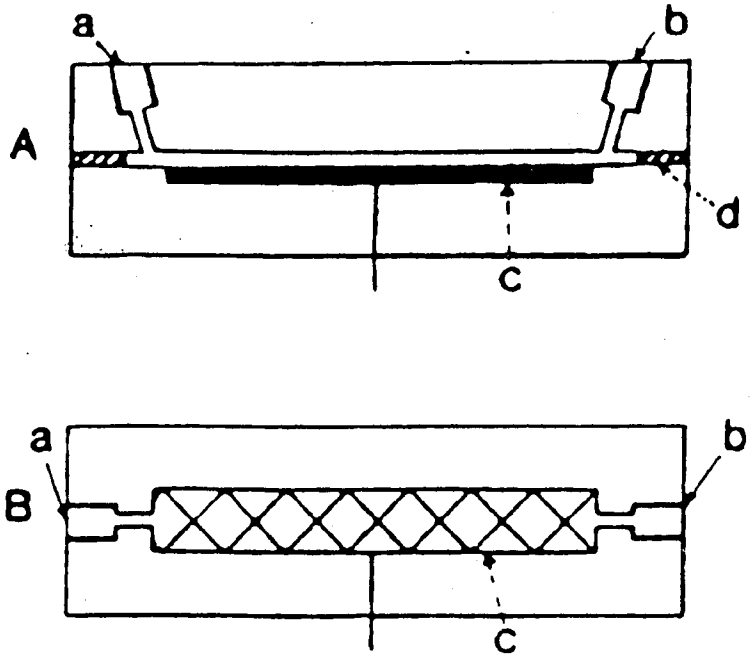

Figure 1-2 Coulometric detector schematics
A, open; B, reticulated; a, entrance; b, exit;
c, working electrode.

renewal of electrode surface by physical means is easy.

The tubular design microcell has been championed by Blaedel and co-workers for a number of years (46,47). The sensitivity of this cell is about the same as channel, but the renewal of electrode is more difficult, and for this practical reason, the tubular cell has not enjoyed the popularity of the channel cell. The wall-jet configuration employs a nozzle or jet through which solution flows. The stream of solution impinges perpendicularly onto the working electrode. The wall-jet was early used by Fleet and Little (48), who reported on the distinct advantages of its cell geometry, and the fact that the electrode seemed to be less susceptible to poisoning, perhaps because of the cleansing action of the solution jet.

Figure 1-2 shows the basic design of coulometric detectors. This type of cell requires a large surface area electrode, since noise and background current are nearly linearly related to electrode surface area, and the additional signal current according to analyte obtained for each increment of area is a decreasing function of the added electrode area. Thus coulometric cells are general expected to have poorer detection limits than amperometric cells.

Two types of coulometric cells are to be discussed here. The first one is a channel-type open working electrode design (49). The electrode required for

complete coulometric conversion is quite large (about 10 cm^2), for standard channel heights and flow rates. Another type of coulometric cell, called a reticulated cell, employs tubular electrodes such as carbon cloth or platinum gauze (50). A new carbon material, reticulated vitreous carbon, was used by Curran (51) and Blaedel (52). Unlike carbon cloth this material is rigid, and does not have the tremendous microscopic surface area of carbon cloth or fiber bundles; thus background currents are relatively low.

In any of the above configurations it may prove beneficial to use more than one working electrode (53), each of which is operated at a different potential. This provides more than one output, thus enhancing selectivity.

The standard procedure for operation of these electrochemcal detectors is to hold the potential of the working electrode constant at a value where the compound of interest will be detected. High background charging current can be avoided if the potential of the working electrode does not have to be altered. However, there are situations where one might wish to change the potential during an analysis. In particular, pulse polarization has advantages under certain circumstances. The pulse method measures a current, mostly the slow decaying Faradaic current, only at certain time after (or before and after in the differential pulse method) a pulse of potential

supplied. In this way the effect of flow rate on the measured current can be minimized. Another advantage of the pulse method, particularly the differential pulse potential application (54,55), is the gain in selectivity. This is because in differential pulse voltammetry, only those compounds whose half-wave potential lies near the applied potential can be detected. The price paid by those who employ differential pulse methods is a decrease in signal-to-noise ratio, because of the lower information-gathering rate employed in the pulse modes.

Most of the studies done by liquid chromatography with electrochemical detector (LCEC) have used the oxidation mode with glassy carbon or carbon paste working electrodes. Compounds such as aromatic amines (56), ascorbic acid (57), phenothiazines (58,59), nitro compounds (60) and phenolic compounds (61)can be easily oxidized without severe interferences. On the other hand, reductive electrochemical detection has major difficulties including the choice of a suitable working electrode and removal of impurities, such as trace metal ions and dissolved oxygen from solvents. Platinum, gold and mercury (thin film or liquid) working electrodes are commonly used for cathodic reactions, but the gold amalgamated mercury electrode (GAME) seems to be the best choice. This electrode has a good negative potential limit (-1.2 V at pH 5.5) as well as reproducible surface chacteristics.

Although regular surface renewal is required, it is the most popular cathodic working electrode being used in LCEC. Compounds that have been detected cathodically are nitrate and nitrite (62), organometallic cations (63,64), sulfur-containg compounds (65) and quinones (66). Table 1-1 is a brief list of compounds that can be detected by ECD.

Organic peroxides have been studied extensively by polarography (5, 11-15), and a range of reduction potentials has been observed depending on the structure of the peroxide and the media being used. For example, in 20% alcohol in water solution hydroperoxides are reduced at 0.00 to -0.35 V vs. SCE, whereas dialkyl peroxides require more negative potentials (-1.00 V), peroxy acids and diacyl peroxides are reduced at positive or slightly negative potentials. Thus variety of compounds containing the peroxy bond could, in theory, be analyzed by ECD following HPLC, since this range of potential readily accessible to electrochemical detection.

Table 1-1 Compounds detectable by electrochemical detector

OXIDATION	REDUCTION
AROMATIC AMINES, DIAMINES	CONJUGATE ACIDS
PHENOLICS	CONJUGATE ESTERS
MERCAPTANS	CONJUGATE NITRILES
HYDROQUINONES	QUINONES
QUINOLINES	NITRO COMPOUNDS
HETEROCYCLIC COMPOUNDS	PEROXIDES
	AROMATIC HALOGENS
	HETEROCYCLIC COMPOUNDS

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CHAPTER II

DETERMINATION OF ORGANIC PEROXIDES WITH LC-EC

I. INTRODUCTION

A major source of air pollution in urban areas is vehicle exhaust. The major components of vehicle exhaust are, of course, the products of the complete oxidation of the fuel, carbon dioxide and water, and the nitrogen that accompanied the oxygen fed to the combustion chamber. Because oxidation is imcomplete, carbon monoxide is always present. Minor constituents, but important ones from an air pollution standpoint, are unburned hydrocarbons, partially oxidized hydrocarbons, nitric oxide, and sulfur dioxide.

Different types of engines give different compositions of exhaust. Internal combustion engines use gasoline as fuel. In this type of engine air and fuel are mixed in a carburetor or by fuel injection, and the mixture is compressed and then ignited by means of a spark. The spent gases are exhausted to the atmosphere. On the other hand, diesel engines operate with excess air at far higher pressure and temperature. The fuel is injected into the cylinder and ignited by the heat generated by compression in the cylinder rather than by a spark. The fuel used is heavier than gasoline and has a higher boiling range. Because of the mechanical design,

internal combustion engines operate at lower air-fuel ratios, especially when engine is in idle or deceleration conditions. This causes relatively high emission levels of hydrocarbons and unburned fuel (1)(Table 2-1). The composition of exhaust from the internal combustion engine is then largely dependent on the chemical composition of the fuel used. Table 2-2 (2) is a typical gasoline composition. The major hydrocarbons present in gasoline also depend on the crude oil from which it is derived and the particular refining process used (Table 2-3). As a whole, the major pollutants emitted by vehicles with internal combustion engines are low boiling point aromatics, olefins and saturated or partially oxidized hydrocarbons, along with other gases. The diesel engine presents an entirely different air pollution problem. Because of the excess air used , very little of the fuel is normally exhausted unburned. However, because of the high pressure and temperature operating conditions and the higher boiling range fuel used, the exhaust has significant quantities of particulates and usually contains higher levels of oxygenated hydrocarbons (3).

Many pollutants present in vehicle exhaust are harmful to human health (4), and others might become harmful through secondary reactions. Smog formation is a good example of these secondary reactions. Basically, photochemical smog arises from the photochemical oxidation

TABLE 2-1.

Effect of Engine Operating Conditions on Composition of Auto Exhaust (uncontrolled cars)* (Ref. 1)

	Idle	Acceleration	Cruising	Deceleration
Air-fuel ratio	11:1–12.5:1	11:1–13:1	13:1–15:1	11:1–12.5:1
Engine speed, rpm	400–500	400–3000	1000–3000	3000–400
Airflow, cfm	6–8	30–35	15–35	6–8
Cylinder vacuum, in. Hg	16–20	0–7	7–19	20–25
Exhaust analysis:				
CO, %	4–6	0–6	1–4	2–4
NO, ppm	10–50	1000–4000	1000–3000	10–50
Hydrocarbons, ppm	500–1000	50–500	200–300	4000–12,000
Unburned fuel, % of supplied fuel	4–6	2–4	2–4	20–60

* rpm—revolutions per minute
ppm—parts per million, by volume
cfm—cubic feet per minute
in. Hg—vacuum, inches of mercury
CO—carbon monoxide
NO—nitric oxide

TABLE 2-2

Specific Hydrocarbons in a Specific Full-Range Motor Gasoline (ref. 2)

Component	Composition % by wt
Propane	0.01
Isobutane	0.37
Isobutylene and butene-1	0.04
n-Butane	4.29
trans-2-butene	0.20
cis-2-butene	0.17
Pentanes	16.59
Pentenes	3.50
Hexanes	9.67
Hexenes	2.70
Benzene	0.81
Heptanes	8.80
Heptenes	7.94
Toluene	12.20
Octanes	9.67
Octenes	—
Nonanes	4.88
Nonenes	—
Ethyl benzene	1.70
Xylenes	4.60
Subs. benzenes (9 carbons)	4.00
Decanes	1.64
Subs. benzenes (10 carbons)	1.85
Undecanes	0.09
Subs. benzenes (11 carbons)	0.11
Naphthalene	0.10
Dodecanes	0.05
Subs. benzenes (12 carbons)	

TABLE 2-3.

Types of Hydrocarbons in Gasoline Stocks

Process	Major hydrocarbon type
Straight-run	Paraffins (straight, branched, and cyclic), C_5 and C_6
Catalytically-cracked	Paraffins and olefins, C_4 to C_9
Hydro-cracked	Paraffins, C_5 to C_6
Alkylate	Paraffins, C_4 to C_9
Reformed	Aromatics, C_6 to C_9; cyclic paraffins
Isomerized	Paraffins (iso and cyclic), C_5 to C_6
Extracted (from reformate)	Aromatics, C_6 to C_9

Taken from Chem. Eng. News, 59 (Nov. 9, 1970)

of gaseous organic compounds, chiefly hydrocarbons. The initial reaction in atmospheric photo-oxidation process is the absorption of ultraviolet light by NO_2. The energized NO_2^* then decomposes (photolyzes) to nitric oxide and atomic oxygen. The atomic oxygen quickly reacts with molecular oxygen to form ozone. In the presence of certain hydrocarbons, especially olefins and a few aromatics, atomic oxygen, ozone and nitric oxide can react to form a variety of products, such as aldehydes, ketones, alkyl nitrates, peroxyacetylnitrate (PAN) (5), and peroxides. A schematic diagram of these reactions is shown in Fig. 2-1. Photochemical smog can cause eye irritation and detrimental effects on the respiratory systems of humans and animals. In addition it can cause damage to vegetation and materials.

In this chapter, we focus our attention on organic peroxides in samples of ambient air and vehicle exhausts. Organic peroxides can be formed by photochemical oxidation of olefins, which constitute a substantial proportion of the hydrocarbons emitted by vehicles. As shown in Fig. 2-2 (6), olefins undergo photo-oxidation in air via ozonides, which can in turn break down to give aldehydes, ketones, alcohols, epoxides, peroxides and peracids. Similarly, epoxides, hydroperoxides, peracids and peroxides can be formed by reactions involving molecular oxygen or more probably activated oxygen species, which might exist in

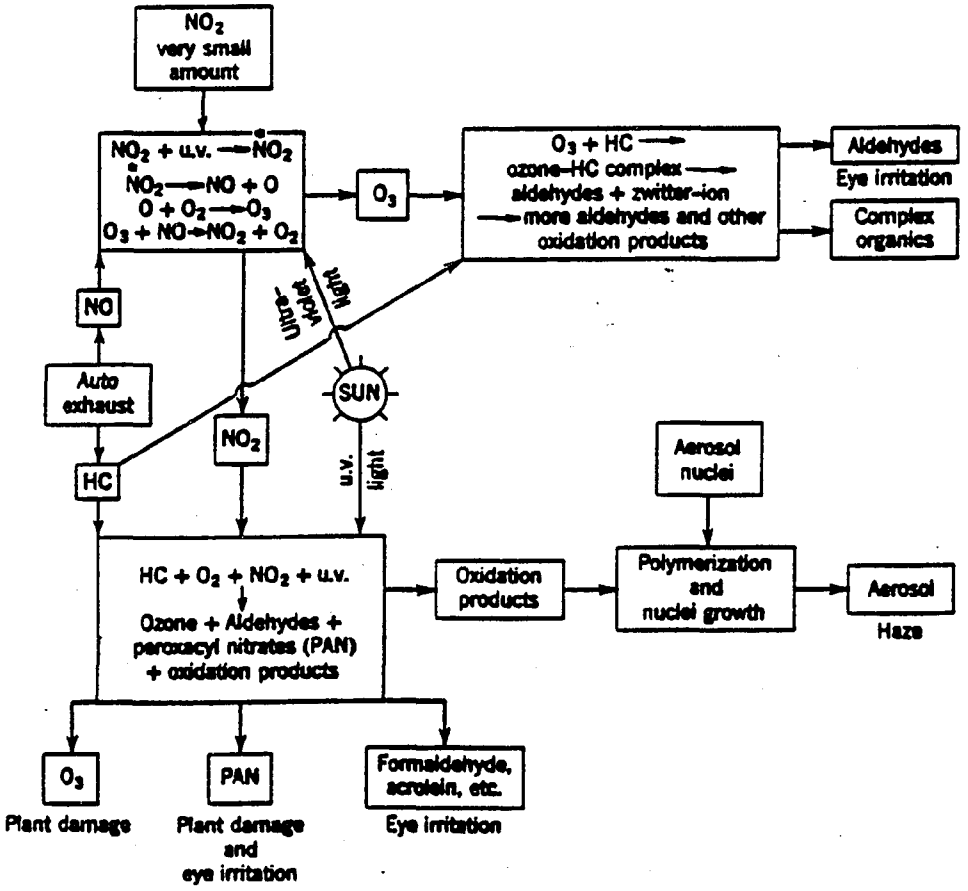

FIGURE 2-1. Diagram showing photochemical smog formation.

(ref. 8)

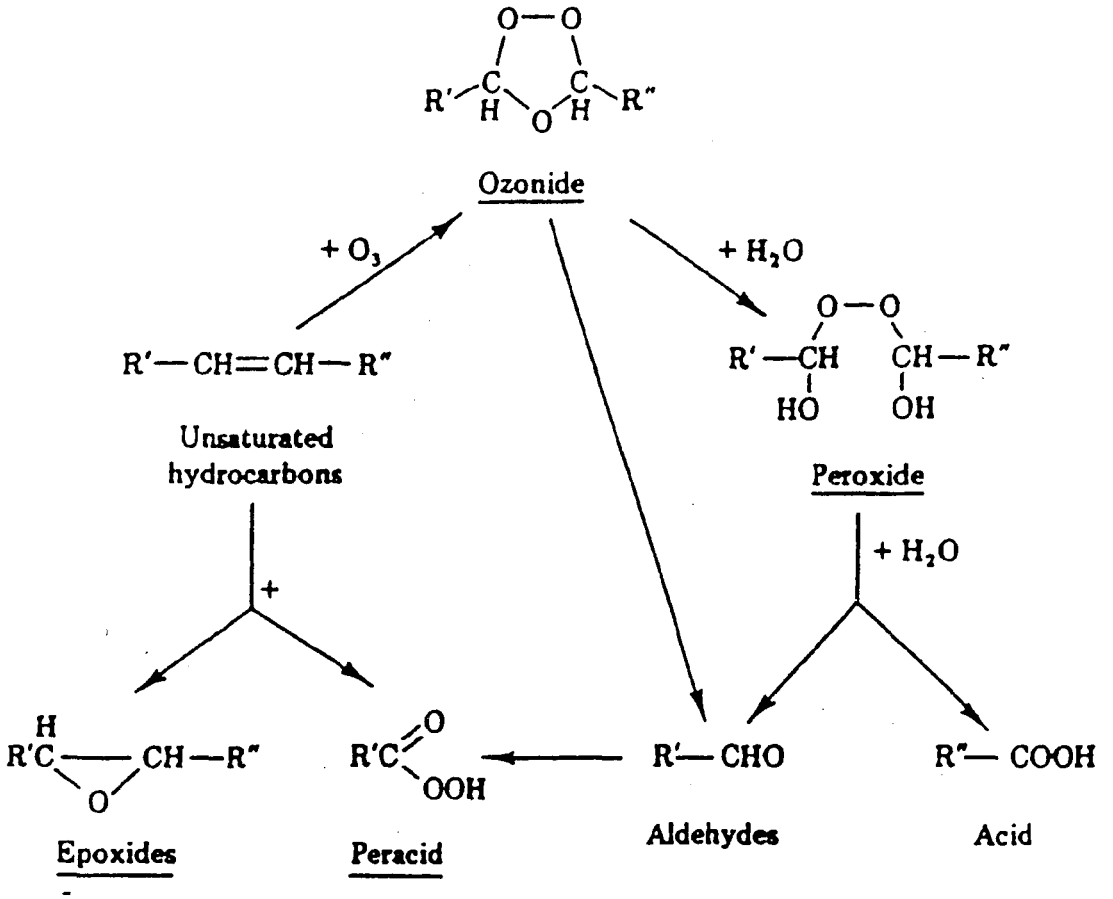

Figure 2-2. Photochemical reactions of carcinogenic significance in polluted air as postulated by Kotin and Falk (ref. 6)

engine chamber during the ignition cycles. Laboratory studies on olefin oxidation and ozonization reactions have led to the isolation of epoxides, peroxides, aldehydes and ketones (7). Some of these compounds, e.g. most peracids, are highly reactive and will not remain in the air for any length of time; others such as hydroperoxides will remain in the air for a sufficient length of time to be detected and potentially to be deleterious to human health.

