Some Fundamental Properties of Developer Solvents in Thin Layer Chromatography Applied to Polymer Separations

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To establish widely applicable rules to choose a developer appropriate for adsorption thin layer chromatography (TLC) of any given polymer sample, a variety of vinyl polymers have been chromatographed with different single and binary solvent on silica gel thin layers. Rules thus deduced are: (i) a chosen developer should be a solvent of sample polymer; (ii) a general theory proposed by Snyder for adsorption chromatography serves as a great help for the purpose. In addition, the development characteristics of binaries, which appears in adsorption TLC of polymers, is investigated experimentally in respect to the molecular weight dependence of R_f (rate of flow).

I. INTRODUCTION

In application of thin layer chromatography (TLC) to polymer separations it is the major question to find out a developer appropriate to a given TLC system. As has been reported during the last half a decade,[1~6] TLC separation of polymeric substances is accomplished according to the different mechanisms, due to each involving (i) the polarity–controlled adsorption process, (ii) the solubility-controlled phase separation process, and (iii) the molecular sieving process. The mechanisms (i) and (ii) lead to separation according largely to composition[1,4,5] and to that according partly to molecular weight and partly to composition,[2~5] respectively; while the mechanism (iii) to separation by molecular size the same as gel permeation chromatography does.[3,6,7]

Principles for choice of developer solvents in separations by the mechanisms (ii) and (iii) are rather simple and have been argued generally as well as systematically in previous papers.[2~6] However, the principle in separations by the mechanism (i) has been described for each polymer species which we had to concern[1,4,5,7] and never been discussed as what holds in general for any given polymer species. Thus one of the aims of this paper is to present the most fundamental property which a developer system or solvent must possess in order to achieve separation by the adsorption mechanism.

The second aim is to investigate whether or not the adsorption-chromatographic behavior of polymers can be interpreted in terms of the Snyder theory[8], the validity of which has been proved for a number of low-molecular-weight compounds. The last subject which we concern is the development characteristics exhibited by binary developers. Recently we have observed a distinct molecular weight dependence of R_f (rate of flow)

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in the separation of atactic and syndiotactic poly(methyl methacrylate) using a binary developer composed of good solvents toward both the sample polymers.[9] This result is incompatible with our previous theory that the R_f value is almost independent of molecular weight so far as developers are good solvents for sample polymer so that adsorption mechanism is mainly operative.[4,5] Thus last part of this paper deals experimentally with a question, under what condition binary developers exhibit molecular weight dependence of R_f even in separation by the adsorption mechanism.

II. FUNDAMENTAL PROPERTY OF DEVELOPER REQUIRED TO ADSORPTION TLC OF POLYMERS

It is well known that the chromatographic behavior of a given sample, *e.g.*, the migration rate, may be decided by a balance of three types of interactions among the adsorbent (A), the developer (D), and the sample (P), being abbreviated as [A-D], [A-P], and [D-P].[1,8] TLC separation by the adsorption mechanism is understood as a dynamic equilibrium which holds as a result of competition among developer and sample molecules in occupying active sites of adsorbent. When the interaction [A-D] is stronger than [A-P], the active sites will be occupied predominantly by the solvent molecules, thus this resulting in the sample migration by a certain distance. In this way, the migration rate of sample on a given adsorbent depends on the interaction [A-D] which may be represented by the polarity assigned to the solvent used.

When any separation of polymer sample by adsorption TLC is intended, the above consideration on the choice of developer can set forth only the necessary condition but not the sufficient condition. The interaction [D-P] should be taken into account in such a way the developer can dissolve well the sample polymer. Poor solvents toward sample

Table I. Chromatographic Behavior of Some Synthetic Polymers*.

