A Comparison of Polymer Separation Efficiency and Resolution by Gradient LC, GPC and TLC

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ABSTRACT

The specific resolution of gradient LC and reversed phase TLC methods for the separation of different molecular weight standards of poly(isoprene), poly(ethylene glycol), poly(ethylene oxide), poly(styrene) and poly(α-methylstyrene) were determined. It was found that gradient LC has an order of magnitude greater resolving power (for high polymers) than gel permeation chromatography (GPC) while TLC had from two to five times the resolving power of GPC in the molecular weight range investigated. This is a direct result of the greater selectivity of gradient LC and TLC techniques. The specific resolution is also dependent on the type of gradient used to achieve fractionation for the LC technique.

INTRODUCTION

Since the first successful fractionation of synthetic poly-

mers using cross-linked poly(styrene) "gels" was demonstrated by

Moore in 1964 (1), gel permeation chromatography (GPC) has

experienced a phenomenal growth and has become one of the more

widely used secondary techniques for determining the molecular

weight and molecular weight distribution of polymers. In spite of

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its relative ease and popularity, GPC gives relatively poor reso-
lution and has a limited peak capacity compared to conventional
LC (2).

Recently an effective method for the fractionation of homo-
polymers by gradient LC and/or reversed phase TLC was demonstrated
(3-6). These techniques are, in principle, modern versions of the
earliest solvent-based fractionation methods (7,8). In addition,
a theoretical and experimental evaluation of this technique and
its "precipitation" mechanism was completed (4-6). The theory
indicates that this technique should have excellent resolving
power over a wide range of molecular weights (6). Although,
resolving power is an important part of any polymer fractionation
method, the ultimate use of a technique for the determination of
molecular weights and molecular weight distribtuions must consider
other factors as well (e.g., accuracy, precision, cost, speed,
etc.). In this study we focus on the relative efficiency and
resolution of homopolymer separation by gradient LC, GPC and TLC.

MATERIALS

KC18F reversed-phase TLC plates (5x20 cm) and Partisil 10,
ODS-3 reversed-phase columns (25 cm long) were obtained from What-
man Chemical Separation Division, Inc. HPLC grade methanol and
methylene chloride were obtained from Baker; Certified ACS grade
1,4 dioxane and ethylene glycol were obtained from Fisher Scien-
tific Company. The following polymer standards were used: (1)
poly(styrene) from Polysciences, Inc. mol. wt. = 10,000,000 (Mw/Mn
= 1.2), mol. wt. = 390,000, (Mw/Mn = 1.04), mol. wt. = 100,000

(Mw/Mn = 1.06) mol. wt. = 35,000 (Mw/Mn = 1.04), mol. wt. = 9,000

(Mw/Mn = 1.08), mol. wt. = 3570 (Mw/Mn = 1.06), mol. wt. = 2,000

(Mw/Mn = 1.06), mol. wt. = 17,500 (Mw/Mn = 1.04), mol. wt. =

63,000 (Mw/Mn = 1.04), poly(isoprene); from Polymer Laboratories,

mol. wt. = 1,360 (Mw/Mn = 1.11), mol. wt. = 11,100 (Mw/Mn = 1.08),

Mol. wt. = 33,300 (Mw/Mn = 1.05), mol. wt. = 113,800 (Mw/Mn =

1.05), mol. wt. = 260,000 (Mw/Mn = 1.07), poly(ethylene

glycol/oxide) from Polymer Laboratories, mol. wt. = 998 (Mw/Mn =

1.06), mol. wt. = 4820 (Mw/Mn = 1.04), mol. wt. = 11,250 (Mw/Mn =

1.07), mol. wt. = 73,000 (Mw/Mn = 1.02), poly(α-methyl styrene)

from Polymer Laboratories, mol. wt. = 19,500 (Mw/Mn = 1.15), mol.

wt. = 87,600 (Mw/Mn = 1.10), mol. wt. = 760,000 (Mw/Mn = 1.10),

and styrene oligomers A1000 from Toyo Soda Manufacturing Company,

LTD, Toso Building, 1-7-7 Akasaka, Minato-Ku, Tokyo, 107, Japan.