Long-term carcinogenicity assays of epoxides, peroxides and hydroperoxides in mice and rats by various routes of adminstration have shown that some of these compounds are carcinogenic (9,10,11). Hence, their detection and elimination as air pollutants should be vigorously pursued.

The determination of organic peroxides starts with sample collecting. In the ambient air sampling procedures, collection of a sample of pollutants in a quantity of air requires the following:

1. Use of an accurate flow device to measure the volume of the air sample.

2. Use of a sample collector, generally a filter or an absorbing solution, to trap the contaminants, preferably quantitatively. The actual efficiency of the collector must be determined experimentally, so that the analyst can calculate the true weight or volume of contaminants trapped.

Particulate pollutants in either ambient air or vehicle exhaust are usually collected by using a high volume sampler with fiberglass filter of high collection efficiency and low resistance. Particles up to 10 um in size are collected; the coarser particles are considered to be nonrespirable particulates and are usually removed by a coarse filter before passage through the system. All samplers should be equipped with sun roofs or sun shields to avoid further photo-oxidation. As organic peroxides are thermally unstable, high temperatures should also be avoided. For the same reason, long sample collecting times are not practical. Thus a collecting time of 12-24 hours is ordinarily used.

Gaseous pollutants can be sampled using different methods. The simplest is grab sampling, rapidly acquiring a small volume of air that can be analyzed for specific pollutants. The container can be made from plastic (e.g. Tedlar), glass, steel or other rigid materials. In adsorption sampling, the air is passed through a solid absorbent such as activated alumina, silica gel, molecular sieve, Tenax, Celite, or charcoal. The adsorbed materials are removed either by volatilization of the trapped materials into another system, or by eluting the adsorbent with a suitable solvent. Scrubbing would use a solution, e.g. an organic solvent or aqueous acid or base. A fourth method of sampling gaseous pollutants is

cryogenic sampling or cold trapping. The procedure involves drawing the air through a trap at a sub-ambient temperature, causing the vapors to condense and be retained. The cold trapping method is best suited for volatile organic peroxide sampling, since the low temperature will minimize decomposition and other unwanted reactions.

Samples collected on high volume fiberglass filters are usually extracted with organic solvents or solvent mixtures to give crude extracts. Some studies have been conducted on the effects of the choice of solvent with regard to the types and amounts of organic matter extracted from atmospheric particulate matter (12,13). For organic peroxides, low boiling point solvents such as ether and acetone are preferred when Soxhlet extraction is used, since at elevated temperatures loss of peroxides during the extraction is greater. Solvents with relatively high boiling points, such as toluene, can be used if ultrasonic extraction is applied. Another good alternative is to use supercritical gas extraction methods. Organic pollutants should be selectively extracted using supercritical carbon dioxide, butane or other suitable fluids. The advantages of supercritical gas extraction method are speed and lack of contamination from organic solvents.

Organic peroxides in the crude extracts have to be

separated from other classes of organic pollutants before further testing. There are different ways to do this clean-up work systematically. Fig. 2-3 shows the separation scheme derived by Hueper and his associates (14). The pollutant sample was first extracted with benzene, and the crude benzene extract was fractionated into weak and strong acidic; basic; and neutral portions. The neutral portion was chromatographed on silica gel and yielded aliphatic, aromatic, and oxygenated fractions. The oxygenated fraction contains at least O-heterocyclic hydrocarbons, epoxides, peroxides, aldehydes, ketones, sugars, quinones, esters and lactones. Using LC-EC we can selectively detect the peroxides of interest with little interference from other electroactive compounds such as quinones and epoxides. A somewhat different fractionation scheme had been derived by Hoffmann and Wynder (Fig. 2-4) (15), who used dilute sulfuric acid instead of dilute hydrochloric acid to extract the basic portion. They suggested the neutral portion should be chromatographed on neutral alumina. to avoid the saponification of esters on acidic silica gel.

In the present study, we have demonstrated the HPLC separation of seven hydroperoxides. Six of them are primary or secondary C_4-C_6 alkyl hydroperoxides. These low molecular weight aliphatic hydroperoxides are likely to be found in ambient air and vehicle exhausts. The other one

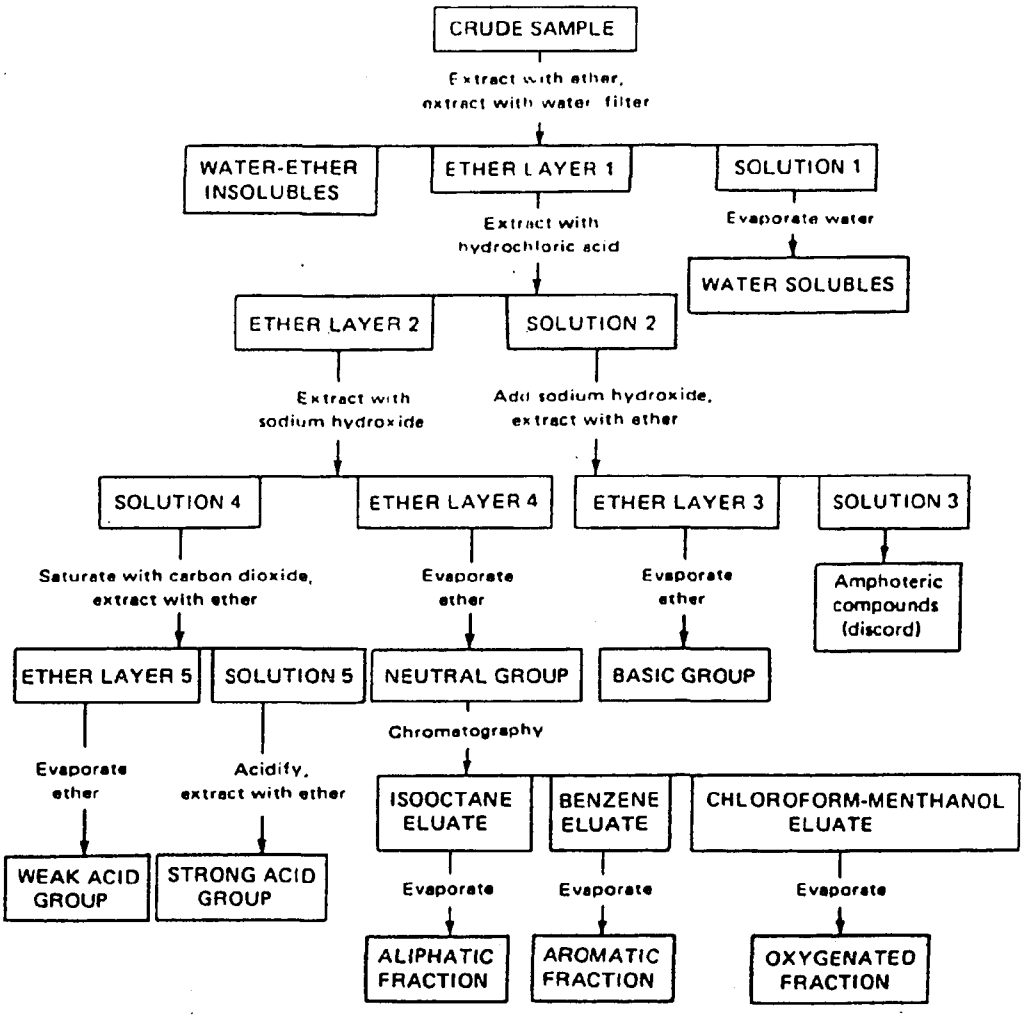

Figure 2-3. **Separation scheme for organic particulate matter by Hueper *et al.*** (ref. 10)

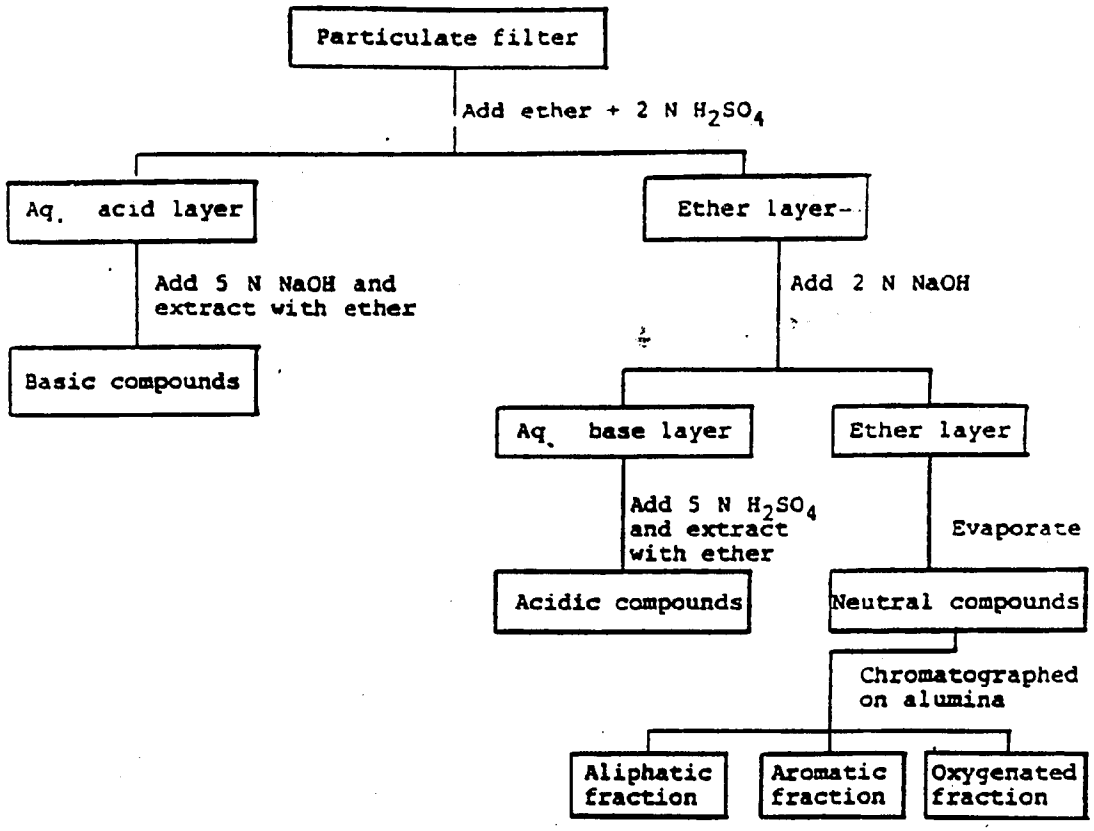

Figure 2-4. Separation scheme for organic particulate matter by Hoffmann and Wynder. (Ref. 12)

is cumene hydroperoxide, which is still being used in industry as process intermediate in phenol production.

II. INSTRUMENTATION:

HPLC-EC

A sandwich-type thin-layer cell was constructed as shown in Figure 2-5. The cell body is made of two pieces of polyacetal (Delrin) plastic, both 1/2" thick and 1" square in size and clamped together with four machine screws. The spacer was cut from 50 um thick Teflon sheet, the center of which was cut out to form the thin layer passage. The total volume of this cell was calculated to be 4.5 ul. The reference electrode is Ag/AgCl in 3 M NaCl electrode. The tip of this electrode is a frit of Vycor porous glass from Princeton Applied Research, Princeton, N. J., which serves as the liquid junction. The working electrode is gold amalgam. It was prepared from a high purity gold rod (1/8" diameter) polished with different grades of diamond paste to a mirror-smooth surface, and dipped into a pool of triple distilled instrument-grade mercury for ten minutes. The excess mercury adhering to the electrode was removed by rubbing with a paper wipe. This procedure provided a rather thick layer of gold amalgam which could be used for about two weeks without serious deterioration of sensitivity. The electrode surface was periodically renewed by dissolving the old mercury film with hot nitric acid and reamalgamating using

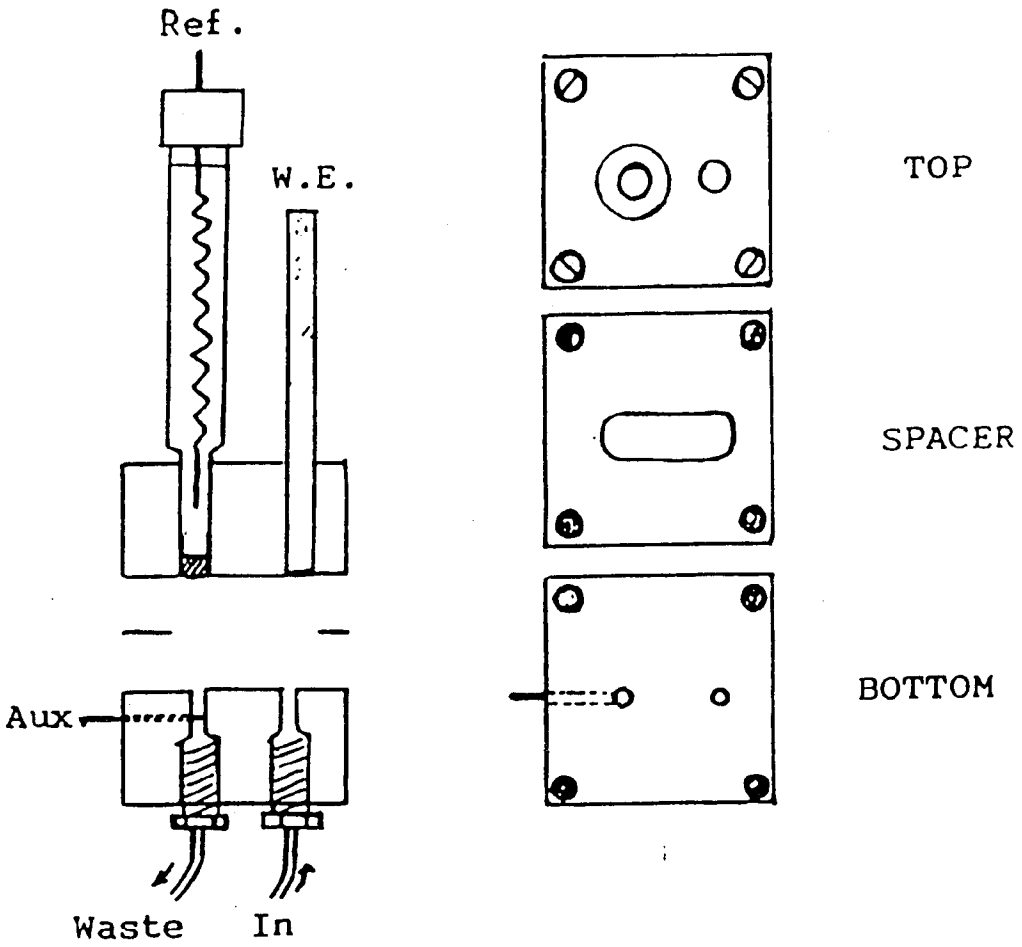

Figure 2-5.
Exploded View of Electrochemical Thin Layer Cell.

the same method. The auxiliary electrode was a piece of platinum wire (1 mm in diameter) epoxied into a hole drilled into the acetal body. One of the plastic blocks was drilled and tapped to provide entrance and exit ports compatible with standard 1/16" stainless steel tube fittings. The whole thin-layer detector cell was then attached to the end of HPLC column via a minimum length of 1/16" o.d. x 0.01" i.d. stainless steel tubing. The detector cell was shielded with a grounded aluminum box to reduce electrical noise pick-up.

As shown in Figure 2-6, the LC-EC instrument was constructed with components from several different manufacturers. The high pressure pump was either a Varian model 8500 syringe pump or a Micromeritics model 750 dual piston reciprocating pump with pulsations dampened by a Bourdon tube pressure gauge (3000 psi) connected to a Swagelok tee immediately downstream from the pump. The injection valve and column compartment was a Micromeritics model 731 with 100 ul sample loop. Whatman C-18 reversed phase columns were used. Constant potential was supplied either by a PAR model 364 polarographic analyzer or a Beckman Electroscan 30 analyzer. The signal current was first amplified by a Keithley 150B electrometer, and then recorded on a Linear 1200 strip chart recorder.

