Prediction and generation of mobile phase composition gradient in miniaturized liquid chromatography system

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1. Introduction

Miniaturization of liquid chromatography instrumentation is not an easy task. Recently we have developed a simple alternative to the complex and bulky binary gradient pumps. It consists only of single syringe pump which is connected with central port of selector valve via capillary which serves as gradient loop [1]. Gradient is created when different solvents are successively introduced into the gradient loop and created boundary is dispersed due to parabolic flow profile. Single boundary dispersion produces nonlinear gradient, while three successively created boundaries between adequate solvents can be dispersed such that almost linear gradient is produced. For this purpose the volume of solvents and flow rate must be set properly.

In this work, the Taylor´s approximation for boundary dispersion [2] is used to evaluate single boundary (Eq. 1) and to determine the coefficients which are used for calculation of gradient profile created from three boundaries according Eq. 2. Computer simulation with simple mathematical model which suggests varying diffusion coefficient in binary mixture and volumetric changes during mixing (Eq. 3) is considered for this purpose as well.

Eq. 1
$$X = (X_1 - X_2)\left(0.5 + 0.5\,\mathrm{erf}\left(0.5(x - ut)\left(\frac{a^2 4 u^2}{192 D_{12}}\right)^{-0.5} t^{-0.5}\right)\right) + X_2$$

Eq. 2
$$X = (X_1 - X_2)\left(0.5 + 0.5\,\mathrm{erf}\left(0.5(x - ut)\left(\frac{a^2 4 u^2}{192 D_{12}}\right)^{-0.5} t^{-0.5}\right)\right) +$$
$$+ (X_2 - X_3)\left(0.5 + 0.5\,\mathrm{erf}\left(0.5(x - ut)\left(\frac{a^2 4 u^2}{192 D_{23}}\right)^{-0.5} t^{-0.5}\right)\right) +$$
$$+ (X_3 - X_4)\left(0.5 + 0.5\,\mathrm{erf}\left(0.5(x - ut)\left(\frac{a^2 4 u^2}{192 D_{34}}\right)^{-0.5} t^{-0.5}\right)\right) + X_4$$

Eq. 3
$$\frac{dC}{dt} = D(x)\frac{d^2 C}{dx^2} + \frac{dD}{dx}\cdot\frac{dC}{dx}; \quad D_{eff} = D\left(1 + \frac{1}{192}\left(\frac{ud}{D}\right)^2\right)$$
$$dx = \sqrt[3]{\frac{\rho_0}{\rho}}\cdot dx_w$$

X	ideal vol.%	a	capillary radius (m)
x	position in capillary (m)	u	linear velocity (m/s)
t	time (s)	D	diffusion coefficient (m²/s)
		C	concentration (mol/m³)
		dx	capillary segment (m)
		ρ	density (kg/m³)

Figure 1. Scheme of experimental setup. (a) syringe pump, (b) gradient loop 0.3×1000 mm , (c) selector valve, (d) solvents, (e) detection capillary 0.075 mm I.D., (f) UV-Vis detector.

2. Experimental

Experimental system (Fig. 1) included (a) syringe pump NE-500 OEM (New Era Pumps) equipped with 100μL glass syringe (SGE, Australia) and (c) 10-port selector valve C55-1000i (VICI Valco). Central port of the selector valve was connected with the syringe via (b) 1 meter long 0.3 mm I.D. PEEKSil tubing (VICI) used as gradient generator. System was computer controlled.

Initially, the gradient loop was filled with terminal solvent. Subsequent selector valve switching and syringe pump withdrawing action at defined speed delivered defined volumes of (d) solvents into the (b) capillary. Thereafter the selector valve was switched to the port occupied with (e) 0.075 mm I.D. capillary. 5 μL/min flow was applied to mobilize the content of gradient loop.

Detection was realized in 0.075 mm I.D. capillary approx. 50 mm from the inlet. (f) Jasco 970 UV-Vis detector was used for absorbance monitoring at 295 nm. Absorbance was converted to the ideal vol. % (% v/v) of acetone via external calibration. Calculations were done in Excel, computer simulations were executed in our own simulator developed in LabVIEW.

