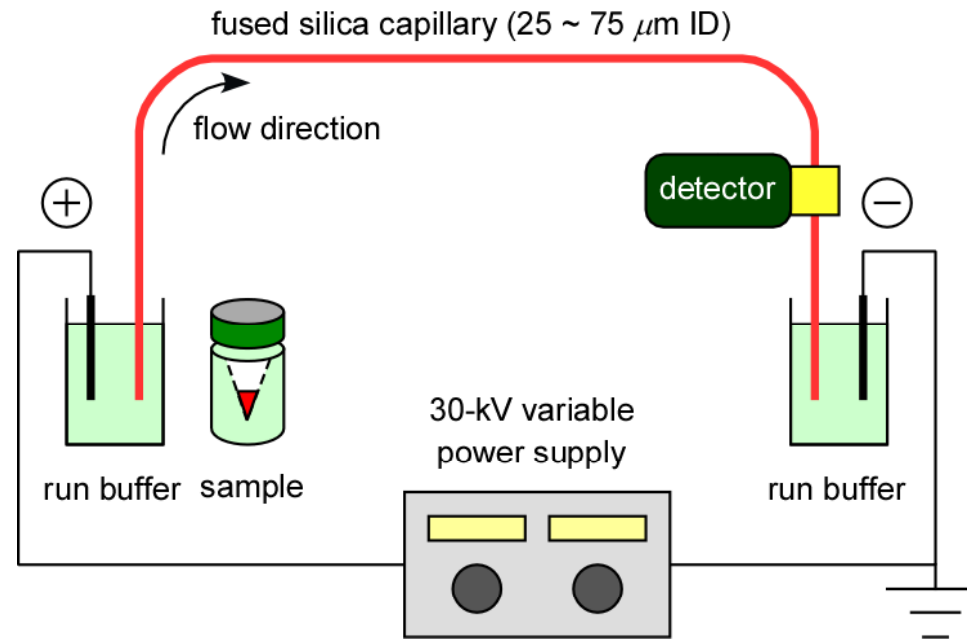


# **Chiral Capillary Electrophoresis of Amino Acids Combined with In-line Single Drop Microextraction**

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Department of Chemistry, Seoul National University, Korea

# Capillary Electrophoresis



## Advantages

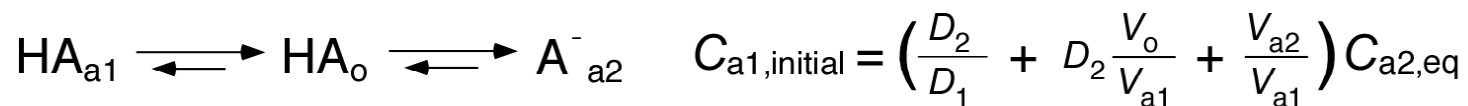
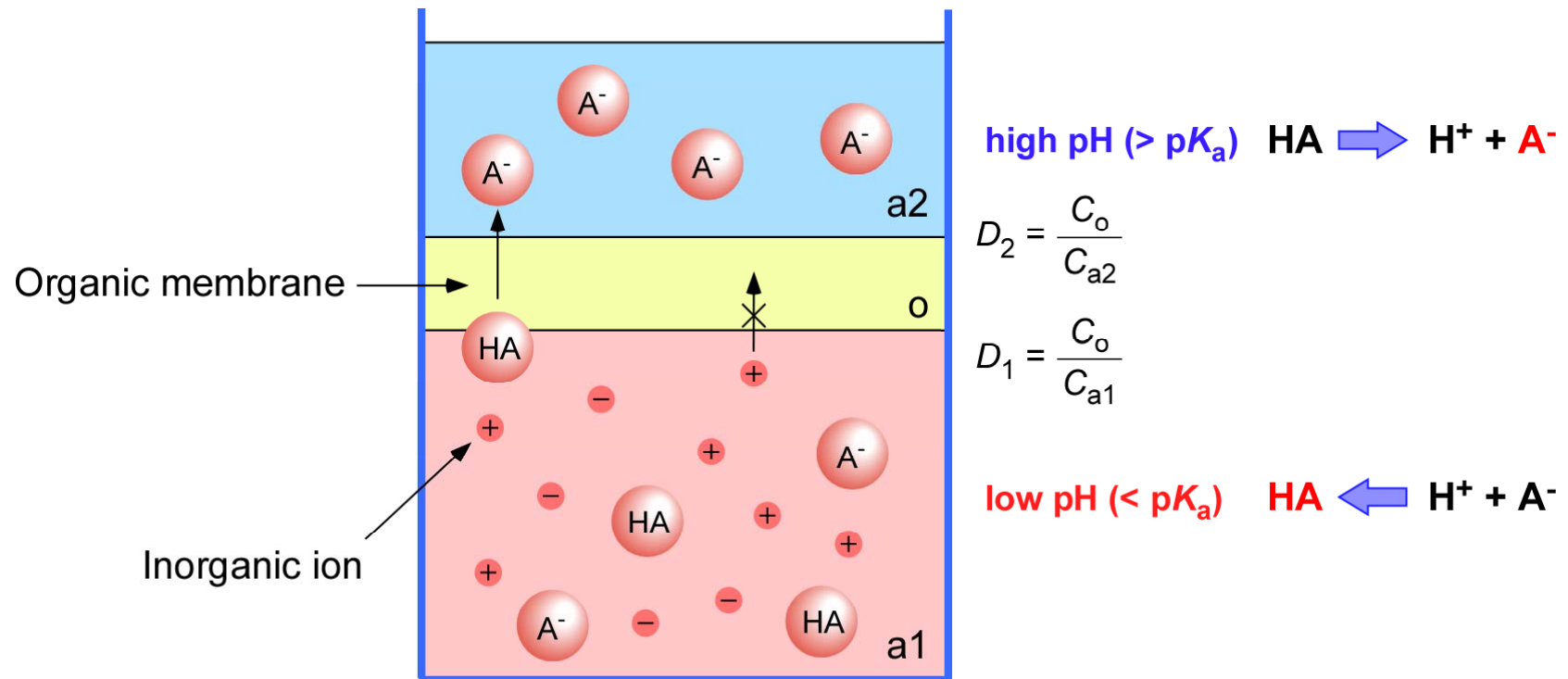
- Fast analysis time: 10 ~ 30 min
- High efficiency:  $N > 10^5 \sim 10^6$
- Small sample volume required: 1 ~ 50 nL
- Numerous modes to vary selectivity and wide applications
- Simple and automated instrumentation