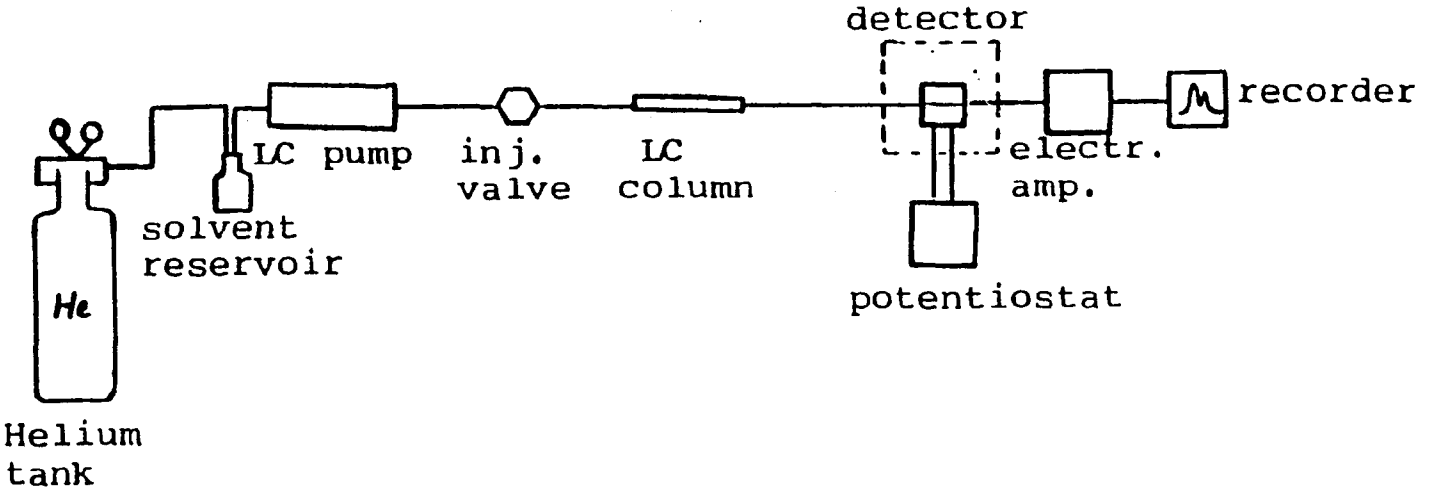

Figure 2-6 Block diagram of HPLC with electrochemical detector

HPLC-UV

A Micromeritics model 786 variable wavelength detector was used either independently or in series with the electrochemical detector.

Voltammetry

A PAR model 364 polarographic analyzer with model 303 static mercury drop electrode was used to obtain voltammograms.

III. EXPERIMENTAL

(1) Chemicals

Burdick & Jackson Distilled-in-Glass HPLC grade acetonitrile and dichloromethane were used without further purification; sodium perchlorate was obtained from G. Frederick Smith Chemical Co.; n-butyl alcohol was from Eastman Kodak; methanesulfonyl chloride, 2-butanol, 2-pentanol and hexyl alcohol were from Aldrich; n-amyl alcohol and 30% hydrogen peroxide were from J. T. Baker; iso-amyl alcohol was from Allied Chemical; cumene hydroperoxide was obtained from K & K Lab. Inc. and was redistilled before using; deionized distilled water was used.

(2) Preparation of primary alkyl hydroperoxides

The three primary hydroperoxides, n-butyl, n-amyl and n-hexyl hydroperoxide, were synthesized according to the method described by Williams and Mosher (16) with minor modifications. A mixture of 22.9 g (0.20 mole) of methanesulfonyl chloride and an equivalent amount of the corresponding alcohol was stirred in an ice-brine-bath, and 31.6 g (0.40 mole) of dry pyridine was added to this mixture slowly over a period of 3.5 hours, while keeping the reaction mixture temperature between 0-5 ^{o}C. Stirring was continued for one-quarter hour; the reaction mixture

was then poured into 125 ml of ice-cold 10% hydrochloric acid and the product extracted with 75 ml of ether. The resulting solution was washed with two-20 ml portions of water followed by a 30 ml portion of saturated sodium bicarbonate solution. The solution was dried over anhydrous potassium carbonate, filtered, and heated on a steam bath to remove the ether. The residue was distilled at reduced pressure to give the methanesulfonate. The alkyl methanesulfonate (0.040 mole) was dissolved in methanol or methanol and water: n-butyl methanesulfonate was dissolved in 25 ml of methanol; n-amyl methanesulfonate was dissolved in 40 ml methanol + 3.5 ml water; n-hexyl methanesulfonate was dissolved in 45 ml methanol + 5.0 ml water. Each mixture was cooled in an ice-bath and combined with 20.0 g (0.16 mole) of 30% hydrogen peroxide, and 5.00 g (0.045 mole) of 50% aqueous potassium hydroxide, in that order. Then the mixture was placed in a 23-25 ^{o}C water-bath for the period of 20 hours. The mixture was cooled in ice and slowly combined with 15.0 g of 50% potassium hydroxide. The alkaline mixture was extracted with 25 ml of benzene to remove unreacted alkyl methanesulfonate, alcohol or dialkyl peroxide. The aqueous layer was cooled in ice, neutralized (pH 7) with concentrated hydrochloric acid, and extracted with four-15 ml portions of benzene. These benzene extracts still contained a small amount of hydrogen

peroxide. The combined benzene extracts were extracted with 20 g of cold 25% potassium hydroxide. The alkaline solution was cooled in ice, neutralized with concentrated hydrochloric acid, and then the neutralized mixture was extracted with four-10 ml portions of benzene. The hydroperoxide solution was dried over sodium sulfate and then stripped of solvent at aspirator pressure. The residue was distilled at reduced pressure to give product.

(3) Preparation of secondary alkyl hydroperoxides

Three secondary alkyl hydroperoxides, 2-butyl, 2-pentyl and iso-amyl hydroperoxides were synthesized also according to the method described by Williams and Mosher (17). The secondary alkyl methanesulfonates were prepared in the same way as the primary alkyl methanesulfonates, except the ether from the ether extracts of alkyl methanesulfonates was removed under reduced pressure at room temperature; the secondary methanesulfonates were distilled in two portions to avoid prolonged heating; and the distillations were made in the presence of 0.5 g of calcium carbonate. The secondary alkyl hydroperoxides were prepared under conditions similar to those employed for the preparation of the primary isomers; however the isolation procedure was modified slightly as following.

After adding 50% potassium hydroxide, the alkaline solution was extracted with 50 ml of hexane and then

neutralized with hydrochloric acid while being cooled in ice, and solution was extracted with six-20 ml portions of 25% potassium hydroxide. The alkaline solution was neutralized with concentrated hydrochloric acid, with cooling, and the liberated hydroperoxide was extracted with three-15 ml portions of ether. The ether solution was dried over sodium sulfate, freed of ether under aspirator pressure and then distilled at reduced pressure to give product.

(4) High performance liquid chromatography

The LC was lab-assembled as described in the instrumentation section. The ECD working electrode potential was maintained at -1.0 volt vs. Ag/AgCl (3 M NaCl), and the UVD was set at 225 nm. The HPLC column was a Whatman Partisil 10/25 ODS-3, 25 cm X 4.6 mm i.d., operated at ambient temperature. The HPLC mobile phase was 30/70 (v/v) acetonitrile/0.05 M aq. $NaClO_4$, purged with high purity N_2 to remove dissolved O_2, and pumped at a flow rate of 1 ml/min. The working standard solution was prepared by dissolving 15 mg 2-butyl hydroperoxide; 15 mg n-butyl hydroperoxide; 18 mg 2-pentyl hydroperoxide; 18 mg iso-amyl hydroperoxide; 18 mg n-pentyl hydroperoxide; 30 mg n-hexyl hydroperoxide and 30 mg cumene hydroperoxide in 3 ml of dichloromethane (DCM); it was stored capped at 5 ^{o}C until ready for injection into HPLC. To carry out an

assay, exactly 1 ul aliquots of working standard solution were injected into LC, the retention volumes of each hydroperoxide was measured, and the peak heights were determined to $\pm$0.5 mm. Average peak heights were calculated for each hydroperoxide. To study the linear dynamic range of response to n-butyl hydroperoxide, seven n-butyl hydroperoxide solutions were prepared, containing 2500, 1250, 250, 125, 25, 12.5, 1.25 ug/ml by dissolving 25 mg n-butyl hydroperoxide in 10 ml DCM and making successive dilutions with DCM. 4 ul aliquots of each were injected into LC 4 times, and average peak heights were determined.

(5) Voltammetry

A PAR 364 polarographic analyzer was used in sampled DC mode with model 303 static mercury drop electrode. Operating conditions as following:

Scan rate: 5 mv/sec

Scan range: 0,0 V to -1.5 V

Sensitivity: 100 uA full scale

Solvent: 30/70 (v/v) acetonitrile / 0.05M aq. $NaClO_4$

(6) Analysis of air filters

Air samples were collected using a high volume sampler with a fiberglass filter (8 in. x 10 in.) over a period of 24 hours. The air particulate sample was first

extracted with acetone for 3 hours, then concentrated to 1 ml and filtered through a 2 um filter before injection into the LC.

IV. RESULTS AND DISCUSSION

The six alkyl hydroperoxides and cumene hydroperoxide were separated with an isocratic acetonitrile-water mixture. Figure 2-7 shows the chromatograms of these compounds using both UVD and ECD. As might be expected, the ECD is much more sensitive than UVD for this type of compound. In general, for alkyl hydroperoxides, the sensitivity of ECD is one or two orders of magnitude greater than UVD. The working electrode was controlled at -1.0 Volt, at which potential all seven hydroperoxides are readily reduced. The voltammograms of four of these hydroperoxides, shown in Figure 2-8, were taken in the same solvent as the LC mobile phase, using the dropping mercury electrode. Although in the 30/70 acetonitrile/H_2O solvent, the reduction waves of some hydroperoxides tested (eg. cumene hydroperoxide) tend to be drawn out, there is no difficulty in detection of these compounds with the ECD.

Any electrochemical experiment performed at negative potentials must cope with interference caused by the reduction of oxygen. We found it convenient to purge the mobile phase with high purity nitrogen for 30 minutes to alleviate the interfering oxygen wave to an acceptable level. The response of the electrochemical detector to hydroperoxides in the 10 - 1000 ng range was linear. Figure 2-9 shows the plot of peak height vs. sample weight

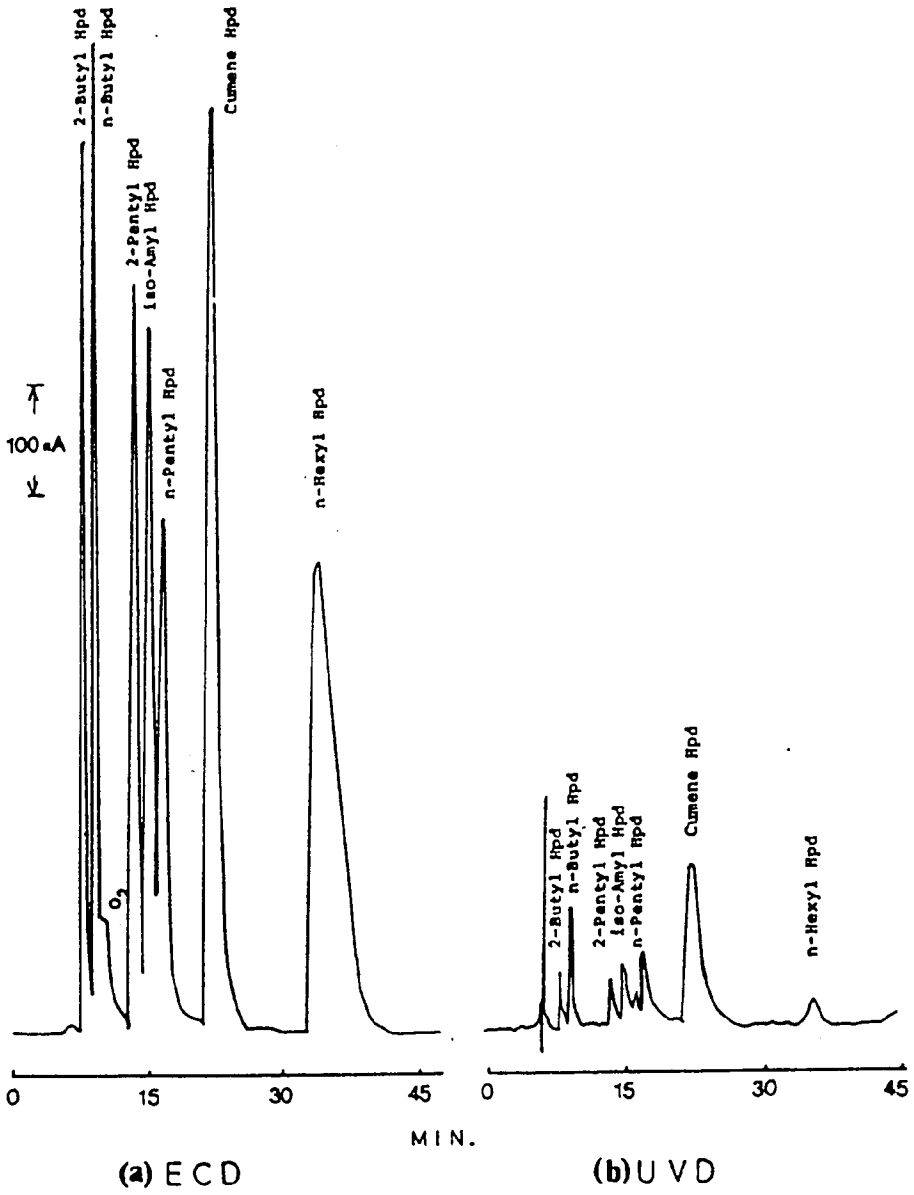

Figure 2-7 LC separation of 7 hydroperoxides with both ECD and UVD. The chromatographic conditions are listed in Table 2-4.

TABLE 2-4. Chromatographic Conditions of Figure 2-7

Mobile phase	: 30% CH_3CN, 70% H_2O, 0.05 M $NaClO_4$
LC column	: Whatman Partisil PX 10/25 ODS-2, 4.1 mm O.D.
Flow rate	: 1 ml/min
ECD potential	: -1.0 V
ECD sensitivity	: 1 uA full scale
UVD wavelength	: 225 nm
UVD sensitivity	: 0.02 ABS full scale
Amount injected	: 5 ug 2-butyl hydroperoxide 5 ug n-butyl hydroperoxide 6 ug 2-pentyl hydroperoxide 6 ug iso-amyl hydroperoxide 6 ug n-pentyl hydroperoxide 10 ug cumene hydroperoxide 15 ug n-hexyl hydroperoxide

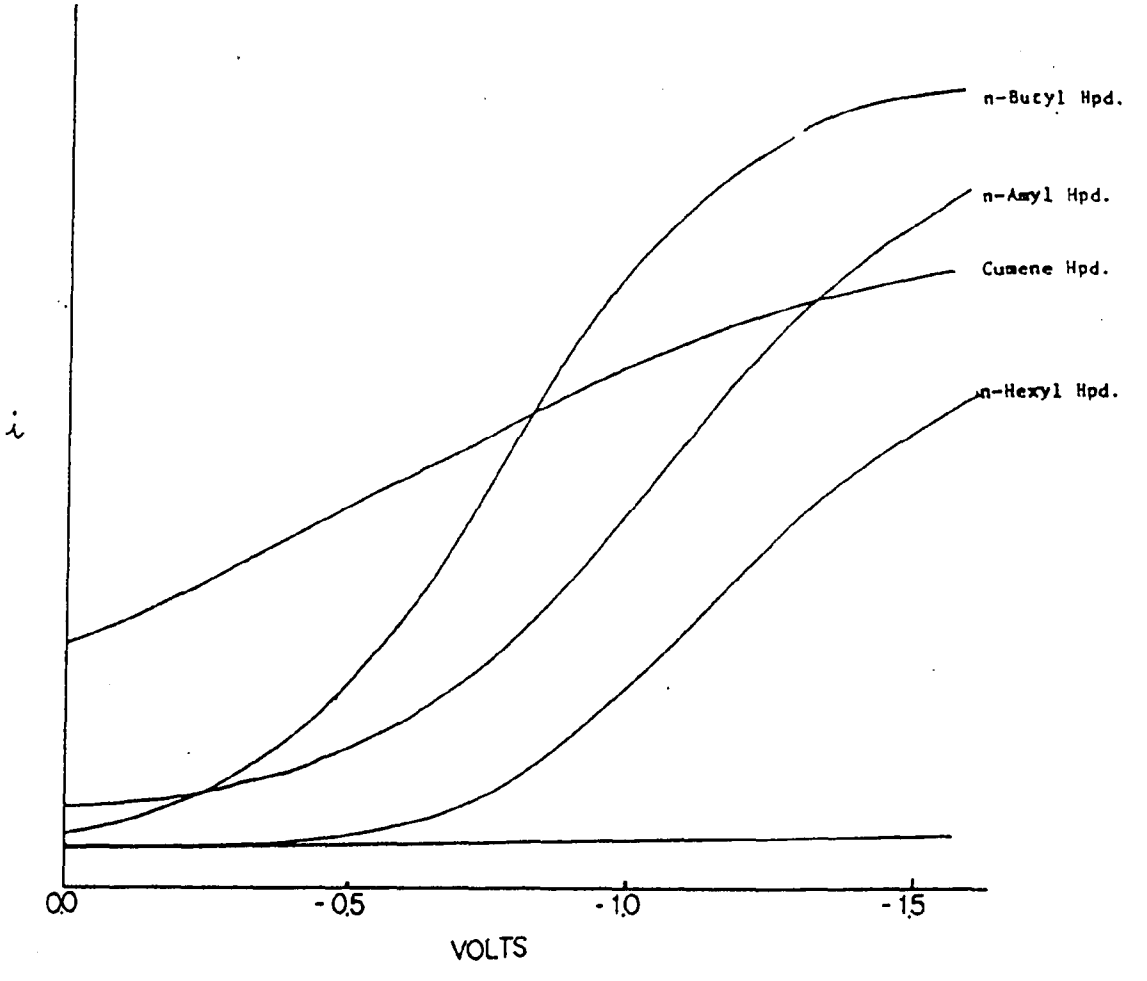

Figure 2-8 Voltammograms of 4 hydroperoxides solvent, 30% CH_3CN, 70% H_2O, 0.05 M $NaClO_4$; scan rate, 5 mv/sec.