METHODS

All polymer standards were dissolved in the "good solvent"

(5 mg/ml). Dioxane was the good solvent for poly(ethylene

glycol/oxide) and methylene chloride was the good solvent for all

other polymers. TLC fractionations were done in an 11 3/4 in.

long, 4 in. wide, and 10 3/4 inch high chromaflex developing

chamber without prior equilibration with the solvent vapor nor

special treatment of the plates. Approximately 1 μℓ of solution

was spotted on the TLC plates and the solvent front was allowed to

move up the plate 10 cm from the spotting line. A Shimadzu dual

wavelength TLC scanner Model CS910 was used in the reflectance

mode to scan the plate. Other TLC chromatographic parameters and

Table I: Chromatographic Parameters of TLC Fractionations

Polymers	Solvents	Mobile phase Composition	Visualization Reagent	Scanning Wavelength (nm)
poly(styrene)	MeCl$_2$:MeOH	79:21 (V:V)	None	255
poly(isoprene)	MeCl$_2$:MeOH	75:25 (V:V)	None	200
poly(ethylene glycol/oxide)	Dioxan:Ethyl-lene glycol	58:42 (V:V)	1%I$_2$/MeOH	405

the wavelengths at which the polymers were scanned are provided in Table I.

All gradient elution HPLC fractionations were done with a Varian 5020 liquid chromatograph coupled to a Water Associates variable wavelength detector. Since methylene chloride has a UV cut off wavelength of 235 nm, only higher wavelengths can be used. The use of composition gradient elution prevents the employment of a refractive index detector. Consequently, only UV absorbing polymers could be detected. This type of fractionation is not limited only to polymers that absorb UV radiation, however. With the recent commercial introduction of the mass detector marketed by Applied Chromatography Systems, the detection of any polymer separated via gradient elution can be easily achieved. The chromatographic parameters for the HPLC fractionation of polymers are provided in Table II.

The resolution of GPC, gradient LC and TLC methods of fractionating polymers are compared using the following equation

Table II: Chromatographic Parameters of HPLC Fractionations

Polymers	Solvents	Gradient	flow rate	UV Wavelength (nm)
poly(styrene)	MeCl$_2$:MeOH	55% MeCl$_2$ $\rightarrow$ 100%MeCl$_2$ in 27.5 minutes	1ml/min	254
poly(isoprene)	MeCl$_2$:MeOH	45% MeCl$_2$ $\rightarrow$ 100%MeCl$_2$ in 25 minutes	2ml/min	240
poly(α-methyl-styrene)	MeCl$_2$:MeOH	72.5% MeCl$_2$ $\rightarrow$ 100%MeCl$_2$ in 27.5 minutes	1ml/min	254

derived for calculating specific resolution (9):

$$\underline{Rsp} = \frac{.576}{\sigma D_2} \qquad (1)$$

where σ is the standard deviation of the polymer standard peak
and D_2 is the slope of the linear portion of the calibration
curve. This equation has been used in GPC to compare the perfor-
mance of GPC columns containing different types of packing
materials (10). It should be noted that the specific resolutions
used in this study (from equation 1) are independent of column
length (9).