Low Concentration Sensitivity  
~10  $\mu\text{M}$  with UV/Vis

Peak broadening of  
High conductivity samples

# Liquid-Liquid-Liquid Extraction

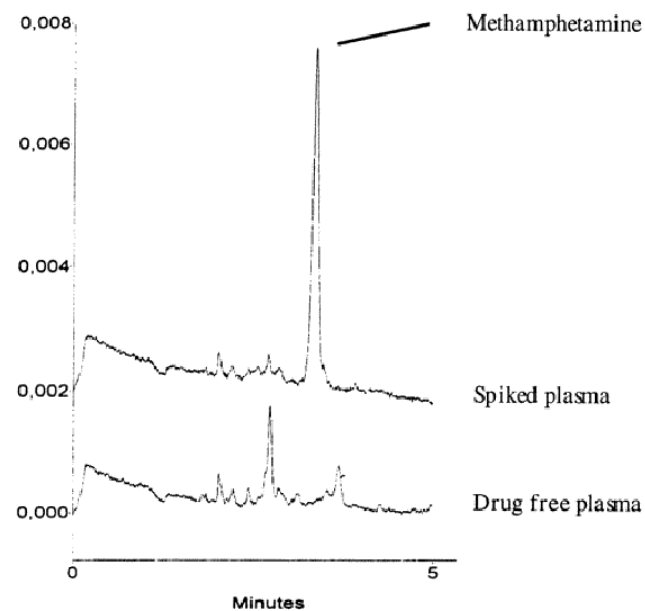
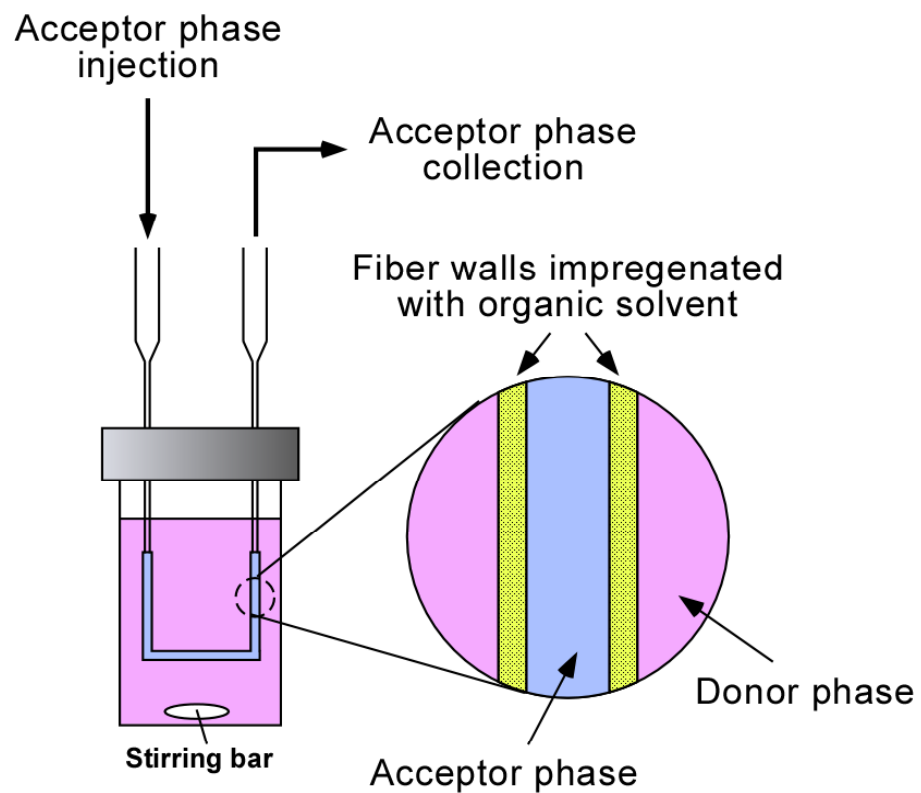
## Enrichment & Desalting