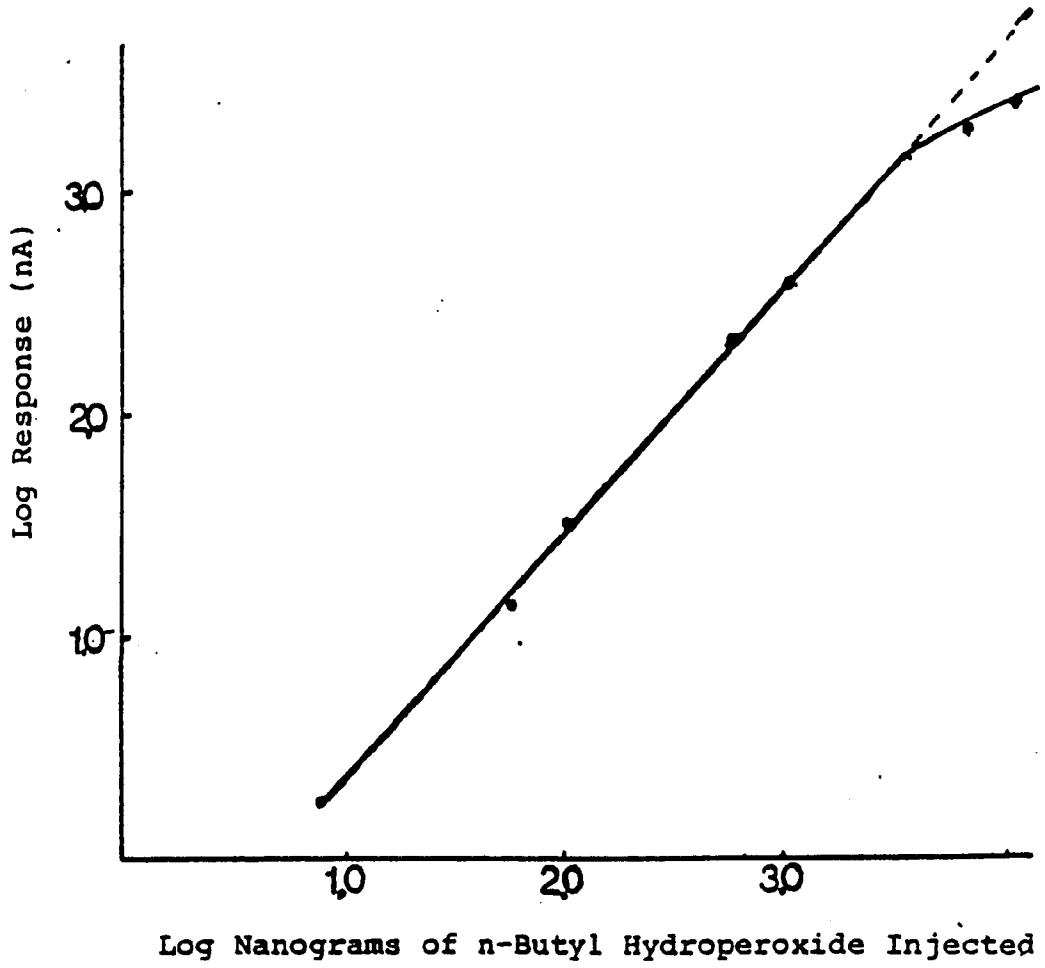

Figure 2-9 LC-EC calibration curve of n-butyl hydroperoxide Mobile phase, 30% CH_3CN, 70% H_2O, 0.05 M $NaClO_4$; potential supplied, -1.0 V; flow rate, 1 ml/min.

injected of n-butyl hydroperoxide over this range. The detection limits of the ECD to the seven hydroperoxides tested are in the range of 2-10 ng. Using 10 ng as a conservative detection limit, the improvement in sensitivity, for the detection of hydroperoxides, over the UVD can be estimated to be at least 2 orders of magnitude.

Since the electrochemical detector is selective, the clean-up steps in sample preparations are usually minimized. Figure 2-10 is the chromatogram of an air particulate sample collected in Queens College. Although this chromatogram does not clearly show the peaks of those hydroperoxides that we are looking for, it still gives a general idea how ECD can be used with LC in "real-world" samples for detection of electroactive compounds. If further study of this analysis is desired, a more concentrated crude acetone extract should be used. This crude extract should be pre-chromatographed on silica gel or alumina to eliminate most of the aliphatic and aromatic fractions. As the solutes come out of the LC, portions should first be extracted with an organic solvent to separate inorganic salts, then be concentrated, and injected into a GC/MS for confirmation of peak identifications.

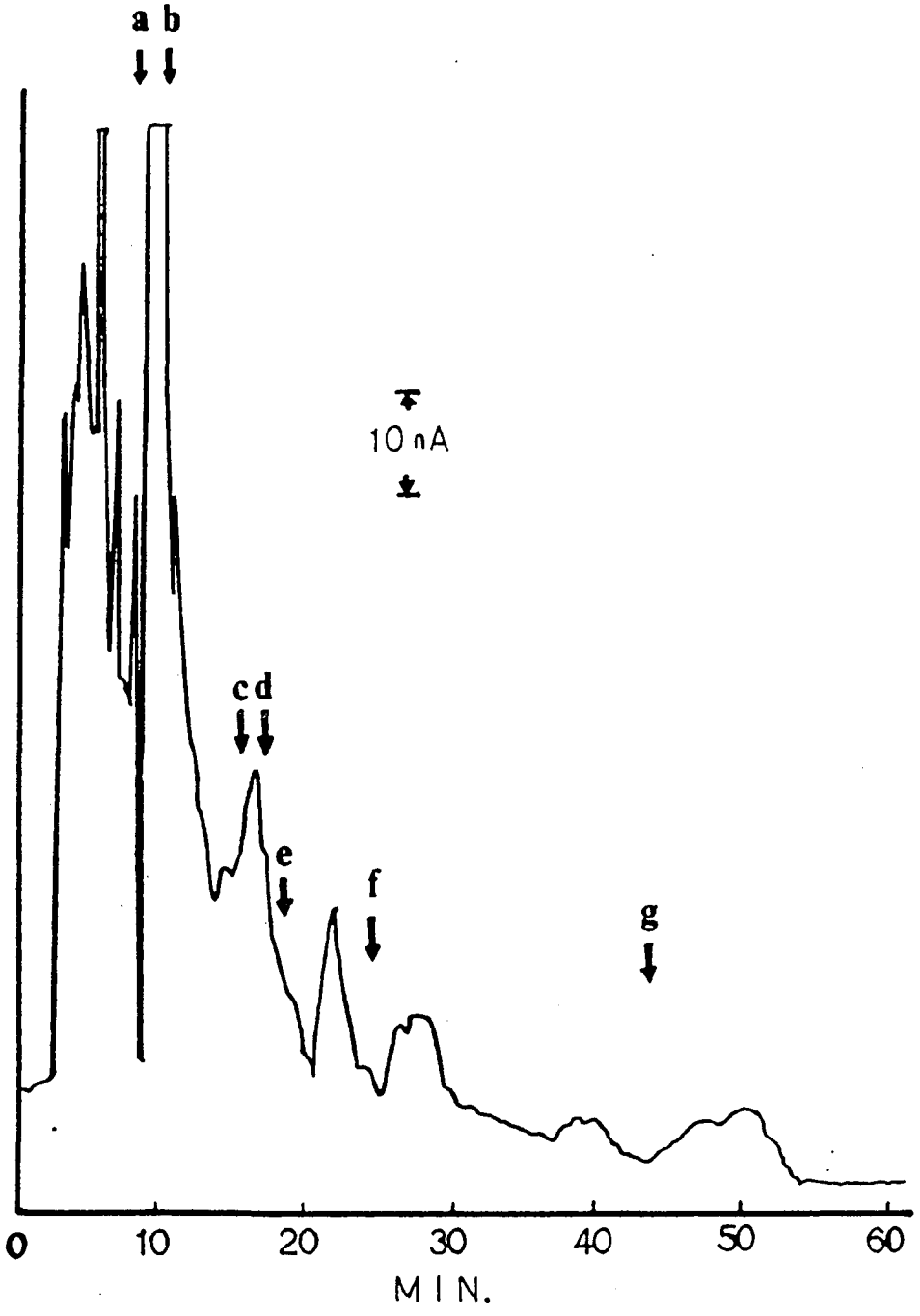

Figure 2-10 Chromatogram of air sample QC-3. The conditions are the same as in Figure 2-7, except with 0.8 ml/min flow rate. a, 2-butyl hydroperoxide; b, n-butyl hydroperoxide; c, 2-pentyl hydroperoxide; d, iso-amyl hydroperoxide; e, n-pentyl hydroperoxide; f, cumene hydroperoxide; g, n-hexyl hydroperoxide.

V. SUMMARY

HPLC with ECD is particularly useful in determinations of organic peroxides. The advantages are selectivity, sensitivity, linear response over a wide range of concentrations, and easy sample preparation. Just as do other LC detectors, the ECD has its limitations. These include (1)routine renewal of working electrode surface is required; (2)general unsuitablility for normal phase LC; (3)extra-high purity solvent is required; (4)solvent gradients can not be used; and (5)highly steady flow is needed.

ECD is capable of routine analysis of organic peroxides, and with careful instrumental preparation and consideration, peroxide compounds in the sub-ppm level can be determined both qualitatively and quantitatively without difficulty.

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CHAPTER III

DETERMINATION OF BENZOYL PEROXIDE IN ACNE PREPARATIONS

I. INTRODUCTION

Benzoyl peroxide is used in acne creams and lotions, because it acts as an irritant to the skin, producing high epithelial cell growth rates and promoting sloughing and expulsion of comedones (1).

A number of analytical procedures for benzoyl peroxide in acne preparations has been published, including spectrophotometric methods (2), iodimetric titrations (3), colorimetric methods using N,N´-di(2-naphthyl)-phenylene-1,4-diamine (4) or other color reagents, polarographic methods (5) and HPLC methods with a UV detector (6,7).

The absorption spectra for benzoyl peroxide and analogous compounds, such as benzaldehyde and benzoic acid, obtained with the spectrophotometric method described in the USP (2) show marked similarity. If benzoyl peroxide is mixed with any of these analogous compounds, an accurate determination of benzoyl peroxide is impossible.

In the iodimetric method, excess standard KI is added and the liberated iodine is back titrated with standard sodium thiosulfate solution. This does not distinguish between benzoyl peroxide and most other

organic peroxides.

As we mentioned in chapter I, the colorimetric and polarographic methods are not specific, therefore the reliablity of these methods for benzoyl peroxide determination is highly suspicious.

In our study, high performance liquid chromatography with a reductive mode electrochemical detector is applied. This method is rapid, precise and accurate, and has better selectivity and sensitivity than the LC-UV method. It has a detection limit of 2 ng per injection and the linear dynamic range is better than 3 orders of magnitude. Four commercial acne products were tested. These results were compared with those of LC-UV and titrimetric methods.

II. EXPERIMENTAL

(1) Chemicals

Benzoic anhydride and benzil were obtained from Eastman-Kodak. Benzoic acid was from Alfa. Burdick & Jackson Distilled-in-Glass HPLC grade acetonitrile and dichloromethane were used without further purification. Benzoyl peroxide standard was obtained from Aldrich and was recrystallized from chloroform solution by addition of methanol. Deionized glass distilled water was used throughout.

(2) Preparation of perbenzoic acid

Perbenzoic acid was synthesized according to the method described by Braun (8) with minor modifications. Sodium metal (2.6 g) was dissolved in 50 ml of absolute methyl alcohol with cooling. The resulting solution of sodium methoxide was then cooled to -5 ^{o}C. A solution of 25 g of benzoyl peroxide in 100 ml of chloroform was prepared, cooled to 0 ^{o}C, and added without delay to the sodium methoxide solution with shaking and cooling. The mixture was kept for five minutes in ice-salt bath with continuous shaking; it turned milky, but no precipitate appeared. The sodium perbenzoate produced was then extracted with 250 ml of water containing much chopped ice. The aqueous layer was washed twice with 50 ml portions of cold chloroform to remove the methyl benzoate.

The aqueous solution contained the sodium salt of perbenzoic acid. The perbenzoic acid was then liberated by the addition of 120 ml of cold 1 N sulfuric acid and was removed from solution by extracting three times with 50 ml portions of cold chloroform. The combined chloroform solution was washed twice with 25 ml portions of water and the chloroform solution was dried with a small amount of anhydrous sodium sulfate for one hour. The sodium sulfate was removed by filtration. The perbenzoic acid-chloroform solution was stored below 5°C before using. The concentration of perbenzoic acid was determined by iodimetric titration.

(3) Sample preparation

Samples of lotion or cream containing ca. 5 mg benzoyl peroxide were accurately weighed into 16 X 150 mm screw cap culture tubes, and were dissolved or dispersed by shaking with 5 ml distilled water. Later, 5 ml CH_2Cl_2 were added and the mixture was shaken for 10 min. on an automatic shaker, then centrifuged for 3 min. at 2000 rpm to separate the layers. The lower CH_2Cl_2 phase was withdrawn with a syringe and transfered to a clean 16 X 150 mm culture tube. This solution was stored capped at 5 °C until ready for injection into the HPLC.

(4) High performance liquid chromatograph

The LC apparatus was the same as described in chapter II for the hydroperoxides separations. The ECD working electrode potential was maintained at -0.15 V vs. Ag/AgCl (3 M NaCl), and the UVD was set at 254 nm.

The HPLC column used was a Whatman Partisil 10/25 ODS-3, 25 cm X 4.1 mm id., operated at ambient temperature. The HPLC mobile phase was 50/50 (v/v) acetonitrile/0.10 M aq. $NaClO_4$, purged with high purity N_2 to remove dissolved O_2, and pumped at a flow rate of 1.5 ml/min.

The working standard solution was prepared by suspending accurately weighed 50 mg benzoyl peroxide in 50 ml distilled water, and a 50.0 ml portion of dichloromethane was added and shaken to extract the benzoyl peroxide. The lower CH_2Cl_2 layer was withdrawn with a syringe and was stored in a 50 ml volumetric flask. To carry out an assay, exactly 4 ul aliquots of standard solution were injected into the LC. The benzoyl peroxide peak heights were measured to $\pm$0.5 mm and the average peak height was calculated. Similarly, exactly 4 ul aliquots of each product extract solution were injected and average peak heights were determined. To study the linear dynamic range, 4 benzoyl peroxide solutions containing 550, 110, 22, and 4.4 ng/ul were prepared by dissolving 5.5 mg benzoyl peroxide in 10 ml dichloromethane and making 3

successive 1:5 dilutions with dichloromethane. 4 ul aliquots of each were injected into the LC 5 times, and average peak heights were determined.

The separation of potential interferences was demonstrated by dissolving 8 mg benzoyl peroxide, 5 mg benzoic acid, 6 mg perbenzoic acid, 7 mg benzoic acid anhydride, 6 mg benzil, and 80 mg benzene in 5 ml dichloromethane. 2 ul aliquots of this solution were injected into the LC. The partition study was done by dissolving 50 mg (accurately weighed) benzoyl peroxide in 50.0 ml dichloromethane. Exactly 4 ul aliquots of this solution were injected into the LC. Also exactly 4 ul aliquots of the working standard solution were injected into the LC. Both injections were repeated 3 times and average peak heights were determined.

The recovery study was carried out by suspending 2 mg (accurately weighed) lotion in 100 ml distilled water with shaking, extracting this mixture with 100 ml dichloromethane and proceeding as above. Next, another 1 gm (accurately weighed) portion of lotion, to which had been added 50 mg (accurately weighed) benzoyl peroxide, was suspended in 100 ml distilled water, and also proceeding as above. Exactly 4 ul aliquots of these dichloromethane solutions and the benzoyl peroxide standard solution were injected into the LC. Each injection was repeated 6 times and average peak heights

were determined.