3. Results and discussion

Fig. 2 shows dispersion of concentration boundary between water and 30% v/v acetone (Fig. 2A), between 30 and 60% v/v acetone (Fig. 2B) and between 60 and 90% v/v acetone (Fig. 2C) in 0.3 mm I.D. capillary at flow rate 50 μL/min as a function of volume introduced. Boundaries between water and 30% v/v acetone (Fig. 2A) and between 30 and 60% v/v acetone (Fig. 2B) exhibit symmetric sigmoidal profile and are well approximated with Eq. 1 ($R^2 > 0.99$). Boundary between 60 and 90% v/v acetone (Fig. 2C) deviates from sigmoidal profile. Explanation for this can be derived from data of Fig. 4 where significant change in boundary diffusion coefficient and dynamic viscosity within given interval of water-acetone binary mixture composition is shown.

Simulations of boundary dispersion between water and 30% v/v acetone and between 30 and 60% v/v acetone agrees well with the experiment (Fig. 2D and E).

Simulated boundary between 60 and 90% v/v acetone (Fig. 2F, red line) reflects contribution of different mutual diffusion coefficients to the asymmetric boundary dispersion. However, discrepancy between experimental and simulated data (Fig. 2F) indicates that it is not just the varying diffusion coefficient but also the effect of steep gradient of viscosity which may induce complicated fluid dynamics within the capillary (e.g. turbulences, non-parabolic flow profile). Such effects are not involved in simplified model represented by Eq. 3.

Fig. 3 shows the dispersion of three boundaries and continuous gradient generation. Non-ideal shape emphasizes the need for optimal conditions represented by the flow rate of solvent introduction and solvent volume. Thus when diffusion coefficient determined from Fig. 2A-C are fitted into the Eq. 2 together with data of Table 1, the smooth gradient profiles are predicted (Fig. 5A) and observed experimentally (Fig. 5B).
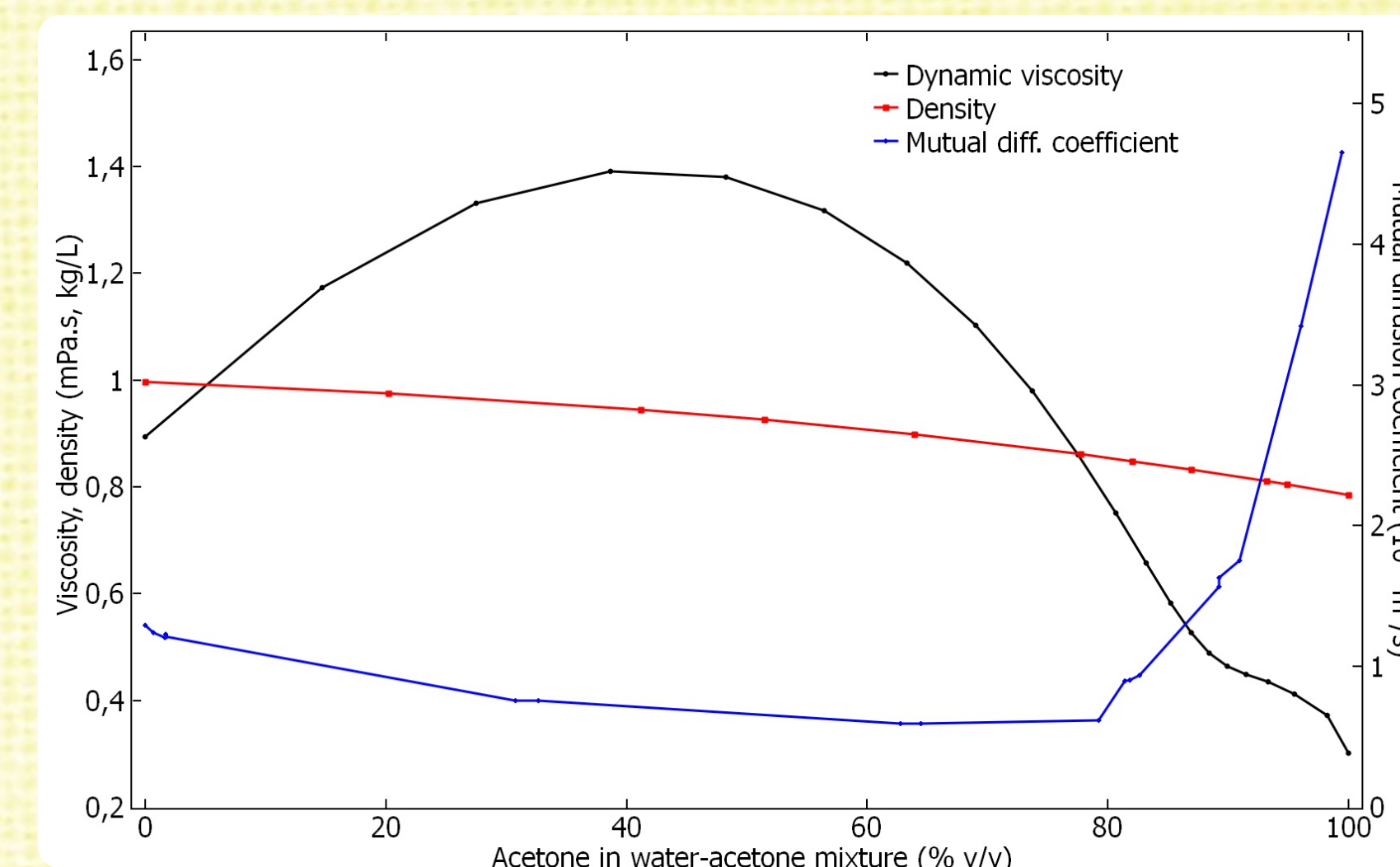