When  $D_2 \ll 1$  &  $V_o \ll V_{a1}$ ,

$$\text{Enrichment factor} = \frac{C_{a2,eq}}{C_{a1,initial}} \approx \frac{V_{a1}}{V_{a2}}$$

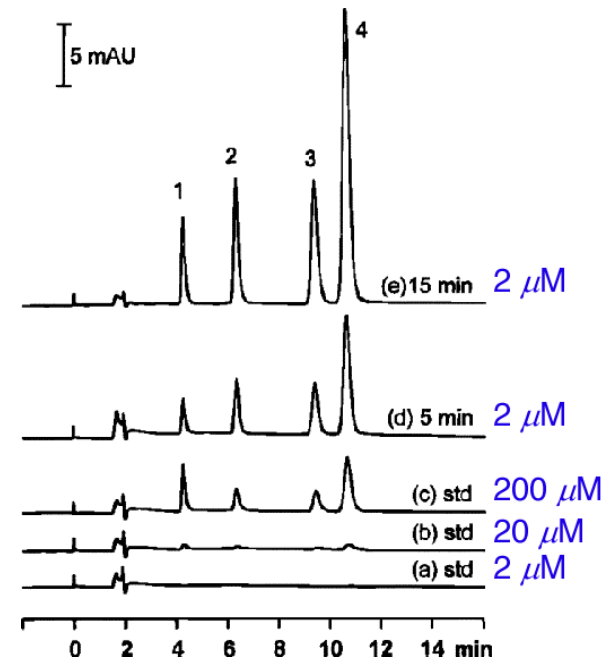
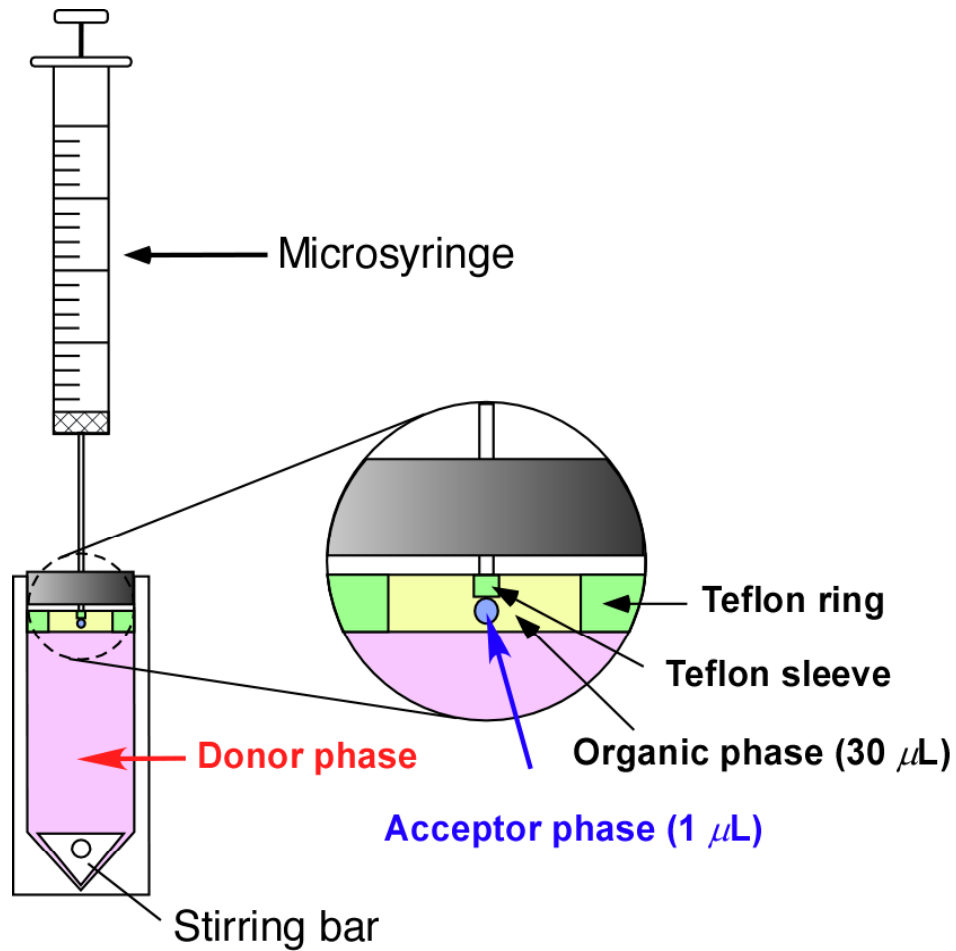
# Liquid-Phase Microextraction