	Cyclo-hexane	CCl_4	$CHCl_3$	methyl isobutyl ketone	THF	butanone	methyl acetate	DMSO	methanol
				R_f					
D.C.	2.02	2.24	4.81	13.11	—	18.51	—	—	32.6
ε^0	0.04	0.18	0.40	0.43	0.45	0.51	0.60	0.62	0.95
PBT	1	1	1	0^n	1	0^n	0^n	0^n	0^n
PMST	0	0	1	1	1	1	0^n	0^n	0^n
PClST	0	0	1	1	1	1	1	0^n	0^n
PST	0	0	1	1	1	1	1	0^n	0^n
PVC	0^n	0^n	0^n	0^n	1	1	0^n	1	0^n
PBMA	0^n	0	0	1	1	1	1	0^n	0^n
PEMA	0^n	0	0	1	1	1	1	1	0^n
PMMA	0^n	0	0	0	1	1	1	1	0^n
PMA	0^n	0	0	0	1	1	1	1	0^n
PVAC	0^n	0	0	0	1	1	1	1	1
PAN	0^n	0^n	0^n	0^n	0^n	0^n	0^n	1	0^n

* (D.C.) means the dielectric constants.
  ε^0 denotes the solvent strength parameter. For the definition, see Eq. (7) and Ref. (8).
  (0^n) indicates that sample polymer remains immobile because of the insoluble nature.

polymer often result in "phase separation" followed by precipitation of polymer so that fractionation by molecular weight occurs even in TLC.[2~5]

The argument mentioned above may be illustrated by a series of following experimental results on the chromatographic behavior of some synthetic polymers, whose molecular weights ranged from 15×10^4 to 60×10^4. The experiment was carried out with nine developer solvents on thin layers of silica gel having the same activity grade. Polymer samples tested were poly(1-butene) (PBT), poly(α-methylstyrene) (PMST), poly(p-chlorostyrene) (PCST), polystyrene (PST), polyvinyl chloride (PVC), poly(butyl methacrylate) (PBMA), poly(ethyl methacrylate) (PEMA), poly(methyl methacrylate)(PMMA), polymethyl acrylate (PMA), polyvinyl acetate (PVAc), and polyacrylonitrile (PAN). The result is summarized in Table I, in which the chromatographic behavior of each polymer is indicated by either $R_f=1$ or 0. The indication, $R_f=1$, means that polymer migrated to a certain extent but not necessarily up to the solvent front. This table was prepared in such a way that the solvents used are arranged from left to right in the order of increasing dielectric constants, $i.e.$, according to the eluotropic series; the polymers tested are, from upper to lower, approximately in the order of increasing dielectric constants again, except for polymethyl acrylate and polyacrylonitrile. From the table it is recognized that the solubility of polymer in each solvent plays a decisive role in TLC separation of polymeric substances, $i.e.$, the interaction [D-P], besides the adsorptive interactions [A-D] and [A-P].

III. VALIDITY TEST OF SNYDER'S THEORY FOR POLYMERS

Separation by adsorption chromatography may be elucidated in terms of the thermodynamic functions of adsorbed and unadsorbed molecules in a chromatographic system. This problem has been discussed in details by Snyder to derive a simple equation which serves as a great help for finding out a suitable eluent (developer) in the study of low-molecular-weight compounds.[8] The aim of this section is to show the validity of the Snyder theory for polymeric substances.

1. Snyder's Theory

A distribution coefficient for adsorption system is defined by

$$K^\circ=(X)_a/(X)_u \tag{1}$$

where $(X)_u$ and $(X)_a$ refer to the concentrations of unadsorbed and adsorbed sample molecules X, respectively. The units of $(X)_u$ and $(X)_a$ are taken as grams (or moles) per milliliter and grams (or moles) per gram, respectively. Denoting the volume of mobile phase by V° and the total weight of adsorbent by W, we can express the fraction of X in the unadsorbed state by

$$f_u=\frac{V^\circ(X)_u}{V^\circ(X)_u+W(X)_a} \tag{2}$$

By introducing K°, Eq. (2) is rewritten

$$f_u=[1+(W/V^\circ)K^\circ]^{-1} \tag{2'}$$

The R_f value may be identified with f_u so that one obtains

$$R_f = [1 + (W/V^\circ)K^\circ]^{-1} \tag{3}$$

From the above relations it is obvious that $R_f = 0$ when $(X)_u = 0$; while $R_f = 1$ when $(X)_a = 0$.