Figure 4. Physical properties of water-acetone binary mixtures [3, 4].

Table 1. Inputs for Eq. 2 and experiment in Fig. 5.

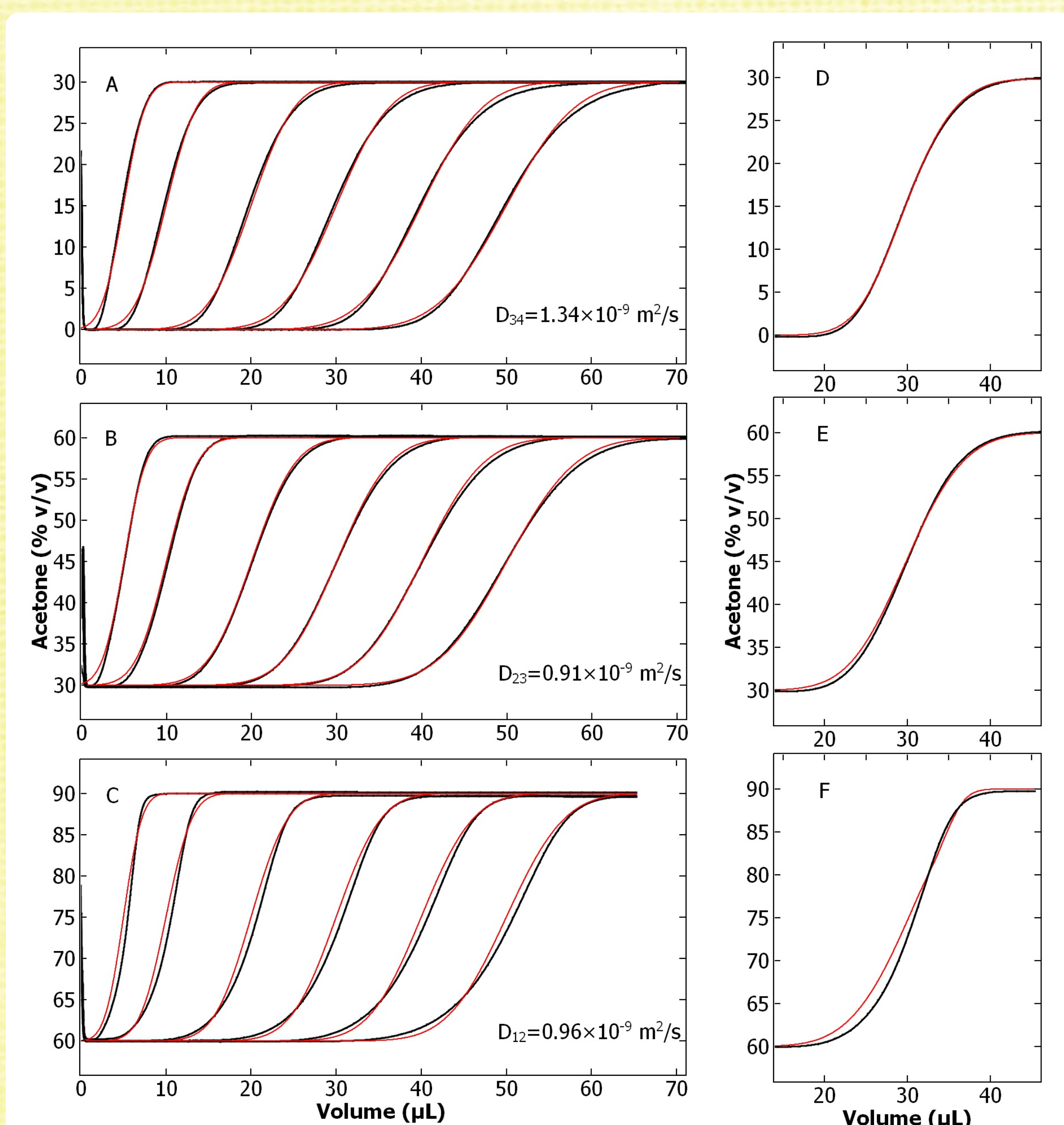| X ideal vol.%		a	b	c	d	e	f	
90	V1			20				μL
	Q1			50				μL/min
60	V2	2	4	6	9	12	17	μL
	Q2	5	10	20	50	50	50	μL/min
30	V3	2	4	6	9	12	17	μL
	Q3	5	10	20	50	50	100	μL/min
0	V4	5	7	9	12	15	20	μL
	Q4	10	30	50	100	150	200	μL/min