(5) Iodimetric titration

For comparison with an independent method (3), each product was assayed titrimetrically in quadruplicate. To an accurately weighed sample of acne cream or lotion equivalent to ca. 250 mg benzoyl peroxide in a 100 ml beaker was added 30 ml of acetone and stirred to dissolve. The mixture was transferred quantitatively into a 100 ml volumetric flask and dilute to the mark with acetone. Exactly 10.0 ml of acetone solution were pipetted into a 125 ml erlenmeyer, 2 ml 20% KI solution added, and let stand 15 min. in the dark. Twenty-five ml acetone were added and liberated iodine titrated with standard 0.01 N $Na_2S_2O_3$ to the disappearance of the yellow I_2 color. The procedure was repeated on a standard solution containing 250 mg pure benzoyl peroxide in acetone.

III. CALCULATIONS

The percentages of benzoyl peroxide in acne preparations determined by LC methods were calculated from:

$$\%B_p = \frac{W_s}{W_p} \times \frac{h_p}{h_s} \times 100\%$$

Where W_s is the weight of pure benzoyl peroxide extracted into 50.0 ml of dichloromethane, W_p is the weight of the product taken, h_s is the average peak height of the benzoyl peroxide peaks from the standard, and h_p is the average peak height of the benzoyl peroxide peaks from the product.

The percentages of benzoyl peroxide recovered from spiked samples determined by the LC method were calculated from:

$$\frac{h_A}{h_B} = \frac{2000\ B_p\ R_b\ P_b}{(1000\ B_p + 50)\ R_b\ P_b} = \frac{2000\ B_p}{(1000\ B_p + 50)} \qquad ----(1)$$

$$\frac{h_B}{h_C} = \frac{(1000\ B_p + 50)\ R_b\ P_b}{100\ P_b} = \frac{(1000\ B_p + 50)\ R_b}{100} \qquad ----(2)$$

$$\frac{h_A}{h_C} = \frac{2000\ B_p\ R_b\ P_b}{100\ P_b} = 20\ B_p\ R_B \qquad ----(3)$$

multiplying eqn (3) by 50:

$$50\ \frac{h_A}{h_C} = 1000\ B_p\ R_b \qquad ----(4)$$

insert (4) into (2):

$$\frac{h_B}{h_C} = \frac{1}{2}\left(\frac{h_A}{h_C} + R_b\right) \qquad \text{or} \qquad R_b = \frac{2h_B - h_A}{h_C}$$

Where subscripts A,B and C represent solutions A,B and C,

respectively. Solution A was prepared by dissolving or dispersing 2.000 gm of acne lotion in 100 ml water and extracted with 100 ml DCM. Solution B was prepared by using 1.000 gm acne lotion and 50 mg benzoyl peroxide and proceeded as with solution A. Solution C was prepared by dispersing 100 mg benzoyl peroxide in 100 ml water and extracting with 100 ml DCM. B_p is the percentage of benzoyl peroxide in acne lotion, P_b is the partition factor of benzoyl peroxide in DCM/H_2O, and R_b is the percentage of benzoyl peroxide recovered. h_A, h_B and h_C represent average peak heights of the solutions A,B and C peaks.

The partition factor was calculated as:

$$\%P_b = \frac{h_1}{h_1 - h_2} \times 100\%$$

Where P_b is the partition factor of benzoyl peroxide in DCM/H_2O, and h_1 and h_2 are average peak heights from solutions 1 and 2. Solution 1 was prepared by dissolving 50 mg benzoyl peroxide in 50.0 ml DCM, and solution 2 was prepared by dispersing 50 mg benzoyl peroxide in 50.0 ml H_2O and then extracted with 50.0 ml DCM.

To calculate the percentages of benzoyl peroxide in acne preparations using the iodimetric titration method, the equations can be written as:

$$\%B_p = \frac{MW \cdot V}{20\ W_S} \times 100\%$$

Where W_s is the weight of acne lotion taken in grams, V is the volume of 0.01 N $Na_2S_2O_3$ used in liters, and MW is the molecular weight of benzoyl peroxide.

IV. RESULTS AND DISCUSSION

The selectivity of the LC system and the specificity of the ECD relative to the UVD are evident in Fig. 3-1. In the chromatograms shown, compounds such as benzoic acid, benzene and benzoic acid anhydride are the decomposition products of benzoyl peroxide, benzil is structurally similar to benzoyl peroxide. These potentially interfering UV-absorbing compounds are well separated from the benzoyl peroxide peak. With the mobile phase employed, only benzoic acid and perbenzoic acid co-elute. If there was a need to separate them, use of 20% acetonitrile in water as mobile phase would produce a base line separation. The ECD responds only to benzoyl peroxide, perbenzoic acid, oxygen dissolved in the sample, and, with much reduced sensitivity, to benzoic acid, at -0.15 V vs. Ag/AgCl reference electrode. Specificity could be increased by lowering the applied potential at the expense of sensitivity to benzoyl peroxide; however, the LC separation obviates the need for this.

The chromatograms of extracts of product 2 using the UVD and the ECD are reproduced in Fig. 3-2. No differences in peak area from a particular detector were noted if the sequence of detectors in the flow stream was reversed, indicating insignificant alteration of benzoyl peroxide in either detector. In practice, either detector could be used alone unless one needed the additional

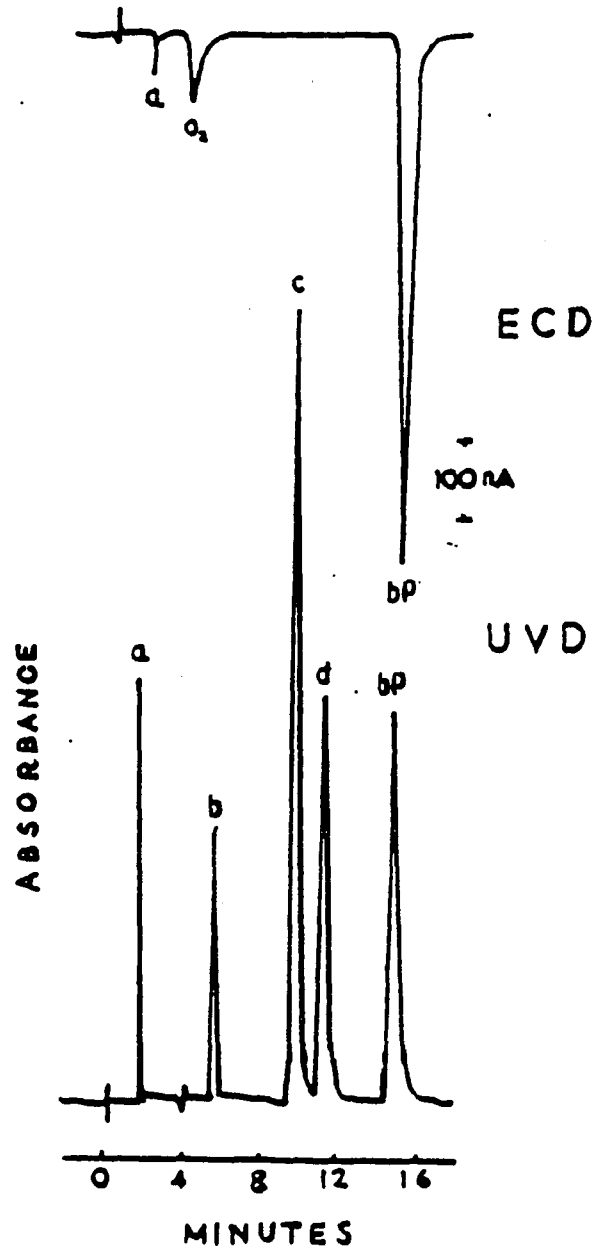

Figure 3-1. Separation of potential interferences using ECD (upper) and UVD (lower). ECD sensitivity, 2 uamp full scale; UVD sensitivity, 0.50 a. u. full scale. Peak (a) benzoic acid; (b) benzene; (c) benzil; (d) benzoic acid anhydride; and (bp), benzoyl peroxide.

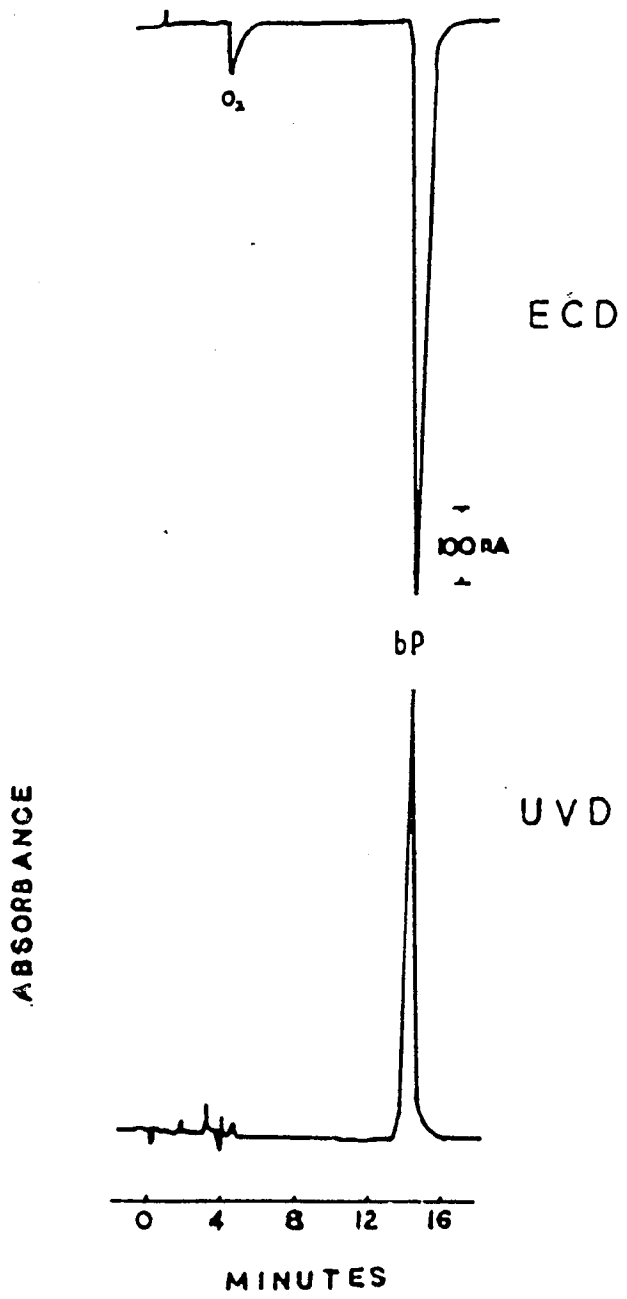

Figure 3-2. Chromatogram of BP in DCM extract of product 2 using EC D (upper) and UVD (lower). ECD sensitivity, 1 uamp full scale; UVD sensitivity, 0.50 a.u. full scale.

sensitivity and specificity of the ECD.

The peak height vs. weight of benzoyl peroxide injected plot is a straight line for both detectors (Figs. 3-3, 3-4) up to at least 2 mg benzoyl peroxide injected. The detection limits expressed as weight of benzoyl peroxide producing a peak twice the noise level are 2 ng per 4 ul injection for the ECD and 20 ng per 4 ul injection for the UVD.

The partition study showed 98.5% transfer of benzoyl peroxide from aqueous to dichloromethane phase, i.e. essentially quantitative extraction. However, this factor was not used in calculating the percentage of benzoyl peroxide in the products, since the working standard solution of benzoyl peroxide was prepared in the same way as sample solutions. Thus the need to use the partition factor is eliminated. The recovery of benzoyl peroxide from the spiked sample was 99.4%, indicating the sample preparation method is adequate.

The results of analysis of 4 acne products by LC-UVD, and iodimetric titration are given in Table 3-1. Product No. 1 through 4 represent Clearsil, Oxy 10, Oxy 5 and Noxzema, respectively. The first 2 products contain nominally 10% benzoyl peroxide and the other 2 nominally 5% benzoyl peroxide, according to the labels. The results of the 3 methods are in good agreement for each product.

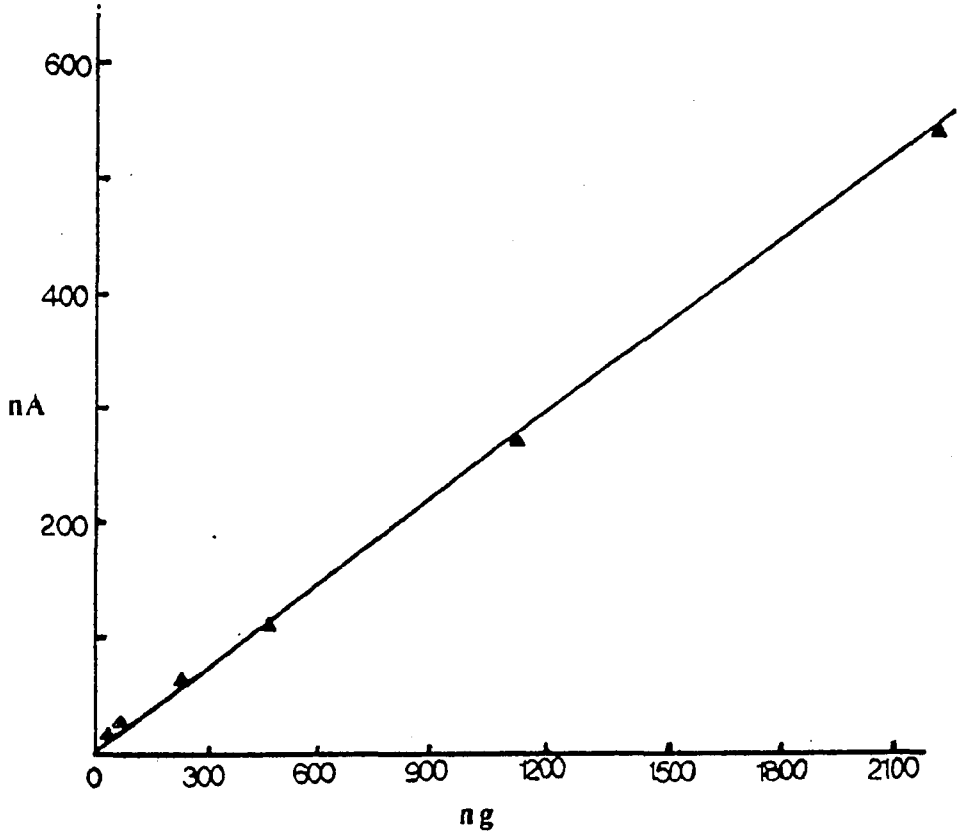

Figure 3-3 LC-EC calibration curve of benzoyl peroxide
The chromatographic conditions are the same as in Fig. 3-1.

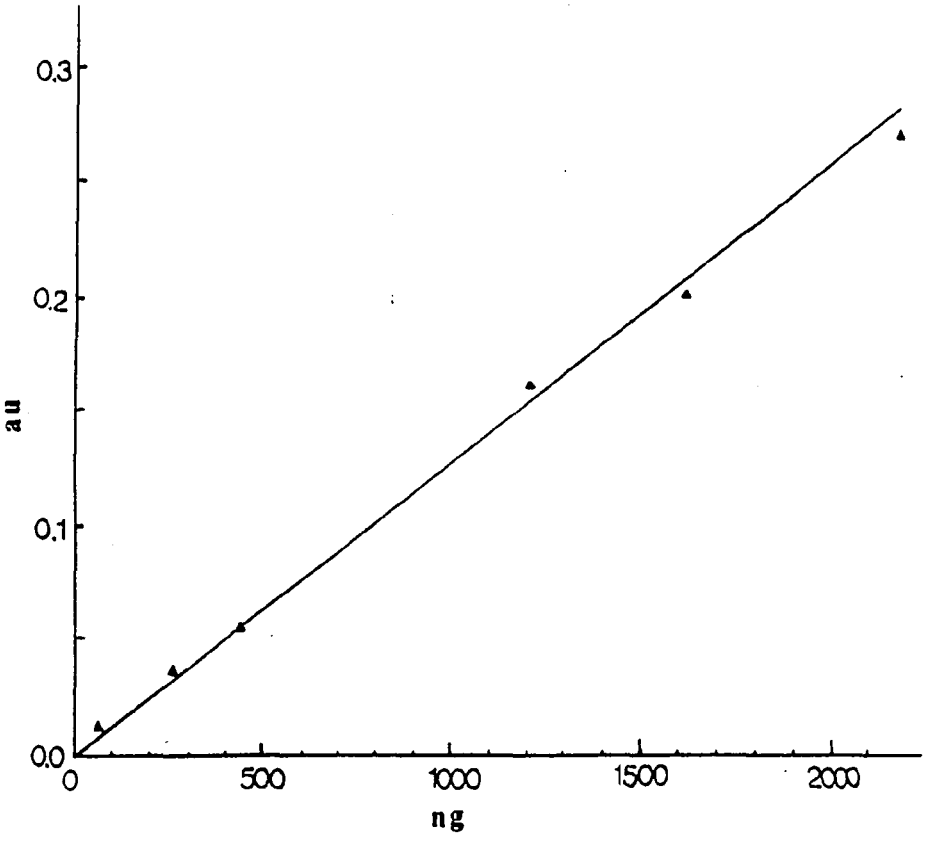

Figure 3-4 LC-UV calibration curve of benzoyl peroxide
The chromatographic conditions are the same as in Fig. 3-1.