Preconcentration of methamphetamine in capillary electrophoresis

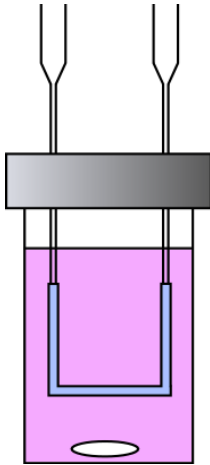
**75-fold enrichment in 45 min, stirring**

# Single Drop Microextraction

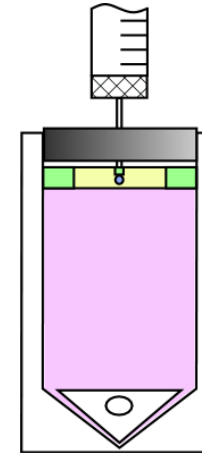


**$\times 500$  preconcentration** with  
15-min SDME, stirring

# LPME vs. SDME



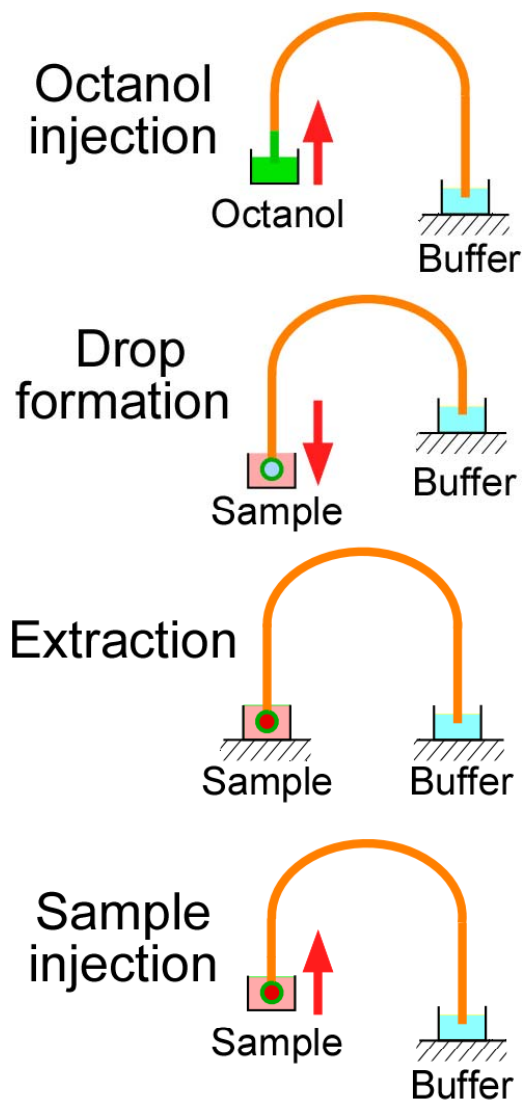
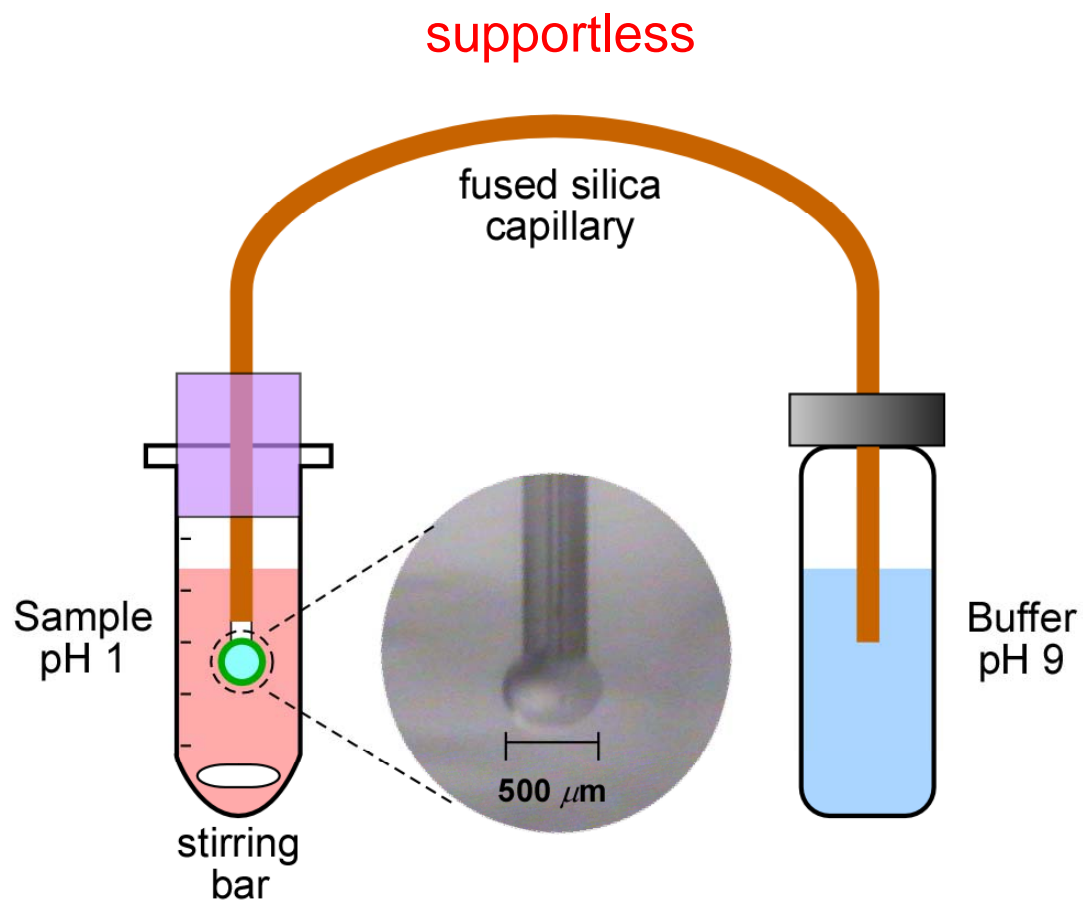
$$\text{Enrichment factor} = \frac{V_{a1}}{V_{a2}}$$