Figure 2. Dispersion of boundary in 0.3 mm I. D. capillary at 50 μL/min. (A-C) Eq. 2 (red lines) fitted to the experimental data (black lines). Diffusion coefficients obtained from nonlinear regression of experimental data. (D-F) Overlay of experimental (black lines) and simulation data (red lines).
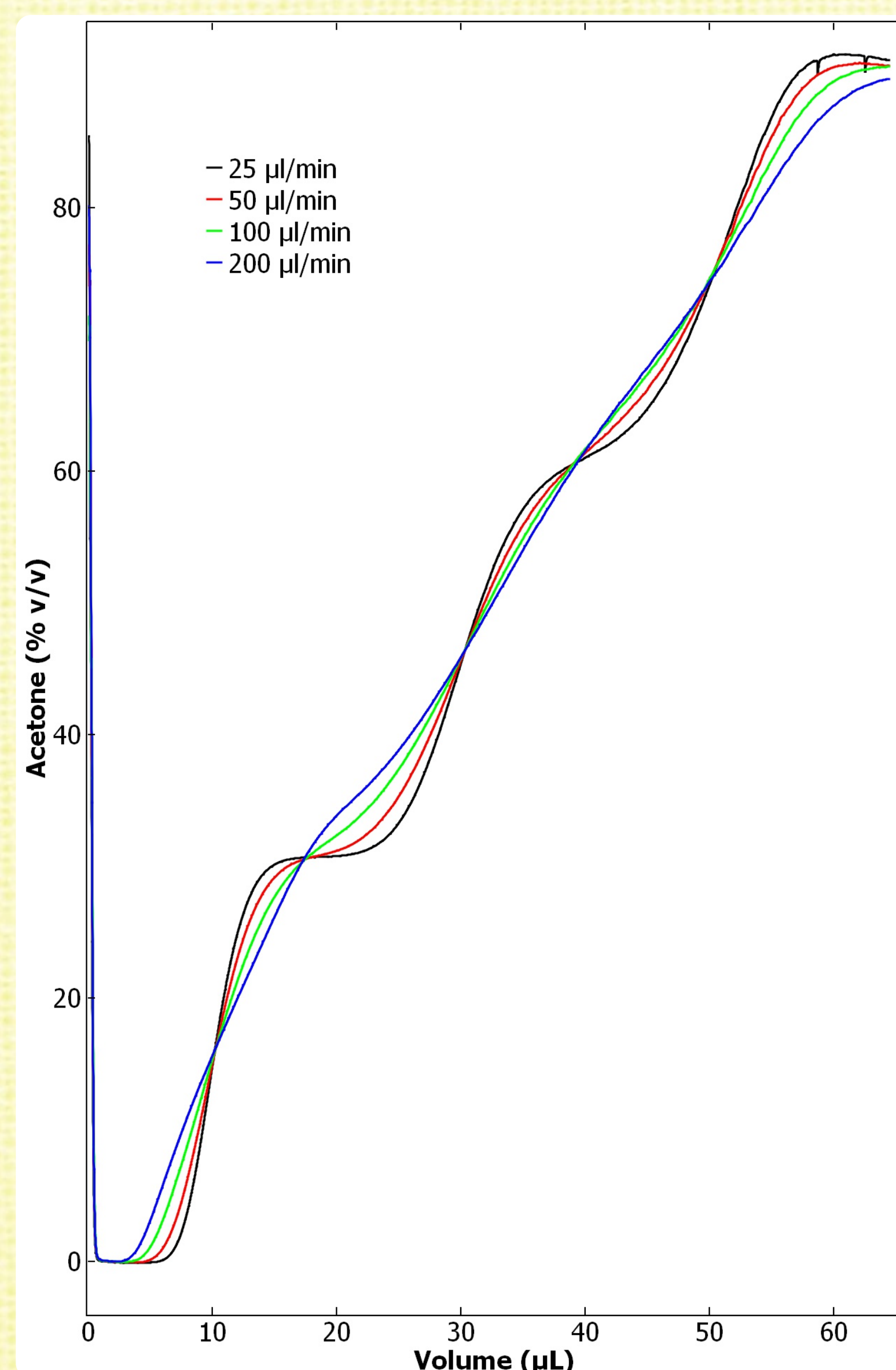