TABLE 3-1

Analysis of Four Acne Preparations for BP

	% BP (standard deviation)		
Product	LC-ECD	LC-UVD	Iodimetry[a]
1	11.3 (0.301)	11.3 (0.288)	11.2 (0.0753)
2	11.6 (0.232)	11.9 (0.313)	11.3 (0.0696)
3	5.05 (0.203)	5.08 (0.262)	5.03 (0.0300)
4	5.17 (0.185)	5.13 (0.214)	5.10 (0.0254)

[a]corrected for 98.21% recovery

V. SUMMARY

The HPLC method is specific and reproducible and offer greater accuracy over current methods; furthermore, the most likely degradation products or impurities, benzoic acid and perbenzoic acid, do not interfere in the assay. The sample preparation is simple and the result is reliable. Suitable modifications of the procedure should make it applicable to benzoyl peroxide determinations in other types of samples.

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PART II

MULTI-MODE NORMAL BONDED-PHASE LIQUID CHROMATOGRAPHY OF POLYSTYRENES

I. INTRODUCTION:

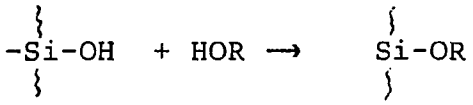
The most widely used column packings for high performance liquid chromatography(HPLC) are those with surface-reacted (chemically bonded) organic stationary phases. Bonded-phase chromatographic(BPC) columns were originally developed to eliminate the loss of mechanically-held liquid stationary phases from liquid-liquid chromatography columns. However, the unique features of these BPC packing now permit applications well beyond this initial intent.

Polar BPC packings are used for normal phase separations in much the same manner as silica packings. Samples of moderate to strong polarity are usually well-separated on the polar BPC packings. Reversed-phase BPC involves a nonpolar stationary phase, such as a C_8 or C_{18} hydrocarbon, used in conjuction with a very polar, such as aqueous methanol, mobile phase to separate a wide variety of less polar solutes.

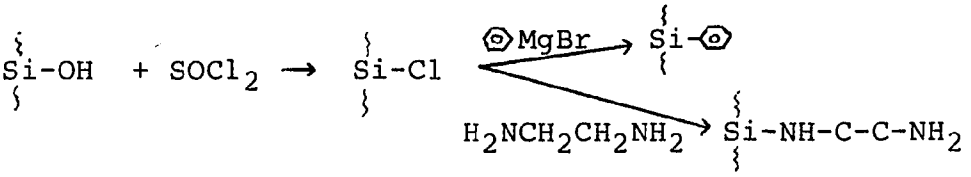
Almost all the reported BPC packings use rigid silica or silica-based supports that provide high column efficiency and excellent mechanical stability under high pressures. Bonded-phase packings are prepared by a variety of synthetic methods. The reactions that have been used to prepare silica-based bonded-phase packings are summarized in Fig. 1. Silicate esters (Fig. 1.1a) are prepared by direct esterification of silanol groups with

Figure 1. Reactions for preparing bonded-phase packings

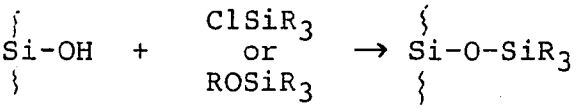
(a) Silicate esters



(b) Silicon-carbon and silicon-nitrogen



(c) Siloxanes



* Taken from Ref. 3

an alcohol (1). The esterification of the siliceous surface with alcohols produces the so-called "brush" packings (2). These suffer the disadvantage of the relatively easy solvolysis of the Si-O-C bond by polar solvents (3).

Other reactions result in covalent Si-C or Si-N bonds being formed (Fig. 1.1b). These materials are prepared by first chlorinating the silanol groups of the support with thionyl chloride. This chlorinated silica can then be reacted with a Grignard reagent or Wurtz and other similar reagent to produce Si-C bonds (4), or with amines to produce Si-N bonds (5). In both reactions the organic is bonded directly to the silica; therefore, if the primary organic layer attached to the silica is itself reactive, such as an aromatic hydrocarbon, its reactivity can in principle be used to introduce further substitution of any desired functionality, such as an ion exchanger (4,6). Hydrolytic and thermal stability are markedly superior for the Si-C and Si-N bonded phases versus the silicate esters. However, Grignard reactions sometimes produce a relatively low concentration of surface organic, and may leave undesired residues. Also, the Si-N bonded-phase are restricted to the pH 4-8 range when aqueous solvents are used.

The most widely used BPC packings are those based on siloxanes. Fig. 1.1c shows that siloxane-type (Si-O-Si-C)

bonded-phase packings typically are prepared by reacting the silanol groups of the support with organochlorosilane or organoalkoxysilane reagents. Bonded-phases of this type are hydrolytically stable throughout the pH range 2-8.5.

The organic bonded-phase siloxane coating can be made as a mono-molecular layer or as a polymerized multi-layer coating. The mono-molecular layer-type packings are usually prepared by reacting organochlorosilanes with the porous siliceous support. This type of packing has a high surface area so that adequate sample retention and capacity can be achieved without polymerization of the organosilane. On the other hand, to achieve a sufficient volume of bonded-phase coating on low-surface-area pellicular particles, polymerization may be required.

It is a recent trend that the separation of a sample of unknown composition is first attempted on a reversed-phase BPC with an alkyl-substituted packings (eg. C_8 and C_{18}). The major advantages of reversed-phase chromatography have been reviewed by Majors (7). These advantages include predictable elution order; relatively inert and fairly reproducible column packings and inexpensive solvents. Although reversed-phase packings are supposedly used in nearly 70% of HPLC applications (7), the usefulness of normal bonded-phase packings should not be overlooked. The major advantages of normal phase chromatography are summarized by Abbott (8) and include

better ability to separate isomers and to obtain class separations; greater mobile phase volatility allowing simpler and more efficient concentration and transfer steps in off-line fraction collection and subsequent structural characterization of solute peaks; lower viscosity of organic mobile phases, resulting in lower operating pressures; greater compatibility of organic mobile phases with on-line coupling to electron-impact mass spectrometry. In addition to above mentioned advantages, in some cases the same normal bonded-phase packing can behave as a reversed-phase substrate, a normal phase adsorbent, and a size exclusion medium, depending on the selection of mobile phase composition (9-11).

In this study, we used a normal bonded PAC phase to study the interaction mechanisms of polystyrene samples of different molecular weight. The PAC bonded-phase is composed of a hydrocarbon chain containing both secondary amino and cyano groups in a ratio of 2:1 (NH : CN) according to manufacturer.

II. EXPERIMENTAL SECTION:

A Varian 8500 liquid chromatographic pump was used with a 25 cm X 4.6 mm Whatman Partisil-10 PAC (10um) column at ambient temperature. The Varian Varichrom UV detector was set at 254 nm. The isocratic mobile phase compositions and flow rates are listed in the figure captions.

Polystyrenes samples were obtained from Pressure Chemical Company, and were dissolved in a solvent of the same composition as the LC eluent, at an approximate concentration of 1 mg/ml; 2 ul aliquots were injected.

Dichloromethane(DCM) and isooctane were Burdick & Jackson Distilled-in-Glass or equivalent HPLC grades and were degassed with a stream of high purity helium.

III. RESULTS AND DISSCUSSION:

Table 1 lists the LC retention volumes and calculated capacity factors k of polystyrene polymers with different molecular weights. These values were obtained with two different compositions of mobile phase operated under the same LC conditions. Mobile phase A is a mixture of DCM and isooctane in a ratio of 51 to 49. We call it a "poor solvent". Thermodynamically speaking, the dissolution process occurs if the free energy of mixing, ΔG, is negative(12):

$$\Delta G = \Delta H - T\Delta S$$

In a good solvent, the entropy of mixing, ΔS, is positive for polymer solutions. If the enthalpy of mixing, ΔH,is negative (meaning there is a net positive attraction favoring solvent/solute pairs), then dissolution will occur at any temperature. When the polymer is in a poor solvent (e.g. ΔH is positive), the thermodynamics of dissolution depends on the temperature. Dissolution can occur at high temperatures since the negative $T\Delta S$ term becomes dominant. In a poor solvent, the PAC bonded-phase behaves as an adsorbent, and the elution sequence follows the order of polymerization. On the other hand, mobile phase B is pure DCM, which is a "good solvent" according to our defination. In this solvent, the elution order of polystyrene polymers reverses and a size exclusion elution order is observed, i.e. the PAC bonded-phase behaves as a

Table 1. Retention volumes and capacity factors of polystyrenes in different solvents

MW	Solvent A (good solvent) V_R	Solvent A (good solvent) k	Solvent B (poor solvent) V_R	Solvent B (poor solvent) k
800	5.49	0.58	6.07	0.74
2K	4.87	0.40	6.96	1.00
4K	4.63	0.33	7.46	1.20
9K	4.13	0.19		

Solvent A: 100% DCM
Solvent B: 51/49 DCM/isooctane
V_R: Retention volume in ml
k: Capacity factor, $k= (V_R-V_o)/V_o$
V_o: Elution volume (ml) of unretained solute, which is 3.48 ml for the column used
MW: Molecular weight of polystyrene

molecular sieve.

Figs. 2 and 3 are plots of theoretical plate height (H) versus mobile phase flow rate (u) for polystyrene polymers in a good or poor solvent, respectively. Each curve in these plots is of the same form, i.e. the plate heights first decrease with the increasing flow rate, then reach a minimum value, and finally increase as flow rate increase.

There are five major processes that contribute to band broadening in modern liquid chromatography. The total theoretical plate height H can be represented as(13):

$$H = H_l + H_e + H_m + H_s + H_{sm} \quad ---(1)$$

where H_l, H_e, H_m, H_s and H_{sm} are the plate height contributions due to longitudinal diffusion, Eddy diffusion, and mobile phase, stationary phase and stagnant mobile phase (the mobile phase contained within the pores of the packing material) mass transfer processes, respectively. The plate height factors given in equation 1 represent a general rather than a comprehensive account of the column dispersion processes, each of these terms can in turn be related to such experimental variables as mobile-phase velocity u, particle diameter of column packing d_p, the sample diffusion coefficients in the mobile phase, D_m, and stagnant mobile phase, D_s, and the thickness d_f of the stationary phase, according to Giddings (14) showed that

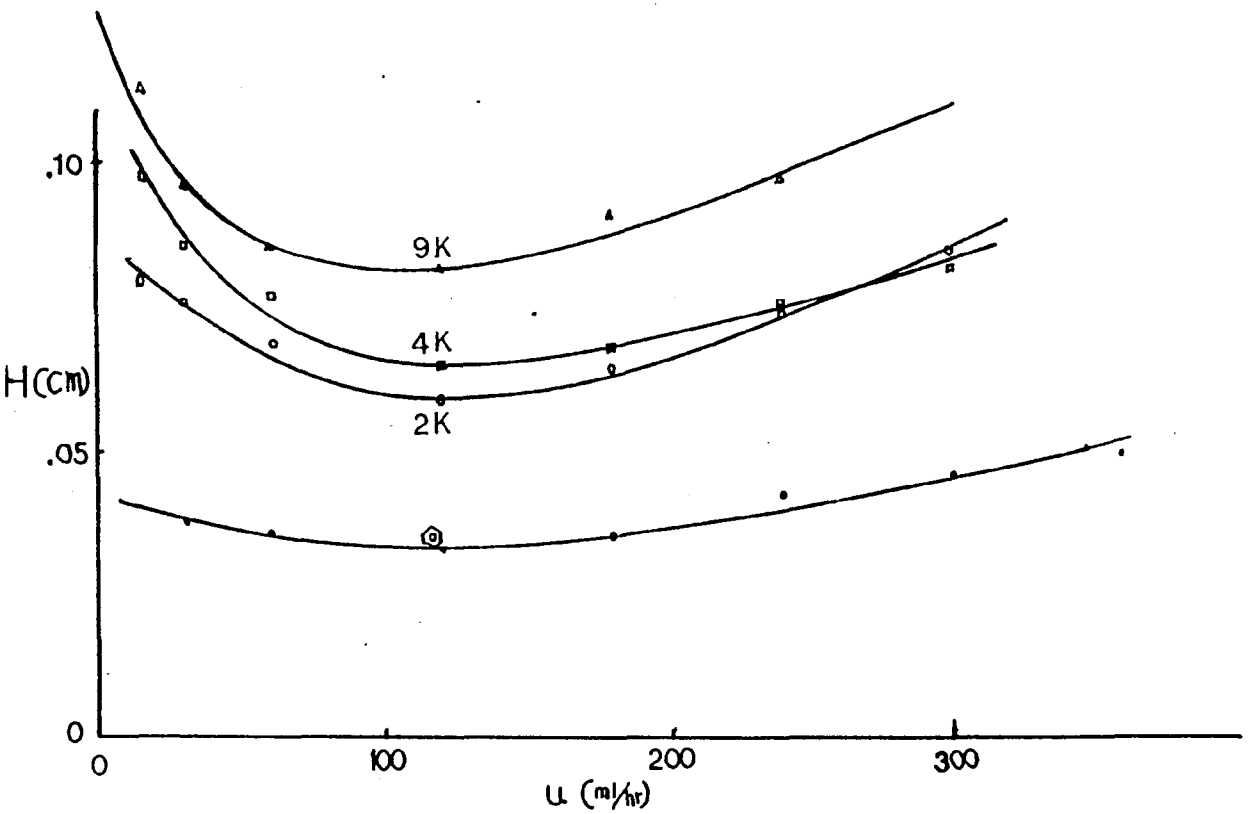


Figure 2. Plots of plate height against the mobile phase flow rate for polystyrenes in the good solvent

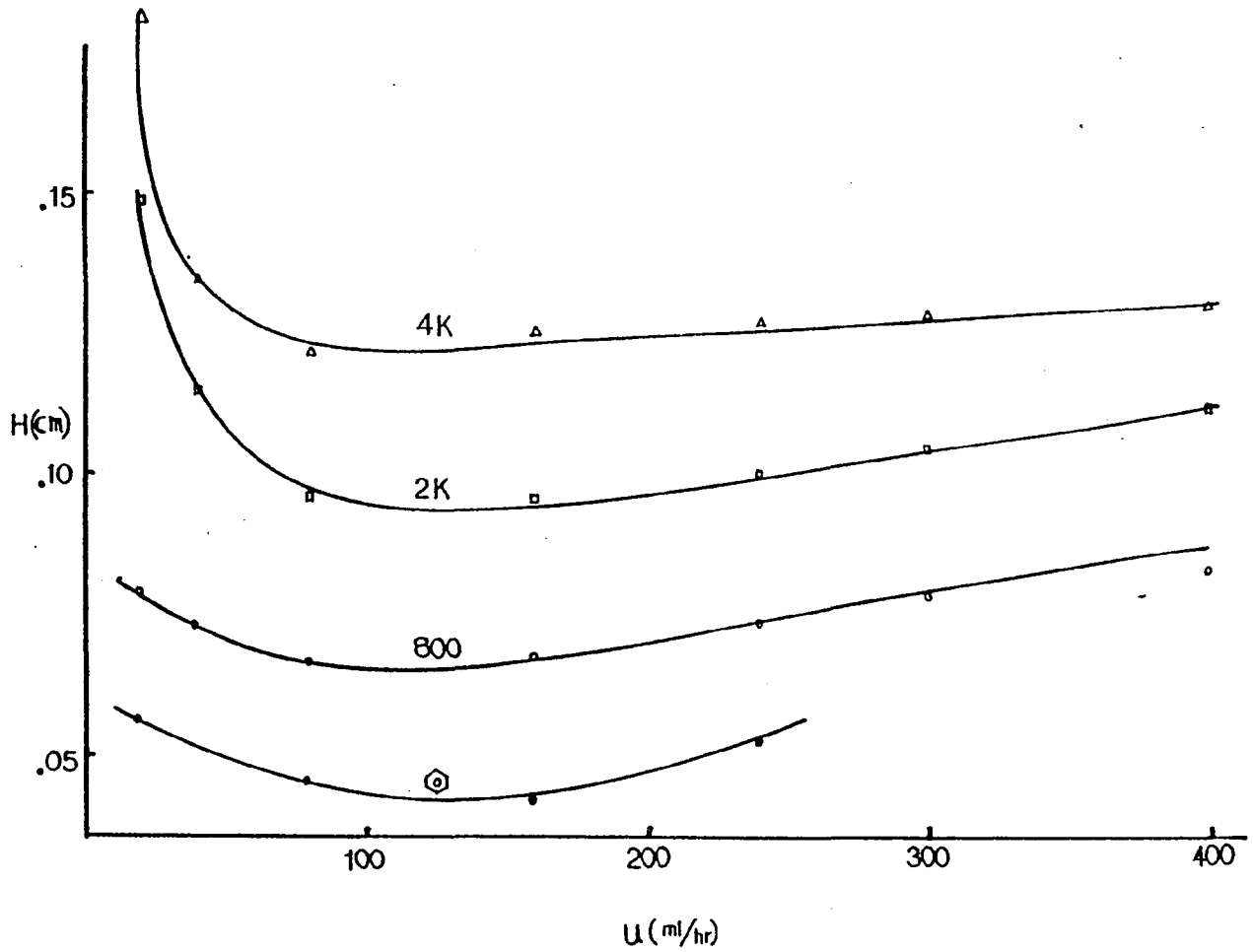


Figure 3. Plots of plate height against the mobile phase flow rate for polystyrenes in the poor solvent

$$H = C_1\frac{D_m}{u} + C_s\frac{u\,d_f^2}{D_s} + C_{sm}\frac{u\,d_p^2}{D_m} + \frac{1}{\dfrac{1}{C_e d_p} + \dfrac{D_m}{C_m u\,d_p^2}} \quad --(2)$$

where C_1, C_s, C_{sm}, C_e and C_m are the coefficients of the respective dispersion terms in the plate height equation 1. Knox (15) showed that for most practical purpose equation 2 may be approximated by

$$H = Au^{0.33} + B/u + Cu \quad ----(3)$$

In equation 3, the parameters A, B and C are largely dependent on the diffusion rate of the solute, the nature of the mobile phase, the packing material particle size and the packing configuration of the column. The first term in equation 3 originated from the coupling term in equation 2 with some simplification. The parameter A characterizes the regularity of the packing; for a good column, A should be small in value. At high velocities, this term approaches a constant value whereas it tends to zero as the flow velocity tends to zero. At very low flow rates, the B/u or longitudinal diffusion term dominates the value of H, so in this region H is inversely proportional to the flow velocity u of the mobile phase. On the other hand, when the system is operated at high flow rates, the B/u term is insignificant, and the Cu or the mass transfer term predominates. Here, H is directly proportional to the flow rate u.

