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Full length article

## Organic solvent modifier and temperature effects in non-aqueous size-exclusion chromatography on reversed-phase columns

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### ABSTRACT

Common reversed-phase columns ( $C_{18}$ ,  $C_4$ , phenyl, and cyano) offer inert surfaces suitable for the analysis of polymers by size-exclusion chromatography (SEC). The effect of tetrahydrofuran (THF) solvent and the mixtures of THF with a variety of common solvents used in high performance liquid chromatography (acetonitrile, methanol, dimethylformamide, 2-propanol, ethanol, acetone and chloroform) on reversed-phase stationary phase characteristics relevant to size exclusion were studied. The effect of solvent on the elution of polystyrene (PS) and poly(methyl methacrylate) (PMMA) and the effect of column temperature (within a relatively narrow range corresponding to typical chromatographic conditions, *i.e.*, 10 °C–60 °C) on the SEC partition coefficients  $K_{SEC}$  of PS and PMMA polymers, were also investigated. The bonded phases show remarkable differences in size separations when binary mixtures of THF with other solvents are used as the mobile phase. The solvent impact can be two-fold: (i) change of the polymeric coil size, and possible shape, and (ii) change of the stationary phase pore volume. If the effect of this impact is properly moderated, then the greatest benefit of optimized solute resolution can be achieved. Additionally, this work provides an insight on solvent-stationary phase interactions and their effects on column pore volume. The only effect of temperature observed in our studies was a decreased elution volume of the polymers with increasing temperature. SEC partition coefficients were temperature-independent in the range of 10 °C–60 °C and therefore, over this temperature range elution of PS and PMMA polymers is by near-ideal SEC on reversed-phase columns. Non-ideal SEC appears to occur for high molar mass PMMA polymers on a cyano column when alcohols are used as mobile phase modifiers.

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### 1. Introduction

Tetrahydrofuran (THF) is one of the most commonly used solvents for non-aqueous size-exclusion chromatography (SEC) because it is a good solvent for a wide range of polymer types. However, this solvent is toxic, attacks polyether ether ketone (PEEK) tubing, forms peroxides that can result in an explosion, and is expensive. During the last 15 years, scientists have been exploring ways to make analytical chemistry “greener” [1]. The main drivers for this are employees’ health, process safety, environmental impact, waste disposal, and costs associated with all of the above. If it is not possible to replace THF by a “greener” solvent, then perhaps its toxicity can be reduced by mixing it with another, less toxic, solvent (*e.g.*, methanol, ethanol, 2-propanol, acetone [2]).

The number of solvents suitable as SEC mobile phases is limited to those that can dissolve polymer analytes (a temperature-dependent solvent property), that are compatible with the SEC column of choice, and that generate sufficient contrast (spectroscopic or otherwise) in the detector [3,4]. The first two requirements are not as stringent for reversed-phase (RP) columns, and the use of mixed solvents in SEC has only been explored recently [5]. Potential benefits of solvents that reduce solute bulkiness (*i.e.*, solvents that reduce the hydrodynamic volume an analyte occupies in solution) are higher resolution and a wider separation range for a given pore volume of a column. Reversed-phase silica columns are compatible with a wide range of organic solvents, and the rigid porous particles neither swell nor shrink as the mobile phase composition is changed. Chemical modifications of the silica surface can result in a loss of pore volume, however. For example, the pore diameter of a narrow pore silica derivatized with a monomeric  $C_{18}$  phase is reduced from 60 Å to approximately 26 Å, and to an even greater extent in a polymeric  $C_{18}$  column – to approximately 18 Å [6]. Nevertheless, pore volume can be manip-

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**Table 1**  
Solvent properties. <sup>a</sup>[12] – Hildebrand units converted into  $(\text{J}\cdot\text{cm}^{-3})^{0.5}$ , or  $\text{MPa}^{0.5}$ , by a factor of 2.045; selectivity factors  $\alpha$ ,  $\beta$  and  $\pi^*$  represent solvent ability to interact as hydrogen ion donor, hydrogen ion acceptor, and by polar and polarization effects, respectively; <sup>b</sup>[14].

Solvent	<sup>a</sup> Hildebrand Solubility, $\text{MPa}^{0.5}$	<sup>b</sup> Polarity Index	<sup>a</sup> Normalized selectivity factors derived from Kamlet-Taft solvatochromic parameters			<sup>a</sup> UV Cutoff, nm
			$\alpha$	$\beta$	$\pi^*$	
Tetrahydrofuran	18.6	4.0	0.00	0.49	0.51	212
Chloroform	18.8	4.1	0.43	0.00	0.57	245
Acetone	19.6	5.1	0.06	0.38	0.56	330
2-Propanol	23.3	3.9	0.35	0.43	0.22	205
Dimethylformamide	24.1	6.4	0.00	0.44	0.56	268
Ethanol	24.5	4.3	0.39	0.36	0.25	205–210
Acetonitrile	24.8	5.8	0.15	0.25	0.60	190
Methanol	29.6	5.1	0.43	0.29	0.28	205

**Table 2**  
Hildebrand solubility parameters,  $\text{MPa}^{0.5}$ , calculated using Eq. (1) and values listed in Table 1 for the mixtures of solvents used as mobile phase.