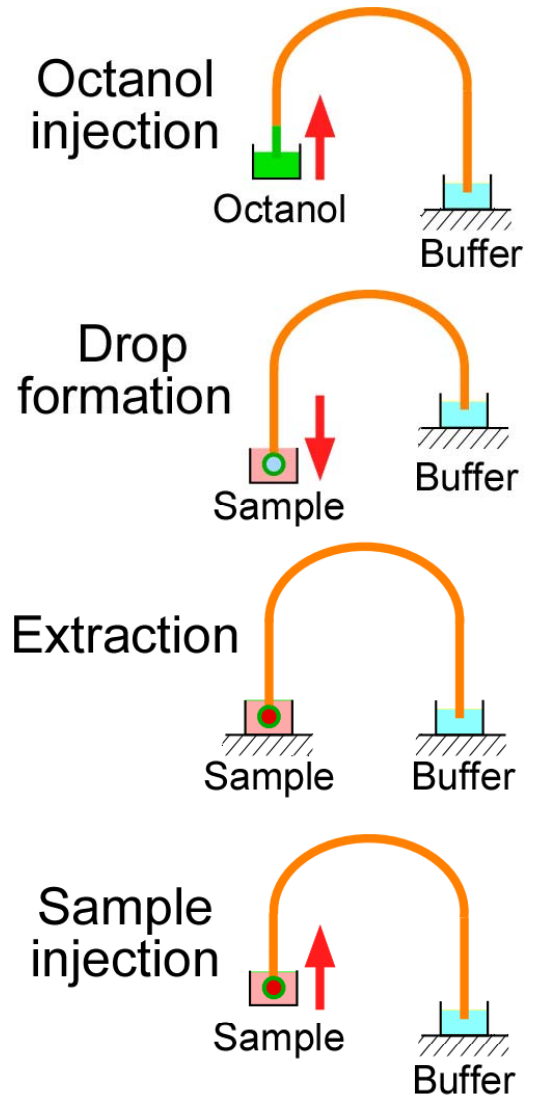
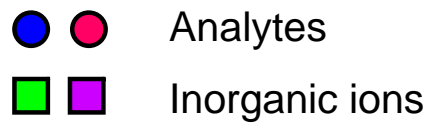
## Volume ratio of aqueous phases

	Donor phase ( $V_{a1}$ )	Acceptor phase ( $V_{a2}$ )	$V_{a1}/V_{a2}$
<b>LPME</b>	2 mL	20 $\mu\text{L}$	100
<b>SDME</b>	2 mL	1 $\mu\text{L}$	2000

