LC Troubleshooting

Solvent Selection, Part II — Physical Properties

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In this second installment of a series about LC solvents, the authors discuss how solvent viscosity, boiling point, miscibility, eluotropic strength, and polarity affect solvent selection during method development.

In a previous installment of "LC Troubleshooting," we discussed several of the variables analysts must consider when selecting solvents for liquid chromatography (LC) (1). LC workers usually select solvents based primarily on their UV characteristics. In this installment, we discuss other important physical properties and their effects on chromatography. In addition, we examine using solvent-strength parameters to optimize separations.

VISCOSITY

Chromatographers often overlook the viscosity of solvents and solvent mixtures. However, because solvent viscosity directly affects system back pressure, it should not be ignored. In addition to conventional 4.6-mm i.d. columns, narrow-bore (2–3 mm i.d.) and microbore (less than 2-mm i.d.) columns are becoming increasingly popular (2). These narrower columns can generate high back pressures even at low flow rates, so solvent viscosity can be critical. Traditional analytical columns may also have high back-pressure problems when the flow rate exceeds 2 mL/min. Higher solvent viscosities result in higher system back pressures when all other parameters are held constant. Whenever the viscosity exceeds 0.5 cP, excessive-pressure problems may occur during a separation. As columns age and particles accumulate on the inlet frit, back pressure will increase. If the system pressure is initially high because of solvent viscosity, you will reach the upper pressure limit of the method more quickly as columns age. By selecting lower viscosity solvents, you can avoid this problem and use columns longer without exceeding the method's back-pressure limit. If a relatively viscous solvent exhibits characteristics that otherwise enhance the separation, use the solvent in a dilute form or as a modifier. Table I lists properties of various solvents.

Many workers are unaware that viscosity changes with solvent composition. It is commonly assumed that as the water content of a solvent mixture decreases and the organic content increases, the viscosity will also decrease. In fact, all solvent mixtures reach a maximum viscosity for mixtures of organic solvent and water. For example, Figure 1 shows that water–acetonitrile mixtures reach a maximum viscosity at 65:35 water–acetonitrile. Alcohols are especially problematic, because at their maximum viscosity mixtures of near 50:50 water–alcohol, the viscosity can be as high as 2.9 cP. This characteristic can create confusion if a pump shuts down during a gradient run as a result of excessive pressure, yet when you restart the system at initial conditions, the pressure is well within limits. When you develop a gradient method, you should monitor the back pressure and allow a sufficient margin for normal back-pressure increases resulting from column aging and accumulation of particles from pump seals or injected samples. If you select low-viscosity solvents you will minimize back-pressure problems.

BOILING POINT

A solvent's boiling point may seem unimportant for LC analyses run at ambient temperature, but the boiling point is a good indicator of viscosity. Low-boiling-point solvents are generally less viscous than high-boiling-point solvents. As a general rule, the solvent boiling point should be 20–50 °C above the separation temperature. Solvents with lower boiling points have higher vapor pressures at ambient temperatures, which can cause pumping problems. Low-boiling-point solvents tend to form bubbles in the piston chamber. This is known as pump cavitation, which can affect pumping precision or even lead to the loss of priming. Also, if a mobile phase contains solvents with widely varying boiling points, heating the mobile phase could cause selective evaporation of solvents with lower boiling points.

MISCIBILITY

When selecting solvents, pay special attention to the miscibility of all components of the mobile phase. Check the solvent miscibility using a tool such as Table I or Figure 2 before selecting any mobile-phase component. For example, as a general rule, hydrocarbon solvents (nonpolar solvents) are insoluble in water, methanol, and acetonitrile. Acetonitrile, tetrahydrofuran, and some alcohols are soluble in water. When solvents are not miscible, very high back pressure will occur if you try to mix them in the LC system. When switching between immiscible solvents, pump 10–20 column volumes (25–50 mL for a 25 cm × 4.6 mm column) of a mutually miscible solvent through the system. Isopropyl alcohol is a good choice to use as an exchange solvent. When a solvent is not miscible and an exchange solvent is not used, the back pressure will increase sharply, quickly reach the system pressure limit, and cause the pump to shut down. Don't forget to switch over all the solvent in the system. Figure 3 shows peak doublets that occurred when the chromatographer forgot to switch the solvent in the autosampler-needle rinse to the exchange solvent before using a normal-phase solvent.