RESULTS AND DISCUSSION

A gradient LC separation of three poly(α-methylstyrene)
standards is shown in Figure 1. A densitometric scan of five
poly(styrene) standards separated by TLC is shown in Figure 2.
Baseline separation is easily achieved for these and a variety of
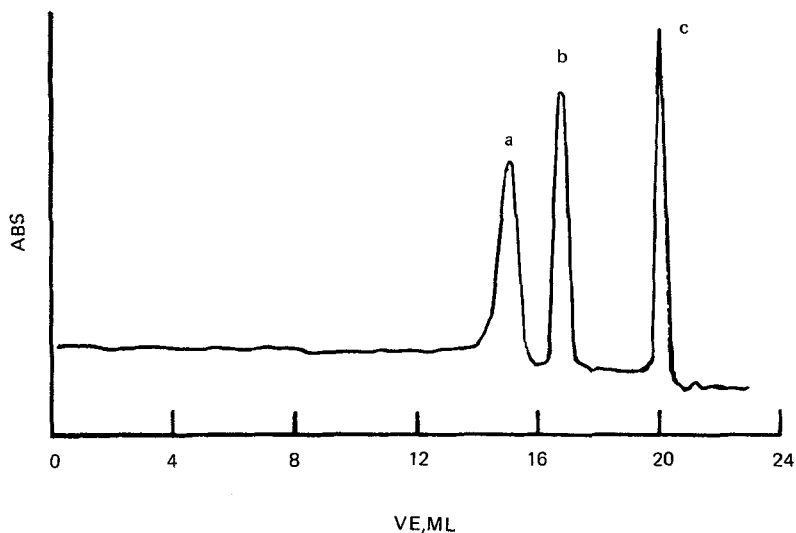
Figure 1:   A gradient LC separation of three different molecular

weight standards of poly(α-methylstyrene). The number

average molecular weight of peak c = 760,000; b =

87,000 and a = 19,500.

other polymers (4). In all cases there is a linear relationship

between the elution volume (Ve) in LC or R_f in TLC with the log

of the molecular weight of the polymer (3-6). Only for very high

molecular weight polymers does the relationship become nonlinear,

as predicted by theory (4,6). As a result of the relationship

between polymer retention and log molecular weight one can easily

use either technique as a comparative method for the determination

of molecular weights. One can also determine the distribution of

molecular weight and different molecular weight averages using

various techniques of peak slicing and integration, as will be
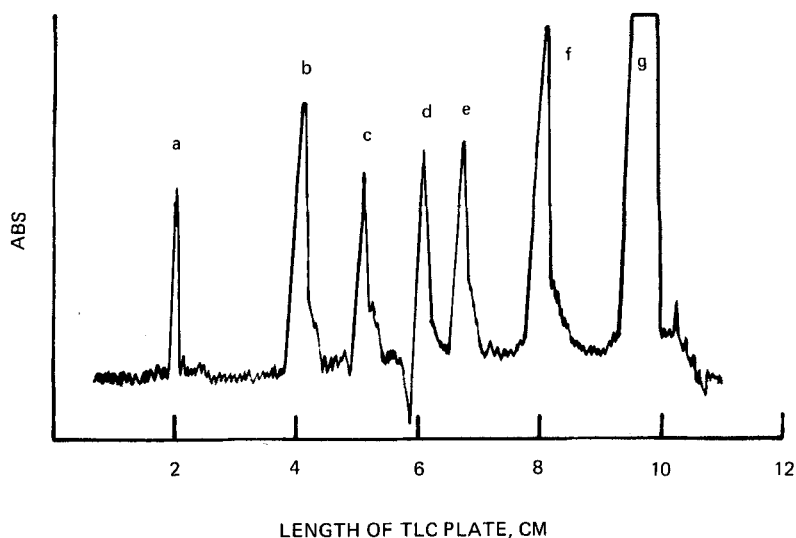
Figure 2: A densitometric scan (260 nm) of five styrene polymers

separated by reversed phase TLC. The number average

molecular weight of peak b = 10,000,000; c = 390,000;

d = 100,000; e = 35,000 and f = 3,570. The "peak a"

is the point on the TLC plate where the standards were

spotted. The "peak g" is the discontinuity at the

solvent front on the TLC plate. Impurities are

frequently concentrated here.

shown in subsequent work. In addition one can determine the spe-
cific chromatographic resolution of each method by the same rela-
tionship originally derived for GPC (see Experimental Section).
Consequently one can compare the resolving power of gradient LC,
GPC and TLC techniques.