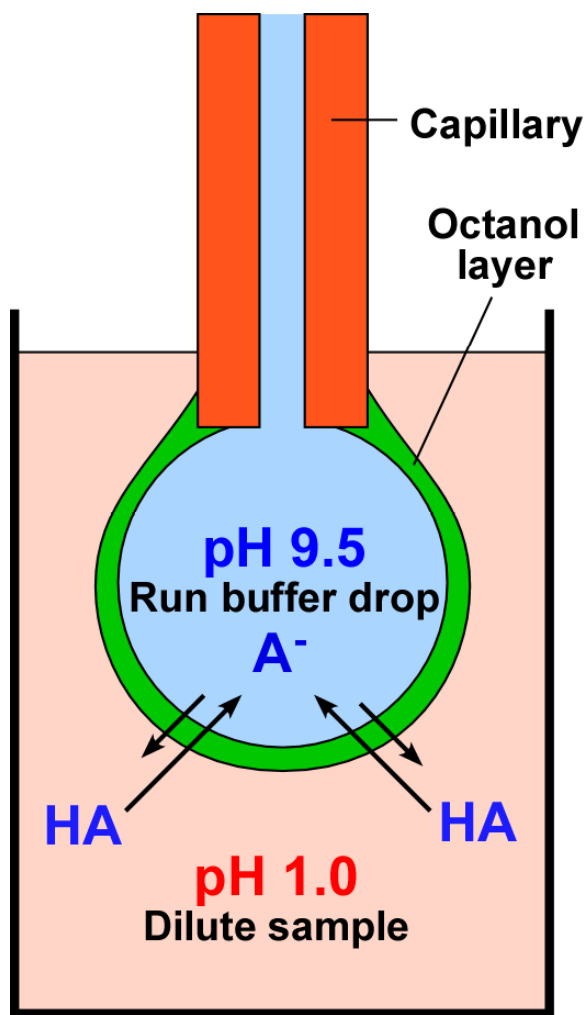
# In-line Single Drop Microextraction



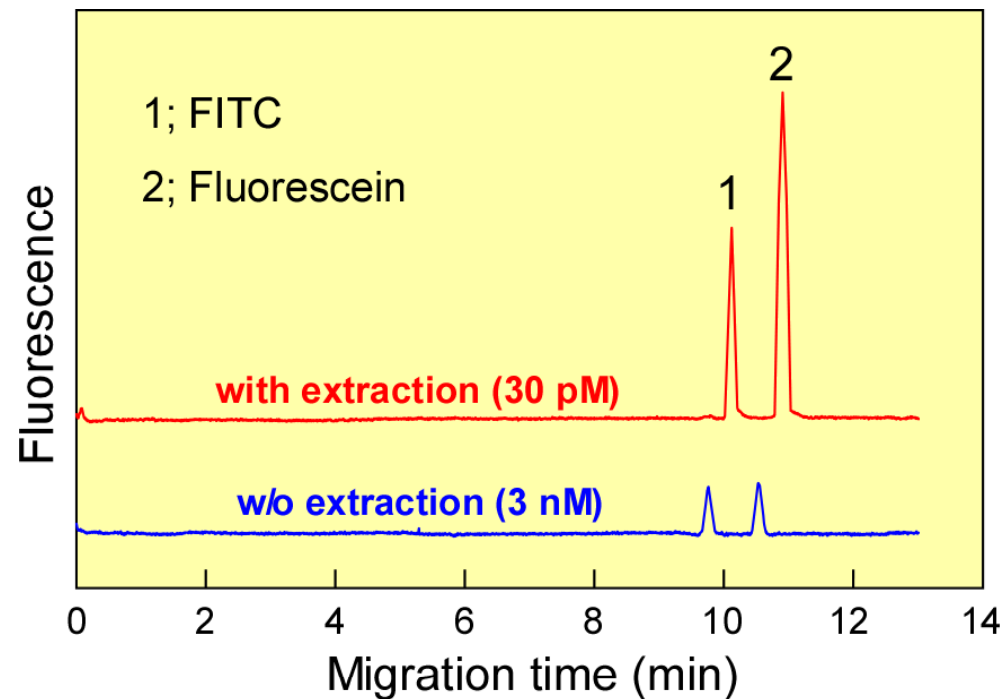
# In-line Single Drop Microextraction



# In-line SDME: Home-made CE



$$V_{a1} = 1 \text{ mL}$$
$$V_o = 5 \text{ nL}$$
$$V_{a2} = 100 \text{ nL}$$



**EF = 490 (fluorescein)**

10-min extraction at 35°C, **stirring**  
20 mM borate buffer (pH 9.5)