You should also be careful when selecting mobile-phase modifiers. Several modifiers have limited solubility in some mobile-phase solvents. For example, aqueous acetate and

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TABLE I: Solvent Properties*

Solvent	UV Cutoff (nM)	Refractive Index (20 °C)	Viscosity (cP)	Boiling Point (°C)	Miscibility Number (*M*)†	Polarity (*P'*)	Eluotropic Values		
							Alumina	C18	Silica
Acetone	330	1.3587	0.36	56.29	15,17	5.1	0.56	8.8	0.53
Acetonitrile	190	1.3441	0.38	81.60	11,17	5.8	0.65	3.1	0.52
n-Butyl acetate	254	1.3942	0.734	126.11	22	4.0	—	—	—
1-Butanol	215	1.3993	2.98	117.5	15	3.9	—	—	—
Chlorobenzene	287	1.5249	0.80	131.69	21	2.7	—	—	—
1-Chlorobutane	220	1.4021	0.45	78.44	—	1.0	—	—	—
Chloroform	245	1.4458	0.57	61.15	19	4.1	0.40	—	0.26
Cyclohexane	200	1.4242	1.0	80.72	28	0.2	0.04	—	—
Cyclopentane	200	1.4064	0.44	49.26	—	0.1	0.05	—	—
Decahydronaphthalene	200	1.4758	2.42	191.7	—	—	—	—	—
o-Dichlorobenzene	295	1.5514	1.32	180.48	—	2.7	—	—	—
Dimethyl acetamide	268	1.4384	0.84	166.1	—	6.5	—	—	—
Dimethyl formamide	268	1.4305	0.92	153.0	12	6.4	—	7.6	—
Dimethyl sulfoxide	268	1.4783	2.24	189.0	9	7.2	0.62	—	—
1,4-Dioxane	215	1.4224	1.37	101.32	17	4.8	0.56	11.7	0.51
Ethyl acetate	256	1.3724	0.45	77.11	19	4.4	0.58	—	0.48
Ethylene dichloride	228	1.4448	0.79	83.48	—	3.5	0.49	—	—
Ethyl ether	215	1.3524	0.24	34.55	23	2.8	0.38	—	0.43
Glyme	220	1.3796	0.46	83.5	—	—	—	—	—
Heptane	200	1.3876	0.40	98.43	29	0.1	0.01	—	0.00
Hexadecane	190	1.4340	—	287.0	—	0.5	—	—	—
Hexane	195	1.3749	0.31	68.7	29	0.1	0.01	—	0.00
Isooctane	215	1.3914	0.50	99.24	29	0.1	0.01	—	—
Isobutyl alcohol	220	1.3959	—	107.7	15	4.0	—	—	—
Isopropyl alcohol	205	1.3772	2.40	82.26	15	3.9	0.82	8.3	0.6
Methanol	205	1.3284	0.55	64.7	12	5.1	0.95	1.0	0.7
2-Methoxyethanol	210	1.4020	1.72	124.6	—	5.5	—	—	—
Methyl *tert*-butyl ether	210	1.3689	0.27	55.2	—	2.5	0.35	—	0.48
Methyl ethyl ketone	329	1.3788	0.43	79.64	17	4.7	0.51	—	—
Methyl isoamyl ketone	330	1.4072	0.80	144.9	—	4.0	—	—	—
Methyl isobutyl ketone	334	1.3957	0.506	116.5	—	4.2	0.43	—	—
Methyl *n*-propyl ketone	331	1.3901	0.51	102.4	—	4.5	—	—	—
Methylene chloride	233	1.4241	0.44	39.75	20	3.1	0.42	—	0.30
n-Methyl-2-pyrrolidone	285	1.4680	1.67	202.00	—	6.7	—	—	—
Pentane	190	1.3575	0.23	36.07	—	0.0	0.00	—	0.00
Propyl alcohol	210	1.3856	2.3	97.2	—	4.0	0.82	—	—
Propylene carbonate	280	1.4210	—	241.7	—	6.1	—	—	—
Pyridine	—	1.5102	0.95	115.25	16	5.3	0.71	—	—
Tetrahydrofuran	212	1.4072	0.55	66.0	17	4.0	0.45	3.7	0.53
Toluene	284	1.4969	0.59	110.62	23	2.4	0.29	—	0.22
1,2,4-Trichlorobenzene	308	1.5717	0.566	213.5	—	—	—	—	—
Trichloroethylene	273	1.4767	0.567	87.19	25	1.0	—	—	—
Trichlorotrifluoroethane	231	1.3557	0.711	47.57	—	0.0	—	—	0.02
Trifluoroacetic acid	210	1.2850	0.926	71.8	—	—	—	—	—
Water	190	1.3330	1.00	100.0	—	10.2	—	—	—
o-Xylene	288	1.5054	0.81	144.41	—	2.5	0.26	—	—

Data were obtained from reference 3 unless otherwise indicated.