A general theory for the distribution coefficient K° has been worked out by Snyder.[8] After a consideration on the thermodynamic equilibrium constant K_{th} for adsorption of a sample molecule X in a linear isotherm system, he derived a relation between K° and K_{th}, which reads:

$$K^\circ = K_{th}V_a \tag{4}$$

where V_a is the adsorbent surface volume which means the volume of an adsorbed solvent monolayer per unit weight of adsorbent. From thermodynamics

$$\log K_{th} = -\Delta G_a^\circ/2.3\,RT \equiv \Delta E \tag{5}$$

where ΔG_a° is the standard free energy of adsorption and RT has the usual meaning. Snyder defined a dimensionless adsorption energy ΔE, equal to $-\Delta G_a^\circ/2.3\,RT$, and approximated ΔE by a difference between two adsorbed phase energy terms, E_{xa} and E_{sa}, which are related to the sample and solvent molecules, respectively, that is to say,

$$\Delta E = E_{xa} - mE_{sa} \tag{6}$$

In the above equation it is assumed that the areas, A_s and A_e, required by an adsorbed sample and solvent molecule on the adsorbent surface, respectively, are different from one another, its ratio being $m = A_s/A_e$. In the derivation of Eq. (6) the difference between the liquid phase energy terms for the sample and solvent molecules is neglected.

It is obvious that the adsorptive interactions [A-D] and [A-P], defined in the last section, are dependent on the activity of adsorbent used for experiment. For example, when silica gel, used as adsorbent, contains a certain amount of water, these interactions will be lowered to a certain extent. Thus to generalize Eq. (6), an activity function for the adsorbent surface, a, which is defined as unity for a standard activity, is introduced, thus ΔE being replaced by $a\Delta E$ for a surface with any activity. Further E_{xa} may be represented by the equivalent quantity S°, which is the sample adsorption energy on the standard activity surface, and characteristic of the molecular structure of given samples. In the similar way the adsorption energy of the solvent per unit area of the standard activity surface, ϵ°, is introduced in place of E_{sa}, which is defined as

$$\epsilon^\circ = E_{sa}/A_e \tag{7}$$

Combination of Eqs. (4) and (5) under such consideration yields

$$\log K^\circ = \log V_a + a(S^\circ - A_s\epsilon^\circ) \tag{8}$$

Experimental check of Eq. (8) combined with Eq. (3) was made intensively by Snyder.[8] It has been proved that Eq. (8) is valid for a variety of chromatographic systems involving low-molecular-weight compounds tested as sample. He has also elucidated S° and ϵ° in terms of the molecular structure of given samples and solvents, respectively, and summarizes the results in his monograph,[8] especially the quantity ϵ° being denoted "solvent strength parameter".

2. Validity Test of Eq. (8) for Polymers

As is clear from Eq. (8), the net energy for the competitive adsorption of sample and solvent molecules onto adsorbent having a certain activity may be expressed by

$$Q_{net} = S° - A_s \varepsilon° \tag{9}$$

When $Q_{net} > 0$, the sample molecules will be adsorbed on stationary phase so that $R_f = 0$, while those will be desorbed when $Q_{net} < 0$ so that $0 < R_f < 1$.

To test the validity of Eq. (8) for polymers, we have chromatographed ten different vinyl polymers on silica gel thin layers having the same activity grade. The samples were developed using binaries of different composition, which were composed of a lower polarity solvent, carbon tetrachloride, and a higher polarity solvent, 2-butanone, and the developer composition, at which a given sample just began to migrate, was determined and expressed by volume fraction of 2-butanone added, being designated V_{MEK}. Under such an experimental condition, values of Q_{net} assigned to all the sample polymers may be regarded as approximately the same, and a linear relation between $S°$ and $\varepsilon°$ will be expected if Eq. (8) be valid.

The result on V_{MEK} thus found is given in Table II. The value of V_{MEK} for polyacrylonitrile (PAN) could not be determined, for this polymer is insoluble in the binaries used. However, a rough estimate of V_{MEK} has been made by investigating the chromatographic behavior of PEMA, Co-[EMA-AN], Co-[MMA-AN] and PMMA using the same binary developer, where Co-[X-Y]'s mean equimolar statistical copolymers of X and Y. The R_f values thus observed showed a decreasing trend in the above mentioned order. This implies, in turn, that the V_{MEK} increases from that for PEMA toward PMMA. Thus we have assigned to PAN an intermediate value between 0.38 and 0.62 observed for V_{MEK} of PEAM and PMMA, respectively, namely 0.5.