10 kV, 75  $\mu\text{m}$  ID, 50/44 cm fused silica capillary

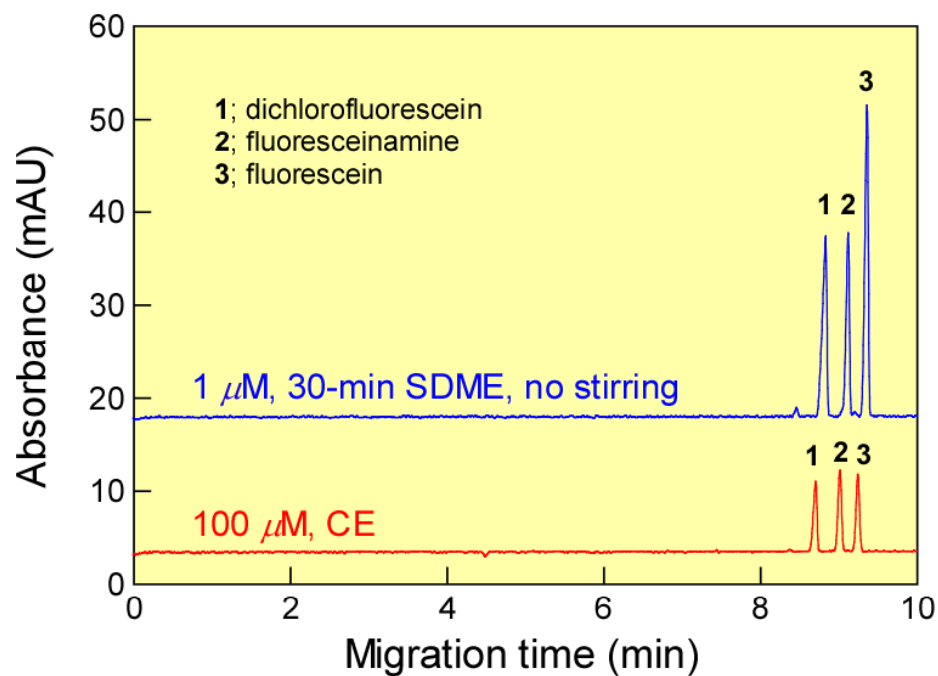
# In-line SDME with a Commercial Instrument

## Without modification; No stirring



### Beckman P/ACE MDQ

40  $\mu\text{m}$  ID, 60/50 cm fused silica capillary  
20 mM sodium borate buffer (pH 9.2)  
20 kV, absorbance at 490 nm



### CE

100  $\mu\text{M}$  sample in a borate buffer (pH 9.2)