* Missing values indicate data are unavailable.

† All pairs whose *M* numbers differ by 15 units or less are miscible in all proportions at 15 °C. Each pair whose *M* number difference is 16 has a critical solution temperature between 25 °C and 75 °C, approximately 50 °C preferably. A difference of 17 or more corresponds to immiscibility or to a critical solution temperature above 75 °C. Miscibility data were obtained from reference 4.

phosphate buffers may precipitate when mixed with organic solvents, causing numerous problems, including irreversible precipitation inside the pumps and the column. When storing columns or turning off the LC system for more than a few hours, thoroughly rinse all buffers from the system with nonbuffered mobile phase.

SOLVENT STRENGTH AND POLARITY

Mobile phases containing unusual solvent mixtures are frequently described in the literature. Figure 4 shows an example of the use of three modifiers in the separation of a standard protein mixture. 2-Methoxyethanol offered the best selectivity for the separation. Eluotropic strength and polarity are two important parameters to consider in selecting comparable solvents for a separation.

Eluotropic strength is a measure of the ability of the solvent to elute a solute from a particular stationary phase. Therefore, the strength of each solvent depends on the choice of stationary phase. For example, acetonitrile and tetrahydrofuran are weak solvents when used with silica or alumina columns but are relatively strong solvents when used in reversed-phase columns. Table I lists some

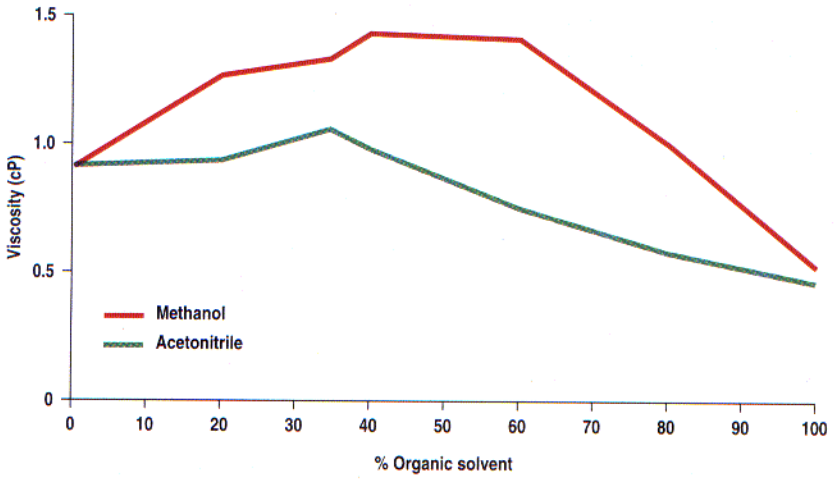

FIGURE 1: Viscosity as a function of solvent composition (water reference). (Reprinted with permission from reference 5.)