In practice of the validity test, one must know the values of $S°$ and A_s for each polymer species. For this purpose we have assumed that the adsorption behavior of a given polymer be represented by that of each corresponding constituent monomeric unit, as has been speculated by Fontana and Thomas[10] as well as by the present authors.[11] Under such

Table II. Chromatographic Behavior of Synthetic Polymers Observed with Binary Developers of Carbon Tetrachloride and 2-Butanone.

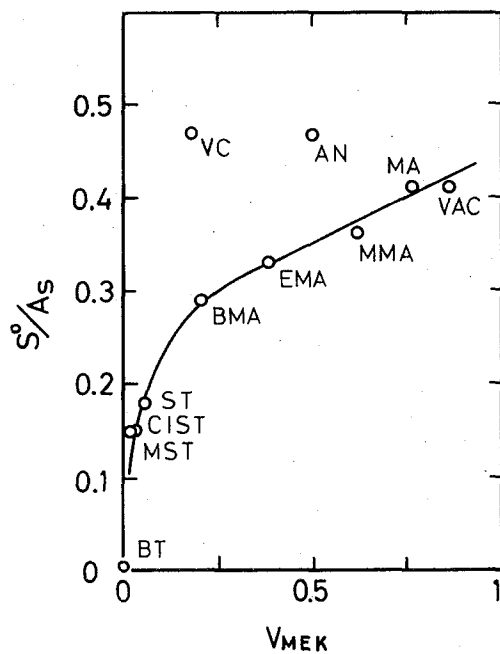| Sample code a)	V_{MEK}	$S°$	A_s	$S°/A_s$
PBT	0.0	−0.08	4.94	~0
PMST	0.02	1.52	9.29	0.16
PCST	0.03	1.45	7.69	0.15
PST	0.07	1.25	8.54	0.19
PVC	0.19	1.69	3.44	0.49
PBMA	0.21	5.14	17.94	0.29
PEMA	0.38	5.25	16.14	0.33
PMMA	0.62	5.36	14.34	0.37
PMA	0.77	5.29	12.74	0.41
PVAC	0.86	5.29	12.74	0.41
PAN	(0.50)	5.22	10.94	0.48

a) For the abbreviations, see Section II.

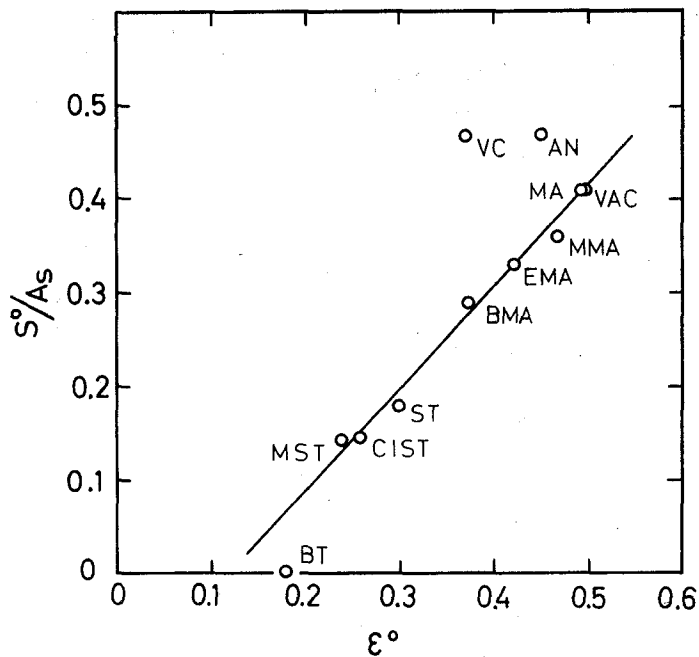Fig. 1. Plot between $S°/A_s$ and V_{MEK}. For the detail, see text.