Specific resolutions (Rsp) of four different molecular weight
poly(styrene) standards were determined for gradient LC and TLC

using equation 1 (see Experimental Section). These values are
listed in Table III along with analogous values for eight dif-
ferent GPC packings. It is apparent that the resolution of both
the gradient LC and TLC methods are better than that of GPC over
the molecular weight range investigated. In fact the Rsp of the
gradient LC technique increases to well over an order of magnitude
of that for values obtained with comparable GPC techniques for
high molecular weight polymers (Table III). The Rsp of TLC
separations remains ~2 to 5 times that of GPC over the entire
molecular weight range. It is also significant that only a single
LC column or a single TLC plate was needed to achieve separation
and generate data over the molecular weight range tested. Con-
versely, the Rsp values obtained for GPC required four separate
columns (and experiments), each optimized for a specific molecular
weight range (Table III).

    Gradient LC produces characteristically high specific resolu-
tions for other polymers such as poly(isoprene) and
poly(α-methylstyrene) as well (Table IV). The relatively high
Rsp for TLC also extends to a variety of different polymers (Table
V). Indeed the TLC-Rsp for poly(ethylene glycol/oxide) approaches
that of gradient LC.

    The reason for the higher resolution of the gradient LC and
TLC techniques is apparent upon examination of equation 1
(Experimental Section). The specific resolution is inversely pro-
portional to the peak width (as expressed by the standard
deviation, σ) and to the slope of the calibration curve (D_2). Con-
sequently a smaller value of D_2 generally indicates greater selec-

Table III.   COMPARISON OF THE SPECIFIC RESOLUTION OF GRADIENT LC, GPC, AND TLC METHODS OF POLY(STYRENE) FRACTIONATION

STATIONARY PHASE	TECHNIQUE	LINEAR MW FRACTIONATION RANGE	D_2	σ(ml)	MW	Rsp
Lichrospher 100[a]	GPC	$3 \times 10^3 - 5 \times 10^4$	1.28	0.229	5,000	1.95[a]
PSM-500[a]	GPC	$5 \times 10^3 - 4 \times 10^4$	2.17	0.147	5,000	1.75[a]
Whatman ODS-3(10μ)	HPLC	$10^3 - 10^7$	0.188	1.000	3,570	3.05
Whatman KC18F	TLC	$10^3 - 10^7$	7.509[b]	0.013[b]	3,570	5.94
Lichrospher 500[a]	GPC	$1.5 \times 10^4 - 1.5 \times 10^5$	1.32	0.275	51,000	1.60[a]
PSM-800[a]	GPC	$1.5 \times 10^4 - 1.5 \times 10^5$	2.25	0.118	51,000	2.15[a]
Whatman ODS-3(10μ)	HPLC	$10^3 - 10^7$	0.188	0.46	35,000	6.70
Whatman KC18F	TLC	$10^3 - 10^7$	7.509[b]	0.015[b]	35,000	5.14
Licrospher 1000[a]	GPC	$3 \times 10^4 - 2 \times 10^6$	1.98	0.142	97,000	2.05[a]
PSM-1500[a]	GPC	$4 \times 10^4 - 2 \times 10^6$	4.56	0.068	97,000	1.85[a]
Whatman ODS-3(10μ)	HPLC	$10^3 - 10^7$	0.188	0.31	100,000	9.95
Whatman KC18F	TLC	$10^3 - 10^7$	7.509[b]	0.018[b]	100,000	4.26
Licrospher 4000[a]	GPC	$10^5 - 7 \times 10^6$	3.84	0.144	390,000	1.05[a]
PSM-4000[a]	GPC	$7 \times 10^4 - 7 \times 10^5$	5.75	0.136	390,000	0.73[a]
Whatman ODS-3(10μ)	HPLC	$10^3 - 10^7$	0.414	0.19	390,000	7.32
Whatman KC18F	TLC	$10^3 - 10^7$	7.509[b]	0.019[b]	390,000	4.03

[a]GPC data taken from: J.J. Kirkland, J. Chromatogr., 125, 231 (1976).

[b]These numbers are unitless and cannot be directly compared to the analogous values obtained for HPLC and GPC. This is because the TLC parameters are normalized by dividing by the distance traveled by the solvent front (see Results and Discussion).