In terms of the reduced parameters introduced by Giddings (14), the reduced plate height $h = H/d_p$ and the reduced mobile phase velocity $v = ud_p/D_m$. We can write an equation in which the constant terms become either universal parameters or in a form that allows numerical comparison, that is

$$h = av^{0.33} + b/v + cv \quad \text{----(4)}$$

where a, b and c are the coefficients of the respective terms. The parameters a and c reflect the importantce of dispersion within the flowing part of the mobile phase, and dispersion due to non-equilibrium between the flowing and stationary parts of the column, respectively. a is expected to have a weak dependence on the capacity factor k , and a strong dependence on the goodness of packing, while c should depend more strongly upon k (16).

With the reduced parameters, H-u plots in Figs. 2 and 3 can be reformed to give the h-v plots shown in Figs. 4 and 5. The diffusion coefficients used to obtain the reduced velocities, listed in Table 2, were calculated using the Wilke-Chang equation (17), and are corrected both for molecular weight differences between polymers and viscosity differences between solvents (18).

The experimental plate height or reduced plate height is a measure of the total chromatographic band broadening. With polydisperse polymers, molecular weight dispersion can contribute to band broadening. For a column

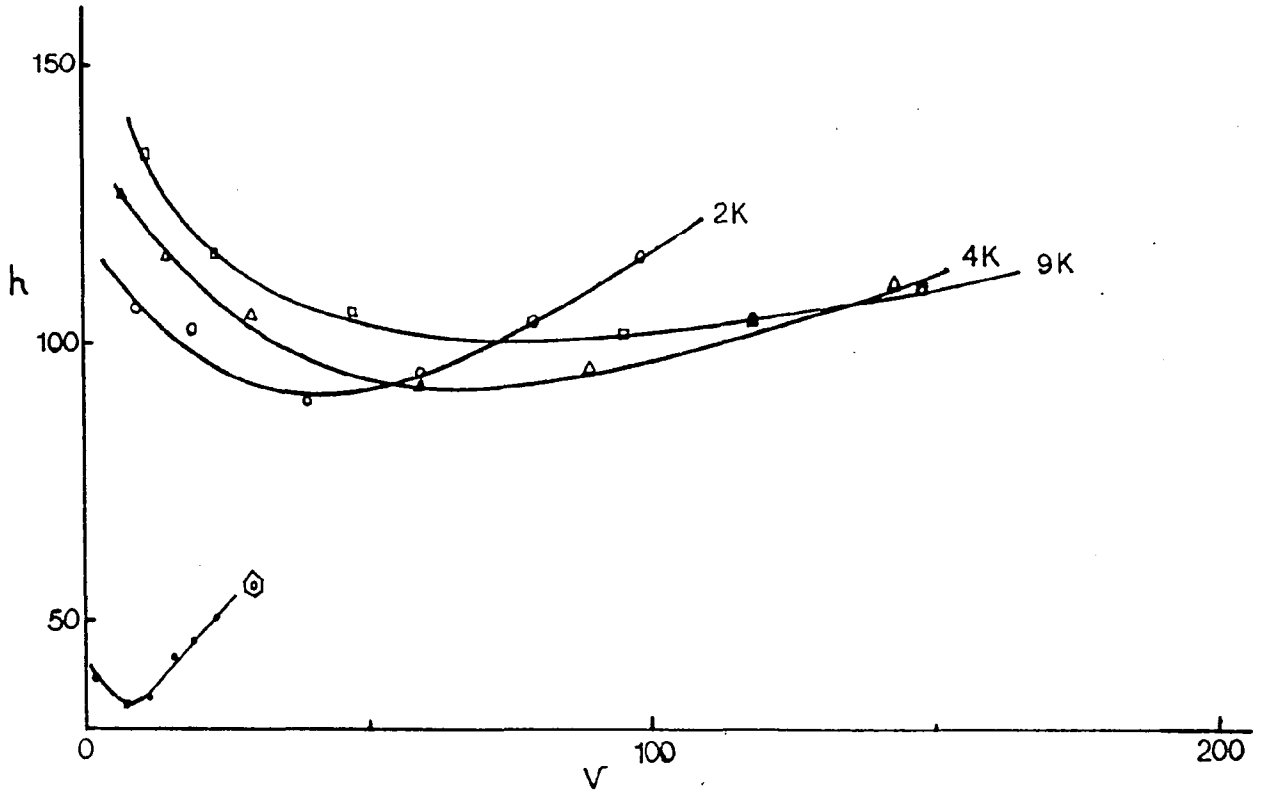


Figure 4. Plots of h against v for polystyrenes in the good solvent

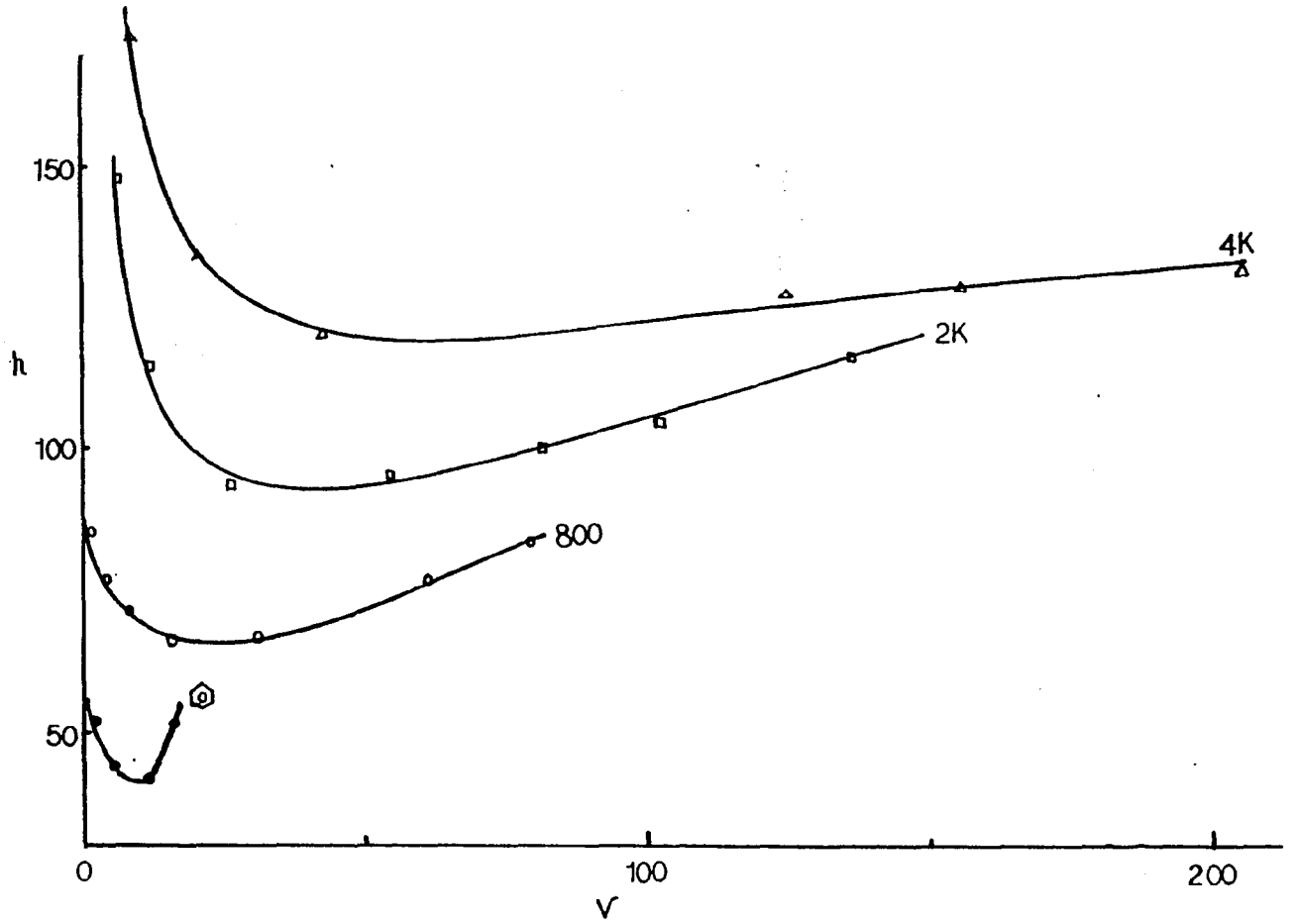


Figure 5. Plots of h against v for polystyrenes in the poor solvent

Table 2. Diffusion coefficients of polystyrenes in different solvents

Molecular wt. of polystyrene	D_m in the "good solvent"	D_m in the "poor solvent"
800	1.09×10^{-5}	1.05×10^{-5}
2K	6.30×10^{-6}	6.08×10^{-6}
4K	4.16×10^{-6}	4.01×10^{-6}
9K	2.56×10^{-6}	2.47×10^{-6}

D_m: Diffusion coefficient of polystyrene in the mobile phase, in unit of cm^2/sec

functioning as size exclusion medium, the polydispersity contribution to band broadening is independent of the mobile phase velocity and can be estimated by using an equation derived by Knox and McLennan (19):

$$h_{poly} = (L/d_p)(P-1)(1+w)(S/V_R) \quad ---(5)$$

where L is the column length; V_R is the elution volume; P is the polydispersity of the polymer, defined by $P=M_w/M_n$; M_w and M_n are the weight- and number-averaged relative molecular masses of the polymer; w is a weak function of (P-1), calculated from

$$w = (11/4)(P-1) + (137/12)(P-1)^2 \quad ------(6)$$

and S is the corresponding negative inverse slope at mass M of the calibration curve for the polymers (shown in Fig. 6) expressed as $-d\ V_R/d\ \ln\ M$, where M is the average molecular weight of the particular polymer.

The calculated h_{poly} values together with the polydispersities of polystyrene samples are listed in Table 3. The h_{poly} values were subtracted from the experimental h values in Figs. 4 and 5, respectively, and the results expressed as h_{kin} (kinetic contribution of band broadening). Logarithmic plots of h_{kin} versus v are shown in Figs. 7 and 8.

Although the magnitude and the relative importance of each plate height contribution from various dispersion mechanisms vary from one LC system to another, the general functional dependence of each contribution to reduced

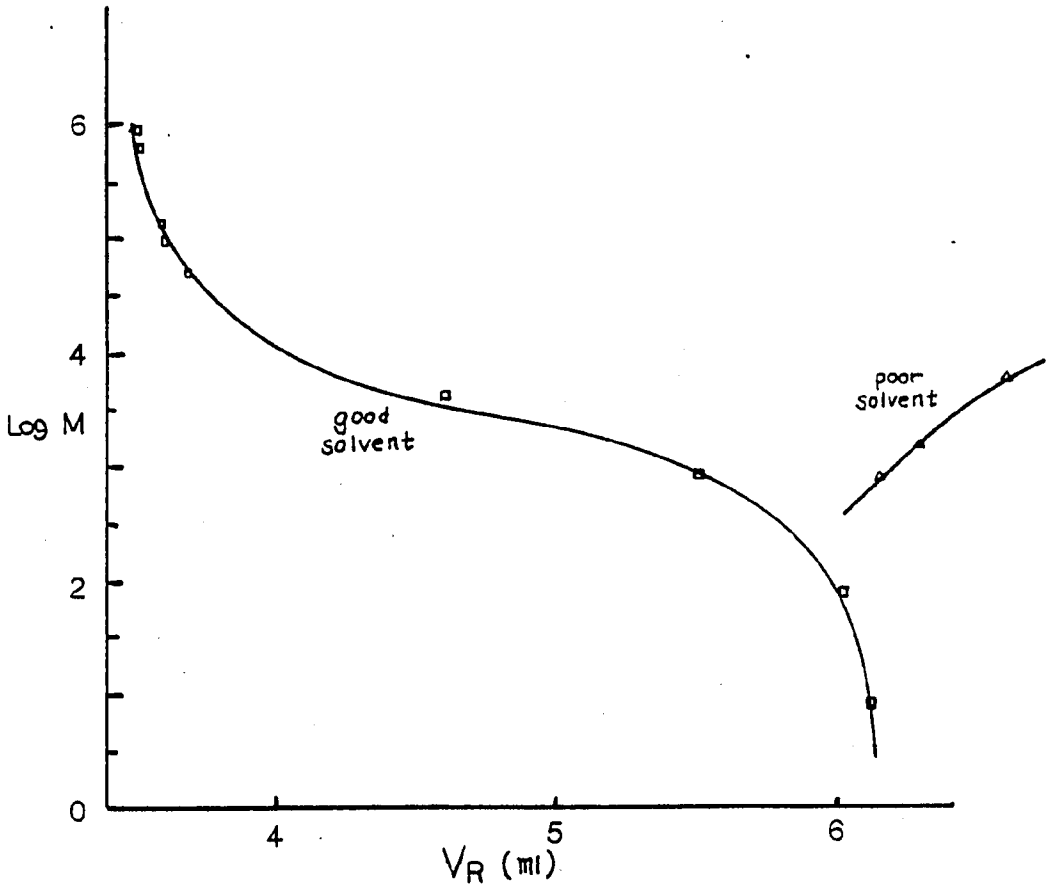


Figure 6. Molecular weight calibration curves for polystyrenes in both good and poor solvents

Table 3. Calculated polydispersity and h_{poly} of polystyrenes

Mol. wt. of polystyrene	P	h_{poly} in the good solvent	h_{poly} in the poor solvent
800	1.30	80	12
2K	1.06	30	3
4K	1.06	36	8
9K	1.06	19	

P : Polydispersity

h_{poly}: Polydispersity contribution of band broadening

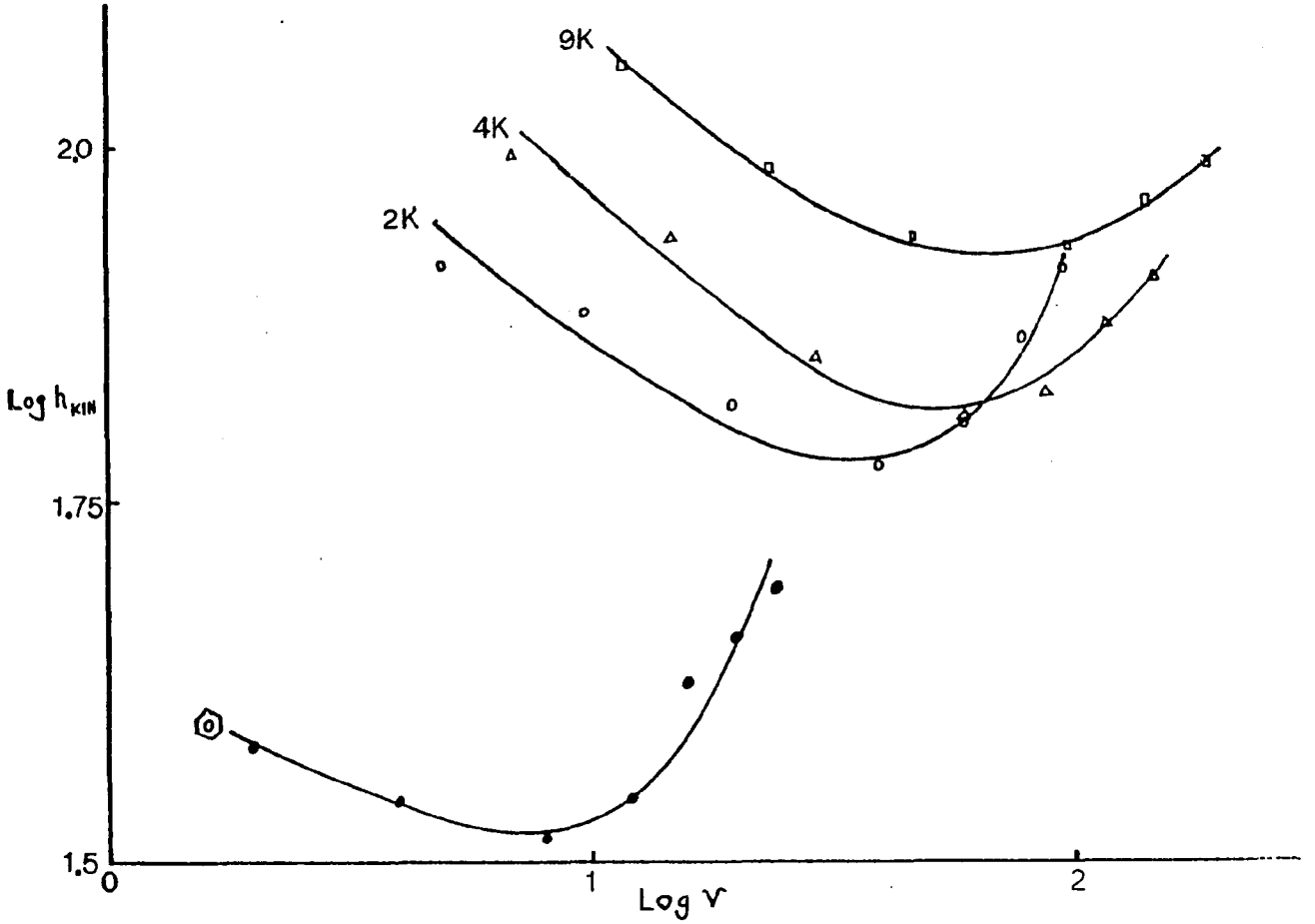


Figure 7. Double logarithmic plots of the reduced plate height(h_{kin}) against the reduced velocity for different polymers in the good solvent