Fig. 2. Experimental validity test of the Snyder theory.

assumption one may calculate $S°$ and A_s on the basis of the simplest additivity rule for adsorption energies and surfaces of each residual group involved in a given sample molecule, proposed by Snyder.[8] The values calculated in this way for our sample polymers

are tabulated in Table II. On the other hand, we have estimated the solvent strength parameter $\varepsilon°$, which will be exhibited by the binary with V_{MEK}, by linear interpolation between those for carbon tetrachloride ($\varepsilon°=0.18$) and 2-butanone ($\varepsilon°=0.51$), which are, however, given for alumina by Snyder.[8] Figures 1 and 2 show a plot between $S°/A_s$ and V_{MEK} and that between $S°/A_s$ and $\varepsilon°$, respectively. It is seen from Fig. 2 that a linear relation holds for polymeric substances, as expected from the theory. However, the behavior of polyvinyl chloride (denoted VC in the figure) and polyacrylonitrile (AN) are out of rule, and this suggests that TLC can hardly be applied to such polymers. In this connection it should be mentioned, despite this result, that statistical copolymers involving either vinyl chloride or acrylonitrile as comonomer may be dealt with as the object of TLC study.

IV. DEVELOPMENT CHARACTERISTICS OF BINARIES

On the basis of our previous findings on the chromatographic behavior of styrene–methyl methacrylate copolymer,[1] it has been regarded as a guiding rule that almost no molecular weight dependence of R_f appears so far as separation occurs according to the adsorption mechanism. However, this rule must be taken with some reservation when binary developers concern. A result incompatible with the rule has been obtained recently in separation of atactic and syndiotactic poly(methyl methacrylate).[9] The observation was that binaries of isopropyl acetate and ethyl acetate exhibited considerable changes in R_f with molecular weight despite the fact that they are both good solvents for the sample polymer. This section deals experimentally with such development characteristics of binaries.

1. Methyl Methacrylate Polymers with Different Molecular Weight

Two fractionated samples of poly(methyl methacrylate) have been used as samples, which were designated PMA-2 ($M_v=41.2\times10^4$) and PMA-3 ($M_v=16.5\times10^4$). The samples were chromatographed on silica gel thin layers with single solvents which dissolve well the polymers. The result is summarized in Table III, from which it is recognized that no solvent, when singly used, gave different R_f for the two samples. However, binaries obtained by some combinations of these solvents exhibited distinct molecular weight dependences of R_f, as shown in Fig. 3. The binaries studied were (a) benzene + acetone (2 : 8 by volume), (b) isopropyl acetate + methyl formate (10 : 6.2), and (c)

Table III. Development Characteristics Exhibited by Single Solvents for Two
Fractionated Samples of Poly-(Methyl Methacrylate).

Developer	Dielectric constant	R_f	
		PMA-2	PMA-3
Benzene	2.3	0	0
Isopropyl acetate	5.7	0	0
Ethyl acetate	6.0	0	0
Methyl acetate	6.7	1	1
Methyl formate	8.2	1	1
Acetone	21.4	1	1

PMA-3

PMA-2

(a)

PMA-3

PMA-2
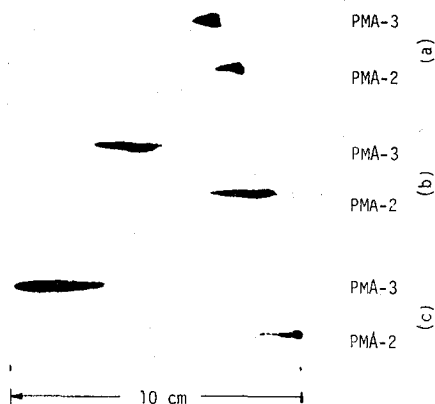
(b)

PMA-3

PMA-2

(c)

|←———— 10 cm ————→|

Fig. 3. Chromatographic behavior of PMA-2 and PMA-3 shown by equieluotropic binary developers, (a), (b), and (c). For the abbreviations, see text.

ethyl acetate + methyl acetate (10 : 2.3). The composition of each binary was adjusted so as to develop the samples intermediate on chromatoplate. Thus the binaries may be regarded as "equieluotropic developer" in a sense of adsorption chromatography. The chromatograms shown in the figure indicate that the molecular weight dependence of R_f, namely, the resolution in respect to molecular weight, is different for different binary. In connection with this result, we should recall that the binary chloroform + methanol did not show any molecular weight dependence for the same samples.[4,5] These results suggest that the resolution in respect to molecular weight increases in such an order as chloroform + methanol < benzene + acetone < isoprophy acetate + methyl formate < ethyl acetate + methyl acetate.