Table IV: Specific Resolution of Gradient LC Fractionations of
            Poly(isoprene) and Poly(α-methylstyrene).

Polymer	MW	σ (ml)	D_2	Rsp
poly(isoprene)	33300	1.20	0.0738	6.50
	113800	0.80	0.0738	9.76
	260000	0.70	0.0738	11.15
poly(α-methylstyrene)	19500	0.39	0.444	3.33
	87000	0.20	0.444	6.49
	76000	0.15	0.444	8.65

tivity.   The standard peak deviation, σ, is thought to represent

the efficiency of the technique.   It is apparent from the values

of D_2 and σ (Tables III, IV and V) that the greater resolution of

the gradient LC method is largely the result of greater selec-

tivity (smaller D_2) and not improvements in peak width (σ) which

tend to be similar or somewhat greater for the low molecular

weight polymers.   Indeed it has been shown that band broadening

for low molecular weight polymers is greater than for high molecu-

lar weight polymers in gradient "precipitation" LC (5). This is a

result of two factors.   First, mass transfer is significant for

low MW polymers (which separate in the transition region) but is

negligible for high MW polymers in this particular form of chroma-

tography (5).   Secondly, polymer standards are not monodisperse.

The greater resolving power of gradient precipitation LC results

in a greater bandwidth due to partial separation of the similar

Table V. Specific Resolution of TLC Fractionations of
Poly(isoprene) and Poly(ethylene Glycol).

Polymer	MW	σ	D_2	Rsp
poly(isoprene)	1360	0.036	2.57	6.27
	11100	0.045	2.57	5.01
	33300	0.059	2.57	3.82
poly(ethylene glycol)	998	0.036	3.57	4.52
	4820	0.024	3.57	6.77
	11250	0.016	3.57	10.15
	73000	0.015	3.57	10.83

molecular weight polymers in a standard. This phenomenon has been

demonstrated to be most pronounced for the lower molecular weight

standards (5). This second factor is not as pronounced in GPC

because of its lower resolving power. Although σ can be used as a

measure of column efficiency, it must be understood that, tech-

nically, it only applies for a pure monodisperse polymer standard.

The standard peak deviation, σ, has been utilized in GPC using

polymer standards of narrow molecular weight distribution because

the resolving power is such that there is generally little dif-

ference between these and an ideal monodisperse standard. One

should note, however, that the use of polydisperse standards in a

technique with higher fractionating power results in the seemingly

ironic situation of having increased fractionation result in

apparent decreased column efficiency (i.e., larger σ). In

reality, of course, this is an artifact of the standard used. This phenomena only affects the lower molecular weight standards for the gradient LC separations (Tables III and IV) (5). Consequently, the value of σ for the lowest molecular weight polymer is artificially high and the Rsp appears somewhat lower than it actually is.

One can compare the Rsp of the TLC technique directly with that of GPC and gradient LC; however, values of D_2 and σ are not directly comparable (Table III). This is because one plots R_f versus log molecular weight (in the TLC case) rather than retention volume. Consequently the quantity σ must be equal to the standard peak deviation divided by the distance the solvent traveled on the TLC plate.

In LC polymer separations the resolution is markedly affected by the type of gradient employed (Table VI). Although band broadening (σ) increases when a more gradual linear gradient is used, the selectivity (D_2) greatly increases. The net result is that Rsp increases with a more gradual gradient but analysis time also increases. One should also note that the linearity of log Mw vs. retention volume plots vary with the gradient used. More gradual gradients sometimes result in nonlinearity for the highest molecular weight polymers.