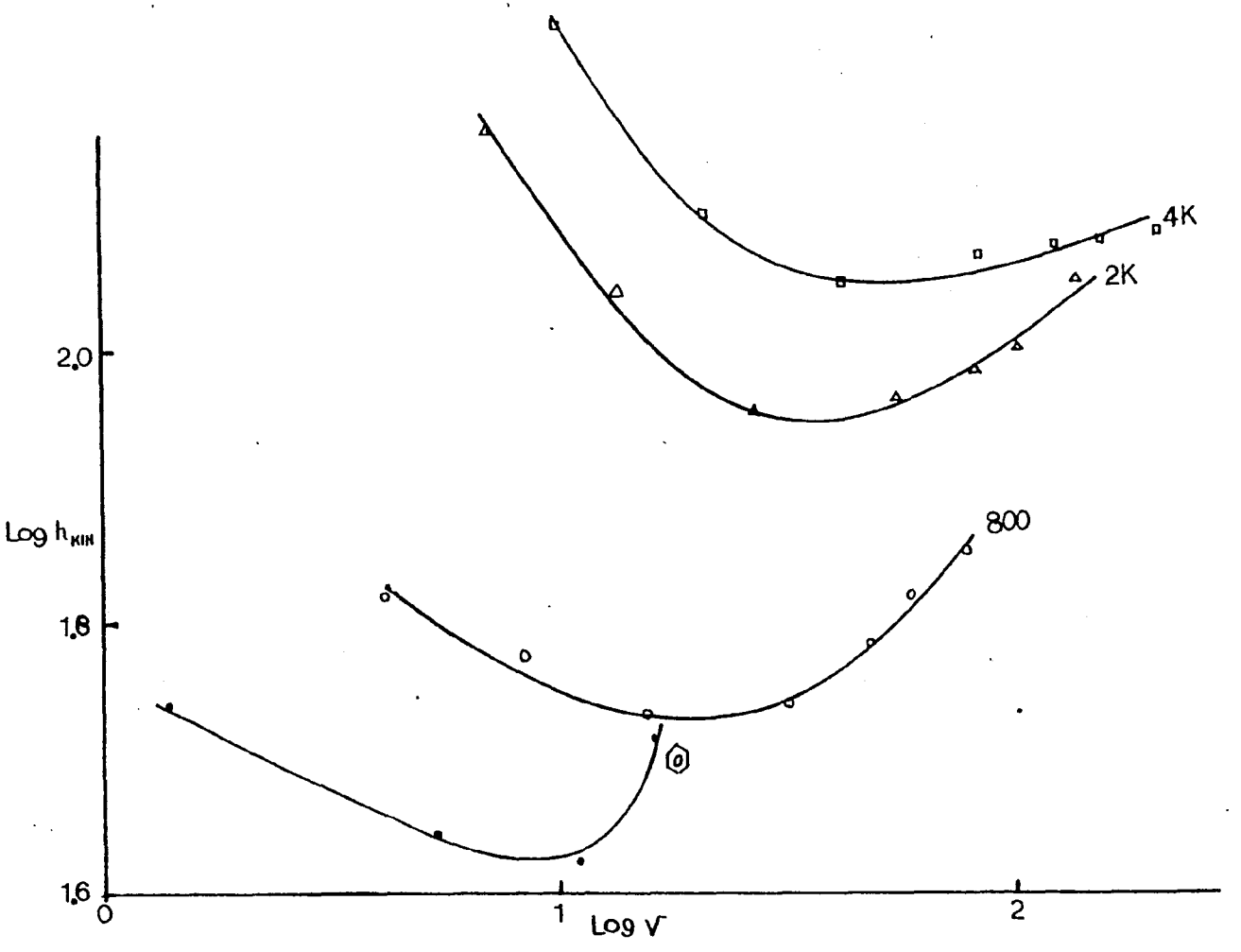


Figure 8. Double logarithmic plots of the reduced plate height(h_{kin}) against the reduced velocity for different polymers in the poor solvent

plate height (h) on reduced flow velocity (v) can be depicted by the plot shown in Fig. 9. Theoretically, values of a, b and c for any particular column can be obtained by curve fitting, but it has to be emphasised that very high quality data are required covering at least a hundredfold range of v before much confidence can be placed on the values obtained. One can, nevertheless, obtain useful qualitative information from the form of the h-v curves.

Values of a, b and c obtained from h-v curves in Figs. 7 and 8 are listed in Table 4. In this table, these values from benzene curves in both solvents were not included, since insufficient data was collected.

In Table 4, in the same solvent, the b values of high-molecular-weight polymers are higher than those of the low-molecular-weight. With the same solute, the value of b is higher when it is in a better solvent. As we mentioned, in equation 4, the b/v term is account for the band broadening causing by axial diffusion of the solute molecules. Knox (20) presented b as $b = 2r(1+k)$, here, r is the obstructive factor. In the same reference, Knox noted that there is some doubt as to whether b is strongly dependent on k. From our results, we found that larger k value does not lead to a greater b value. Therefore, we know that the obstructive factor r is the most important factor that determines the value of b. The values of b in

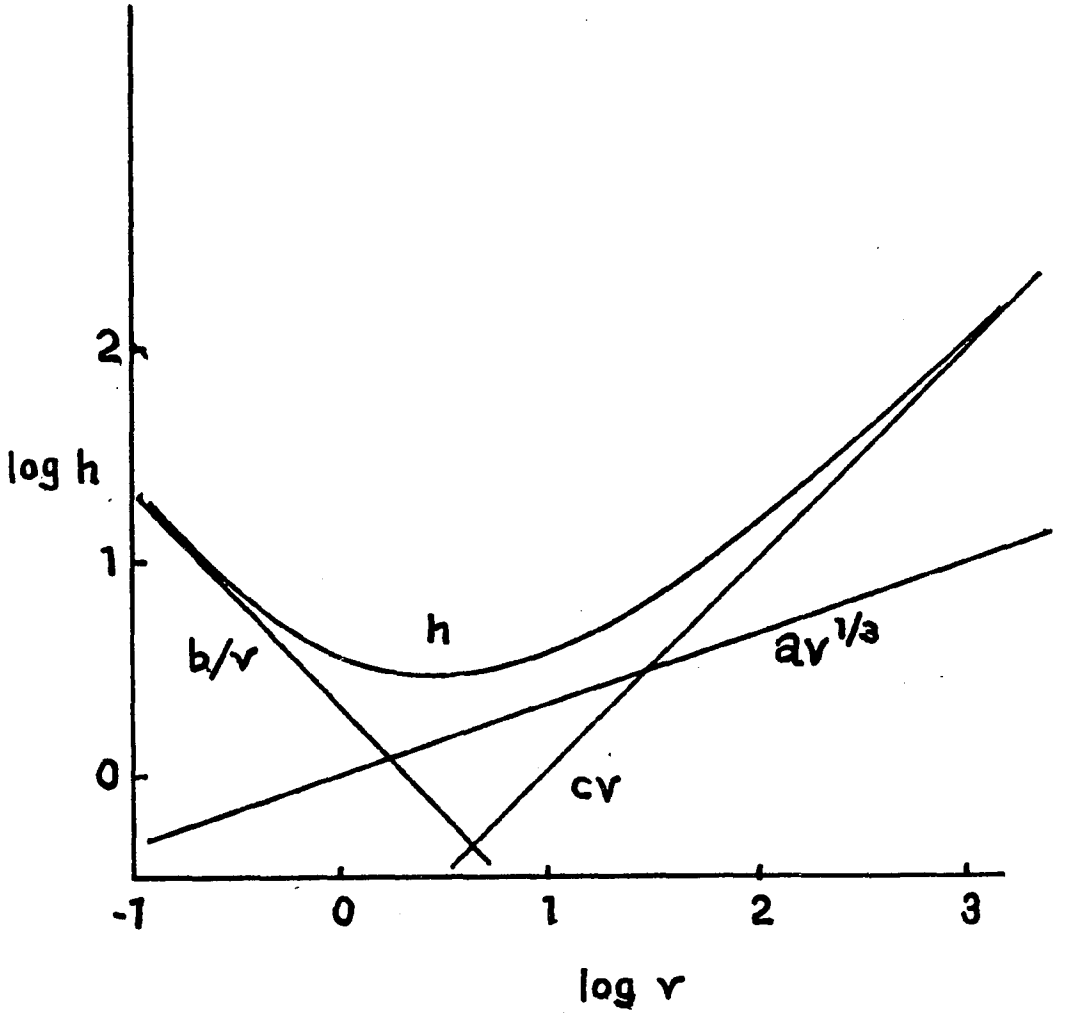


Figure 9. Double logarithmic plot of reduced plate height, h, against reduced velocity, v, showing contributions from factors a,b and c (taken from Ref. 18)

Table 4 are much higher than Done and Knox's (16) results. To explain this observation, we assume that some very slow process can occur to the polymer molecules during their migration along the column. Knox and McLennan (21) suggested that these processes might be the partial penetration of otherwise excluded polymer molecules into the outermost pores of the packing material. Since this would require partial unravelling of the polymer chain, the longer the molecular chain the more likehood that molecule could be trapped in this way. In other words, the obstructive factor r is effectively greater in value for large-molecular-weight polymers. By the same theory, when the same molecule is in a better solvent, its chain become more extended, which will give it a better chance to partially penetrate the pores of the packing, and a higher b value results. Table 5 is a list of particle sizes of different polystyrenes in tetrahydrofuran (a good solvent). Comparison of these values to the average pore size of the PAC column packing, 40 A, shows that these polymers are in the same size range as the column packing. Thus the partial penetration model postulated is plausible, and explains the high b values in Table 4.

The c values in Table 4 represent the zone dispersion from slow mass transfer of solute in the stationary phase. A general expression for c used by Knox (20) is

Table 4 Calculated a, b, c and $k/(1+k)^2$ values of polystyrenes in different solvents

(1) Polystyrenes in the good solvent

MW	a	b	c	$k/(1+k)^2$
2K	4.86	780	0.55	0.204
4K	8.24	1130	0.21	0.187
9K	15.8	1186	0.081	0.134

(2) Polystyrenes in the poor solvent

MW	a	b	c	$k/(1+k)^2$
800	14.7	237	0.072	0.244
2K	22.9	675	0.024	0.250
4K	29.7	1000	0.011	0.248

MW: Molecular weight of polystyrene

Table 5. Particle sizes of polystyrenes in tetrahydrofuran

Mol. wt.	R_g (A)	r (A)
1,250	9	9
2,250	13	12
4,000	18	16
10,300	32	28
20,400	47	42
51,000	81	72
97,200	119	105
160,000	159	141

*Taken from Ref. 12

R_g: Radius of gyration

r : Radius of equivalent hard sphere

$$c = q \frac{k}{(1+k)^2} \frac{D_m}{D_s} \frac{d^2}{d_p^2} \qquad ---(7)$$

where q is a configuration factor and d is the average pore depth of the column packing. With the same column, q and d_p are constants.

In the poor solvent, as listed in Table 4, there is little difference among the $k/(1+k)^2$ values of the three polystyrenes. In this case the differences among their c values can only arise from the differences in either their d values or D_m/D_s ratios, or both. To find this out, assume for the moment d is a constant; the value of c should be directly proportional to the ratio of D_m/D_s. Van Krefeld and Van den Hoed (22) and Knox (21) found that large-molecular-weight polymers have higher D_m/D_s ratios in columns packed with different pore size packings. Their results showed that c can not be proportional to D_m/D_s ratio only, since this will lead to a reversed order in c values in our poor solvent case. In other words, the pore depth d can not be a constant for all the polymers studied here.

In most liquid chromatography, the size of solutes is much smaller than the average pore diameter of the column packing. In theory, d is the maximum diffusion range of the solutes in the stationary phase. In this study, because the sizes of the polymers are so close to the average pore size of the packing, the larger the solute

molecule the less deep the pore that can be reached by the molecule. This means that d is effectively smaller in value for large polymers. In other words, the small polymer molecules have extra space inside the pores to travel within. This will give rise to additional band broadening and a higher value of the c parameter. In the literature, the same correlation was observed by Giddings (23). In his study, polystyrenes of the same size range were eluted from a column with a 40 A pore size. Theoretically, when the pore size becomes very large compared to the particle sizes of the solutes, the parameter d becomes a constant, and the value of c will be proportional only to the ratio of D_m/D_s. In this case, the large polymers will have greater c values, as seen by Kirkland (24). In his study, a combination of 6 columns was used, with average pore sizes up to 3500 A. In another experiment, when packings of medium pore size were used, the c values of different polymers were very close, because of the compensatory effects between the variations of d and D_m/D_s. This phenomenon was observed by Knox and McLennan (21) when they were using a 120 A pore size column.

Above mentioned was the case for which polystyrenes are eluted using a poor solvent. In a good solvent, the values of $k/(1+k)^2$ are different for different polymers. Since small polymers have higher values of $k/(1+k)^2$, their

c values become even larger, resulting in a more rapid increase in reduced plate height at high flow velocities, as seen in Fig. 7.

If we compare the c values of the same solute, e.g. PS2K (polystrene with average molecular weight of 2000), in the two different solvents, we find that the polymer has a smaller c value in a poor solvent. In such a solvent, the polymer will have a smaller particle size, i.e. it should have large d value, which results in a reversed order of c values compared to the values listed in Table 4. Also, the difference in $k/(1+k)^2$ (Table 4) would lead to the same result. In equation 7, besides d and $k/(1+k)^2$, the only parameter left that could possibly give a polymer a smaller c value in the poor solvent is the D_m/D_s ratio, which must obey

$$\frac{D_m{}^g}{D_s{}^g} > \frac{D_m{}^p}{D_s{}^p} \qquad \text{-----(8)}$$

here superscripts g and p indicate that is in the good or poor solvent, respectively. In a chromatography system, if the kinetics are controlled solely by the solute diffusion rates, there should have no change in the D_m/D_s ratio arising from a change of solvent . Therefore, equation 8 implies that there are other processes in the system which influence solute mass transfer in the column. Most likely, these are slow solute adsorption-desorption processes. Since the adsorption-type interactions will obstruct the

transfer of solute particles, and since the degree of retardation will depend upon the size and shape of the solute, the nature of the column packing and the goodness of the solvent. Therefore, due to the adsorption effect, the ratio of D_m/D_s can vary for the same polymer, in different solvents. In general, the desorption rate of a solute is dependent on the solvent. Thus a good solvent will always reduce the adsorption-desorption effect. If we assume the D_m/D_s ratio of a polymer in the good solvent is unaffected by the adsorption processes, then the change in the D_m/D_s ratio of the same polymer in the poor solvent will give us an idea how the adsorption affects the solute transfer in the column. In equation 8, the D_m/D_s ratio of a polymer becomes smaller when it is in a poor solvent, since the adsorption-desorption process will obstruct the mass transfer rates in both mobile and stagnant mobile phasees, thus it will reduce both D_m and D_s. In this case, the only situation that a polymer can have a smaller D_m/D_s ratio in a poor solvent is to have a smaller D_m value and a relatively unchanged D_s value. In other words, the influence of adsorption-desorption process is more important in the mobile phase than that in the stagnant mobile phase.

The normal bonded PAC column used in this study was not developed to separate high molecular weight polymers. The average pore size of the packing is too small to

separate the polystyrenes effectively, this leads to high values of a and b in equation 4. Since the purpose of this study is solely to study the mechanism, these abnormal values are tolerable.

IV. SUMMARY:

The versatility of normal bonded-phase liquid chromatography makes itself a useful tool in chemical separations. The potential of normal bonded-phase LC has not been fully explored. Better information about the interaction mechanisms between the packing and solutes will lead to the use of this technique more frequently and more effectively.

Normal bonded-phase packings not only retain the advantages of normal phase liquid chromatography but also increase the inertness and the sample compatibility of normal phase packings. Althought the complexity of the interaction mechanisms of the normal bonded-phase column packings makes it hard to predict chromatographic behavior. This multi-functional activity also gives normal bonded-phase a potential leading edge in the separation of complex mixtures. The PAC phase is only a example, as more bonded phases with diversified functional groups become available, the versatility of LC should become more recognizable.

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