Solvent <i>j</i>	% of solvent in THF mobile phase									
	10	20	30	40	50	60	70	80	90	100
	Hildebrand Solubility, $\text{MPa}^{0.5}$									
Tetrahydrofuran	–	–	–	–	–	–	–	–	–	18.6
Chloroform	18.6	18.6	18.7	18.7	18.7	18.7	18.7	18.8	18.8	18.8
Acetone	18.7	18.7	18.9	19.0	19.1	19.2	19.3	19.4	–	–
2-Propanol	19.1	19.5	20.0	20.5	–	–	–	–	–	–
Dimethylformamide	19.2	19.7	20.3	20.8	21.4	21.9	22.5	–	–	–
Ethanol	19.2	19.8	20.4	21.0	–	–	–	–	–	–
Acetonitrile	19.2	19.8	20.5	21.1	–	–	–	–	–	–
Methanol	19.7	20.8	21.9	23.0	–	–	–	–	–	–

ulated by using a solvent that decreases the conformational order of the bonded ligands [5,6] and thus increases the volume of the pores.

There are three regions that can be distinguished within the chromatographic column [7–9]: (i) the solvated bonded-phase region; (ii) the interface region, where the ends of the bonded-phase meet the mobile phase; and (iii) the bulk mobile phase region. The thickness of the interface region may have a further impact on the pore volume of the column and depends on multiple parameters (e.g., mobile phase composition, the identity and bonding density of the stationary phase ligands, the morphology of the silica substrate – endcapped or not [10]). Preferential sorption of mobile phase components by the stationary phase ligands can result in a monolayer or multilayer of adsorbed solvents in the interface region [10].

The resolution of an SEC separation can be improved by minimizing peak dispersion (increasing chromatographic efficiency) and maximizing internal pore volume. For a given experimental set-up, these can be manipulated by the choice of solvent, temperature, and flow rate (the roles of solvent and temperature can be closely linked, as regards thermodynamic and elutropic strength, viscosity, etc.). As previously discussed in [5], solvation interactions between a solvent and a surface-functionalized stationary phase can influence the conformational order of the bound ligands, which in turn can impact the phase ratio (ratio of pore to interstitial volume,  $V_i/V_0$ ) of the column.

In this work, we contrast the effect of neat THF to that of binary mixtures of THF with a variety of common high performance liquid chromatography (HPLC) solvents (acetonitrile, methanol, dimethylformamide, 2-propanol, ethanol, acetone, and chloroform) on reversed-phase stationary phase ( $C_{18}$ ,  $C_4$ , phenyl, and cyano) characteristics relevant to size-exclusion: total permeation volume,  $V_t$ ; interstitial volume,  $V_0$ ; pore volume  $V_i$ ; and phase ratio,  $V_i/V_0$ . The solvent effect on the elution of narrow dispersity, linear polystyrene (PS) and poly(methyl methacrylate) (PMMA) polymers is also investigated, as is the effect of column temperature (within

**Table 3**  
Column Parameters. Column length and internal diameter (mm)  $150 \times 4.6$ ; particle size ( $\mu\text{m}$ ) 3; pore size ( $\text{\AA}$ ) 300; Distributor: MAC-MOD Analytical.

Column No.	Column ID
1	ACE 3 $C_{18}$ -300
2	ACE 3 $C_4$ -300
3	ACE 3 Phenyl-300
4	ACE 3 CN-300

a  $50^\circ\text{C}$  range) on the SEC partition coefficient  $K_{SEC}$  of PS and PMMA polymers.

## 2. Theory

There are various general schemes that categorize the solvation properties of liquids, including empirical estimates of a solvent's strength and selectivity for HPLC applications [11]. A solvent's ability to elute analytes from a column (elutropic strength) is estimated by the solvent (elution) strength parameter. This strength depends on the mobile phase composition (e.g., nature and concentration of solvents), type of stationary phase, column temperature, and solute properties. Thus, elution strength is not a sole property of the solvent, but is a composite property of the variables involved [12]. Nevertheless, elution strength is a practical concept in liquid chromatography (LC) that is used to control the retention of analytes in RP-HPLC, where elution strength is associated with the ability of solvents with high elution strength to sweep away the retained solutes, i.e., to elute solutes faster, or in other words, to minimize the elution volumes of solutes. Thus, elution strength is related to the ability of a solvent to interact with the stationary phase. For the purpose of this SEC study, the strength of a solvent will be identified by its ability to minimize the solute-accessible column volume (i.e., to minimize the total permeation volume), which is a result of the ability of a solvent to interact strongly with the stationary phase. While solvents can have similar elution strengths, they can also differ in selectivity, resulting in differences in the

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