It is well known that conventional reversed phase LC can be used to separate styrene oligomers (9). Separation is achieved by a conventional partition-type mechanism and is superior to anything possible with GPC in this limited molecular weight range (9). This technique of nonaqueous gradient LC separates oligomers

Table VI: Effect of Different Mobile Phase Compositional Gradients on the Specific Resolution (Rsp) of Poly(styrene).

| Gradient	3570			9000			35000			100,000			390,000		
	σ(ml)	D_2	Rsp	σ(ml)	D_2	Rsp	σ	D_2	Rsp	σ	D_2	Rsp	σ	D_2	Rsp
45 to 100% MeCl$_2$ in 55 minutes	1.58	0.090	4.00	1.13	0.090	5.65	0.55	0.090	11.65	0.75	0.090	8.55	0.21	0.144	19.04
45 to 100% MeCl$_2$ in 27.5 minutes	1.00	0.181	3.15	0.69	0.181	4.60	0.46	0.181	6.90	0.31	0.181	10.25	0.19	0.414	7.32
45 to 100% MeCl$_2$ in 18.3 minutes	0.67	0.338	2.55	0.40	0.338	4.25	0.19	0.338	8.95	0.18	0.338	9.45	0.14	0.833	5.10
45 to 100% MeCl$_2$ in 13.75 minutes	0.56	0.509	2.00	0.28	0.509	4.05	0.18	0.509	6.25	0.14	0.509	8.1	0.11	0.737	7.13
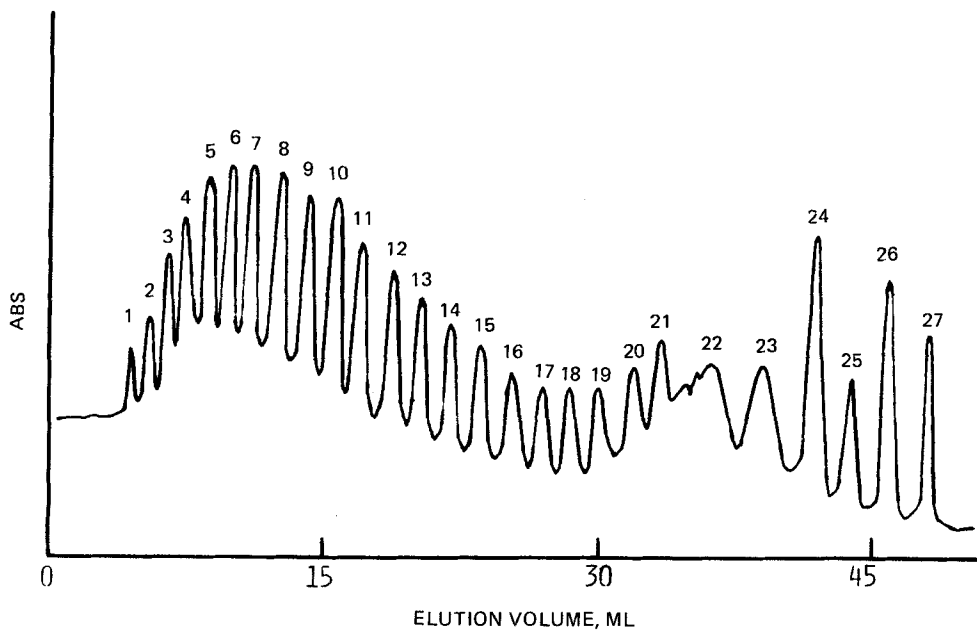
Figure 3: A combined phase (C_{18}) gradient LC separation of

styrene oligomers and polymers. The mechanism of

separation changes from traditional partitioning for

the oligomers to a precipitation-dissolution process

for the polymers. Peaks 1 through 20 are oligomers

where the peak number is equivalent to the degree of

polymerization (i.e., peak 2 = dimer, peak 8 =

octamer, etc). Peaks 21 through 27 are polymer stan-

dards of low polydispersity (see Experimental

Section). The number average molecular weight of peak

21 = 2,000; 22 = 3,570; 23 = 9,000; 24 = 17,500; 25 =

35,000; 26 = 100,000; 27 = 390,000.

in an identical manner and then continues to fractionate polymers with molecular weights well over a few thousand MW via a precipitation mechanism (3-5). Consequently, either gradient LC or TLC can be used to separate a variety of synthetic polymers over a wide range of molecular weights in a single efficient, high resolution run (Figure 3).

ACKNOWLEDGEMENT

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