Figure 3. Generation of continuous 0-90 % v/v acetone gradient after successive introduction of 20, 20, 20, and 10 μL of 90, 60, 30 % v/v acetone and water into the 0.3 mm I. D. capillary at flow rate 25-200 μL/min.
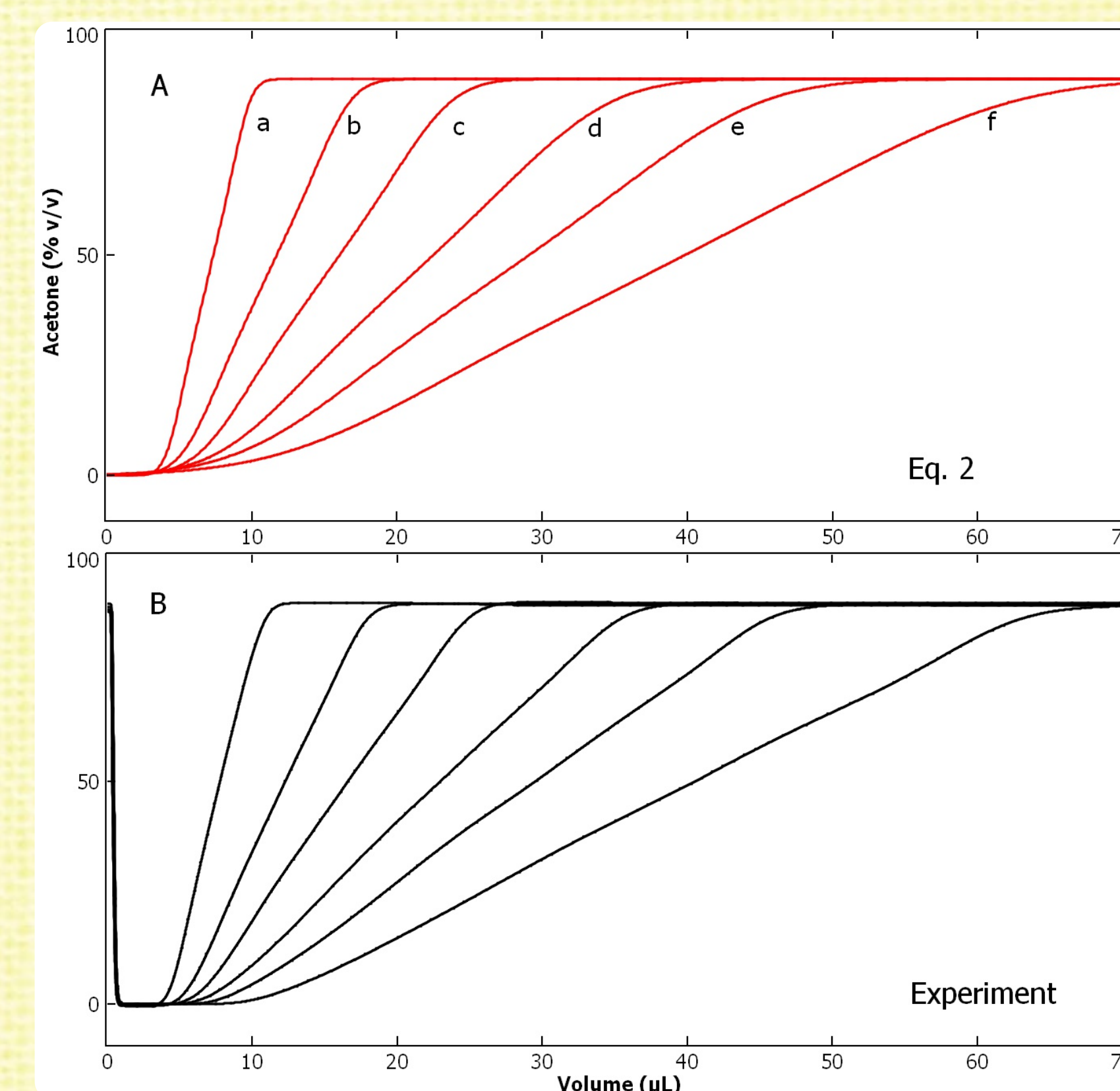

Figure 5. Optimized gradient profiles 0-90 % v/v acetone after dispersion of three concentration boundaries in 0.3 mm I. D. capillary. Volumes and flow rates according Table 1. (A) Calculations according Eq. 2, (B) experiment.

4. Conclusion

Taylor´s approximative solution and one-dimensional computer simulation of boundary dispersion in capillary were considered for prediction of gradient profile generated in our simplified liquid chromatography system. The results revealed that gradient created by successive introduction of 60% v/v acetone, 30% v/v acetone and water into the capillary filled with 90 % v/v acetone is well predicted with Eq. 2. Simulations based on one-dimensional mathematic model which accounts mutual diffusion coefficient of binary mixtures were also found useful. However, numerical calculations take too long and predictions for systems with significant gradient of viscosity are not precise. Instead, experimental determination of diffusion coefficients from boundary dispersion between individual solvents and fit into the Eq. 2 is much more effective approach. Study continues with the water-acetonitrile and water-methanol binary mixtures.

Acknowledgement

References

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