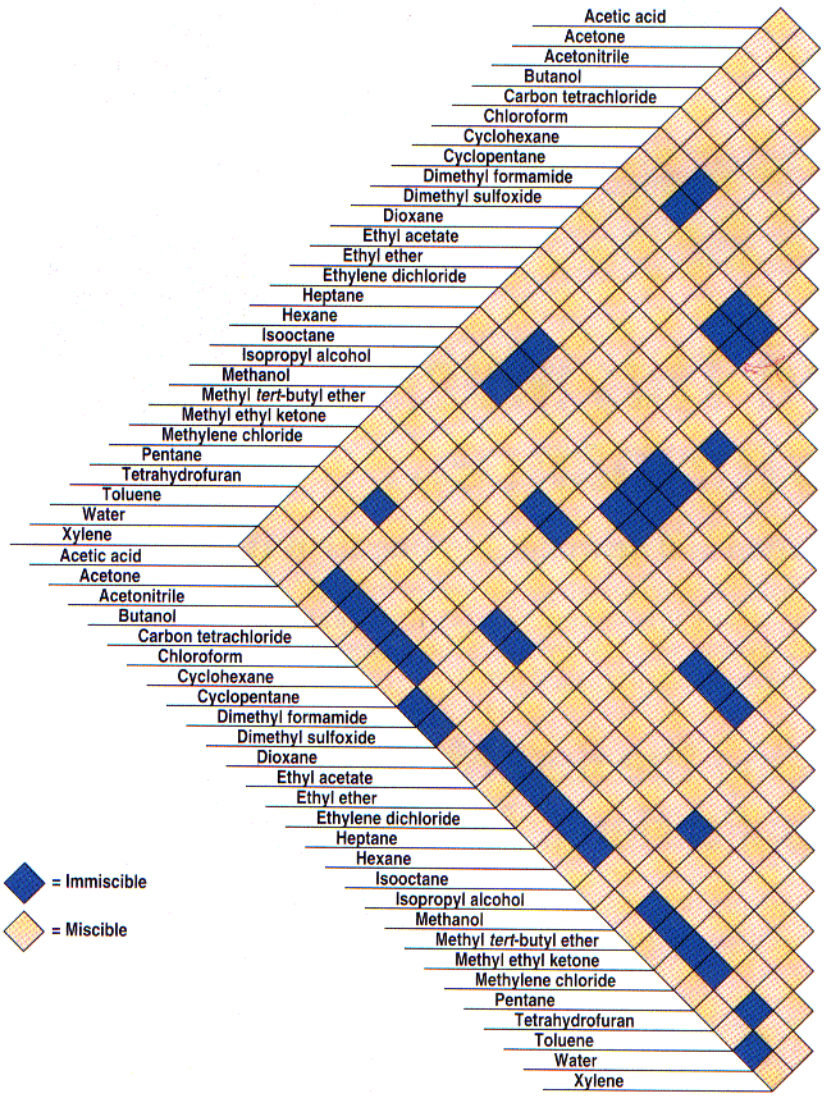

FIGURE 2: Solvent miscibilities. (Data were obtained from reference 3.)

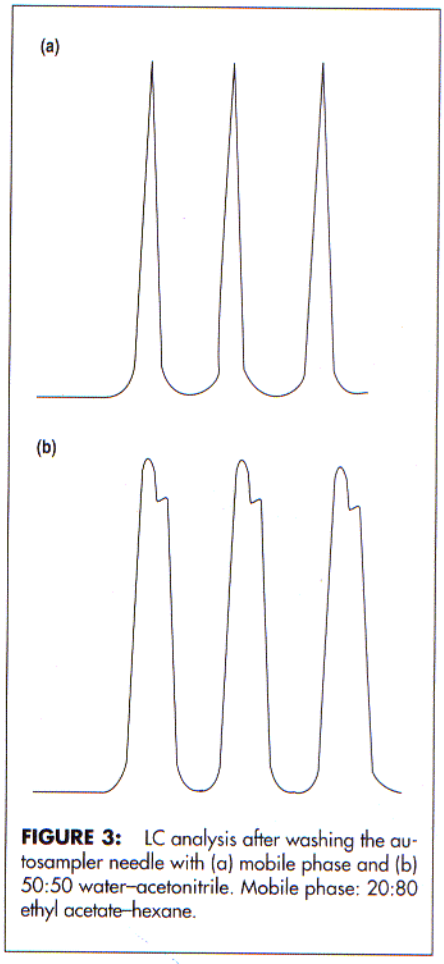

FIGURE 3: LC analysis after washing the autosampler needle with (a) mobile phase and (b) 50:50 water–acetonitrile. Mobile phase: 20:80 ethyl acetate–hexane.

physical properties of other solvents. When you optimize reversed-phase methods, use eluotropic strength tables as a guide for adjusting the mobile-phase strength.

The solvent polarity table, based on solvents' ability to share electrons, is useful when you choose the polarity of your solvent system to optimize separation. Polarity is of particular interest when developing or optimizing normal-phase separations. If the solvent is extremely polar, slight changes in concentration can drastically affect the selectivity — a less polar solvent is often more desirable. Determine how sensitive a new separation is to small changes in mobile-phase composition before you invest too much time developing a method with a particular solvent. You don't want to finalize a method only to find that the retention times of the compounds of interest change significantly with only a 1–2% change in solvent concentration. If solvent polarity is the problem, a higher concentration of a less polar solvent may achieve the same separation and still allow you to fine-tune the separation conditions without adversely affecting retention times. Degassing can cause problems with the more volatile solvents. For example, volatile solvents in premixed normal-phase eluents can boil off with excessive helium sparging, changing retention times significantly. As you validate each method, be sure to test for reasonable and expected changes in mobile-

phase composition and adjust operating procedures to minimize future problems.