The above observation leads us to a consideration that the resolution exhibited by binary developer must be dependent largely on the difference between the eluting strengths of each component solvent. Provided the dielectric constant is a measure for the eluting strength, we shall define $\Delta\mu$, which is the difference between dielectric constants of solvents composing each binary. Further we define ΔR_f as the difference in R_f found for the
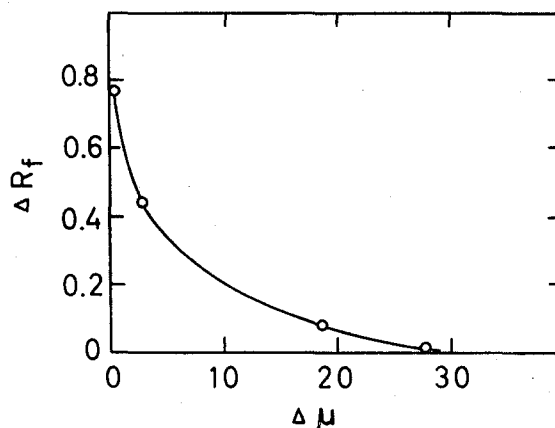
Fig. 4. Relation between ΔR_f and $\Delta\mu$ observed for poly(methyl methacrylate) fractions with binary developers (refer to Fig. 3).
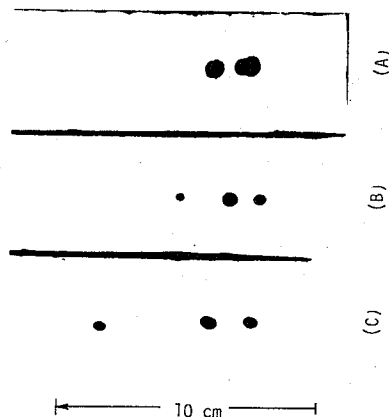
Fig. 5.   Chromatograms obtained for three dyestuffs with equieluotropic developers designated as (A), (B), and (C).   For the abbreviation, see text.

samples with each binary.   Figure 4 shows a plot between ΔR_f and $\Delta\mu$, which indicates a rapid increase in ΔR_f with decrease in $\Delta\mu$.   Thus it should be kept in mind that even when a TLC development proceeds according to adsorption mechanism, the molecular weight dependence of R_f appears if binary developers with small values of $\Delta\mu$ are employed.

2.   Low-Molecular-Weight Compounds–Dyestuffs

In relation to the above finding, low-molecular-weight compounds such as dyestuffs, namely, Butter Yellow, Sudan Red G, and Indophenol were chromatographed using equieluotropic developers, i.e., (A) n-heptane + butanone, (B) n-heptane + chloroform, and (C) benzene.   The chromatograms are shown in Fig. 5.   The resolution is enhanced in the order (A), (B), and (C), and it may be evident that $\Delta\mu$ for (B) is smaller than for (A), and (C) may be regarded as a binary with infinitesimally small $\Delta\mu$.   Thus the relation between ΔR_f and $\Delta\mu$ holds again for low-molecular-weight compounds.

It should be noted that Snyder's theory represented by Eq. (8) in Section II cannot explain such resolutions in TLC separation as shown in Figs. (3) and (5).   The reason is as follows: The resolution exhibited by a given developer for two samples X and Y, whose $K°$ are K_x and K_y, respectively, may be represented by $\log(K_x/K_y)$ on the basis of Eq. (8), but this measure for the resolution is independent of any property of developer within the framework of Eq. (8).   At present we may only point out that some demixing effects and/or changes in the phase ratio might be concerned with the phenomena.

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