When using the solvents' eluotropic strength or polarity index for method development, remember that to increase analyte retention, decrease the eluotropic strength. Conversely, shorter retention times require an increase in the eluotropic strength. For example, in normal-phase separations you would decrease mobile-phase polarity to increase retention and increase mobile-phase polarity to decrease retention. Figure 4 shows how substituting solvents with similar polarity will often change the selectivity by affecting analyte retention.

SUMMARY

Solvent viscosity and miscibility are important characteristics to consider during method development. If the solvent is too viscous, back-pressure problems are likely as the column ages. Strange-looking chromatograms often appear when mobile-phase components are not completely miscible. Reproducibility suffers when miscibility problems occur. Simple mobile phases — those containing only two or three components — are the least prone to preparation problems. As the number of mobile-phase components increases, the probability of problems resulting from human or instrument error increases. You often can gain additional selectivity by substituting all or part of one solvent with another of similar polarity.

The next column in this series will discuss solvent degradation and lifespan, and how to identify solvents with degradation potential. We will consider why solvents degrade, the reactions involved, their by-products, and their influences on separations. We will also examine the use of solvent stabilizers to minimize problems.

REFERENCES

(1) C. Seaver and P. Sadek, *LC•GC* **12**(10), 742–746 (1994).
(2) R.E. Majors, *LC•GC* **12**(12), 890–898 (1994).
(3) "Burdick & Jackson Solvent Guide; Third Edition," Burdick & Jackson (Muskegon, Michigan, 1990).
(4) B.A. Bidlingmeyer, *Practical HPLC Methodology* (John Wiley and Sons, New York, 1992), p. 245.
(5) L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography* (John Wiley and Sons, New York, 2nd ed., 1979).
(6) J.W. Huber and C.L. Seaver, *J. Liq. Chrom.* **10**(11), 2337–2348 (1987).

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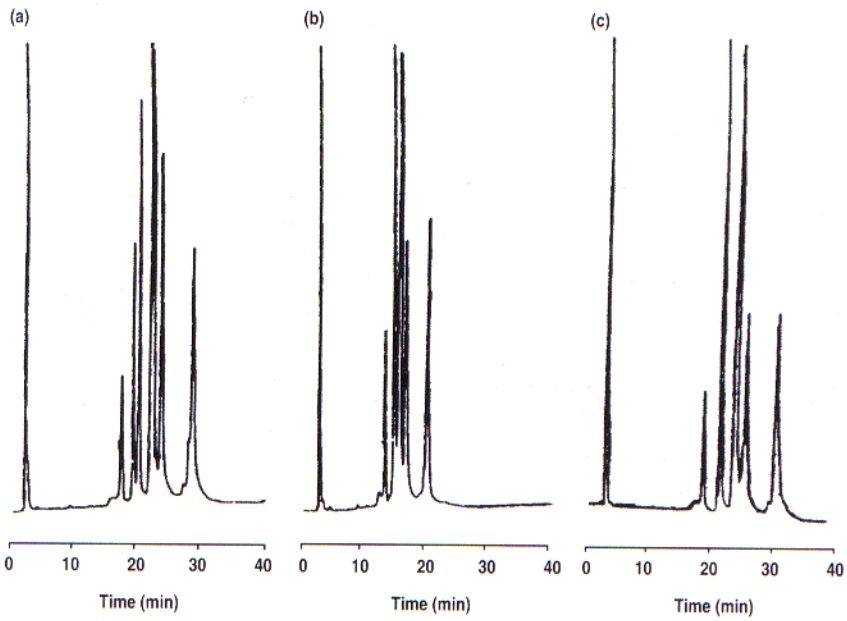

FIGURE 4: LC analysis of a protein sample using (a) 2-methoxyethanol, (b) acetonitrile, and (c) methanol as mobile-phase modifiers. Mobile phase A: 0.1% trifluoroacetic acid in water; mobile phase B: 0.1% trifluoroacetic acid in 50:50 (v/v) organic modifier–acetonitrile; linear gradient: 5–95% B over 30 min; flow rate: 1 mL/min; detection: UV absorbance at 280 nm (0.064 AUFS); sample: 10 µL of 200–300 µg/protein. Sample: uracil (void volume indicator), bovine insulin, ribonuclease A, lysozyme, cytochrome c, and bovine serum albumin. (Reprinted with permission from reference 6.)