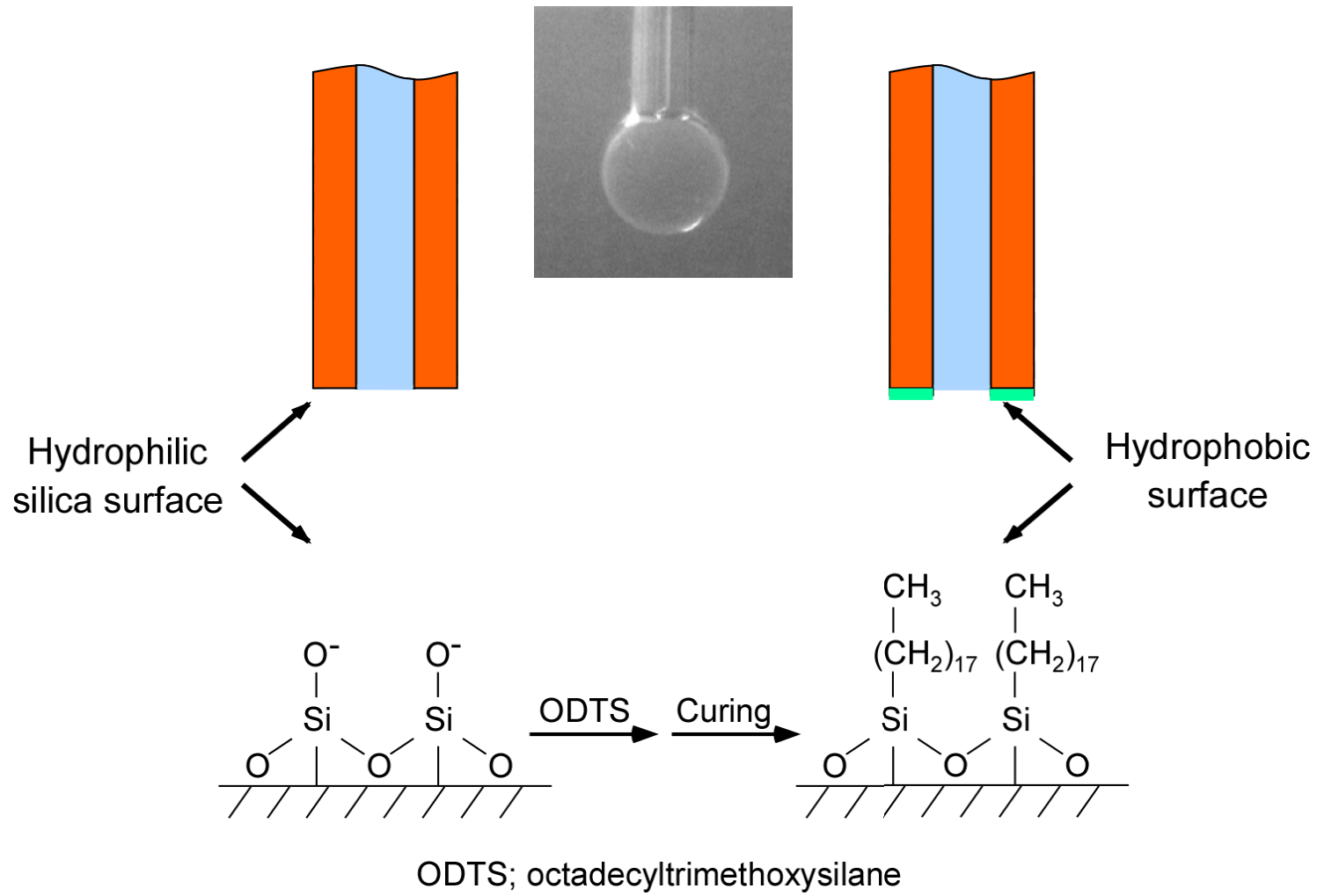
### SDME

1  $\mu\text{M}$  sample in a glycine buffer (pH 1.5)  
Organic phase: octanol, 6 psi  $\times$  5 s injection  
Drop formation: 1 psi  $\times$  36 s  
Extraction: 30 min, no stirring, 25°C

**EF = 360 (fluorescein)**

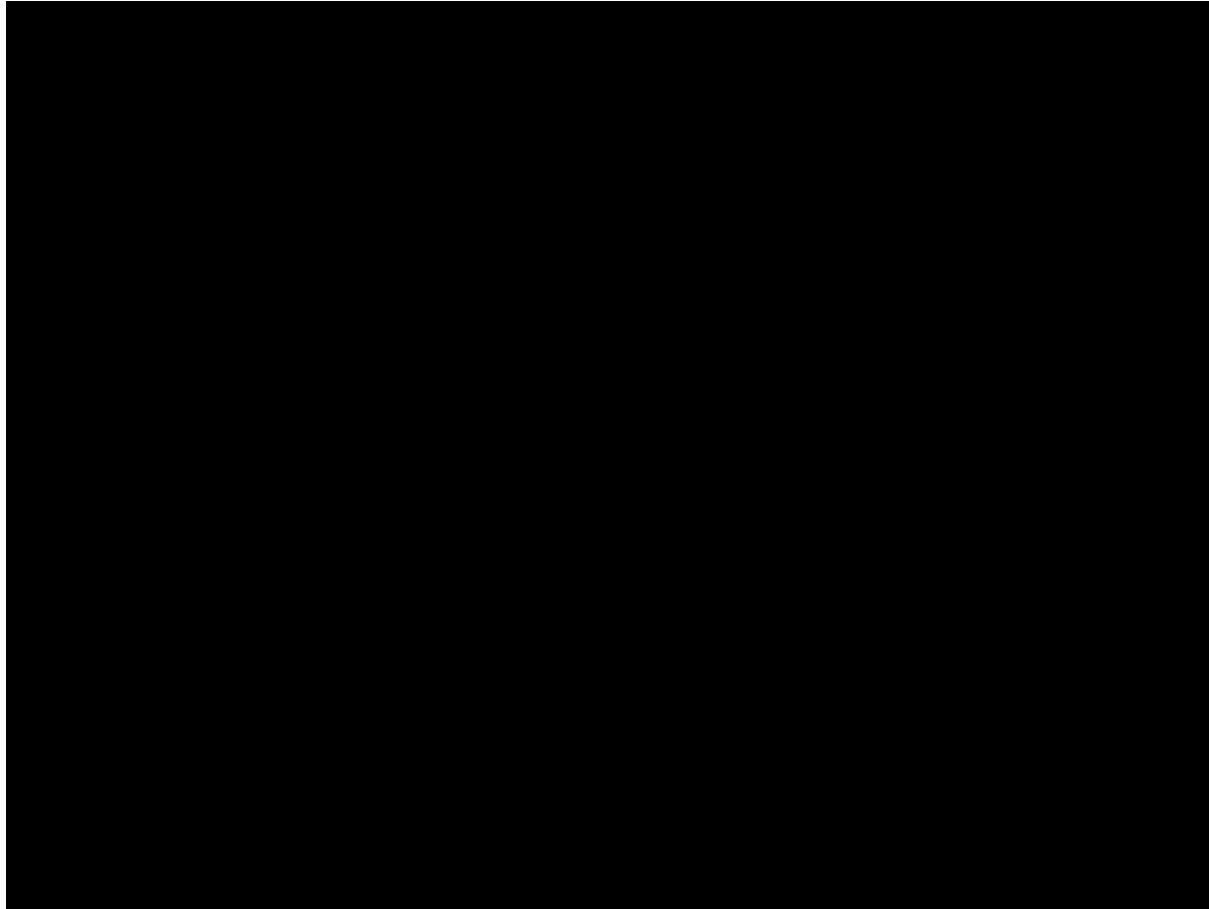
30-min SDME, no stirring

# Hydrophobic Coating



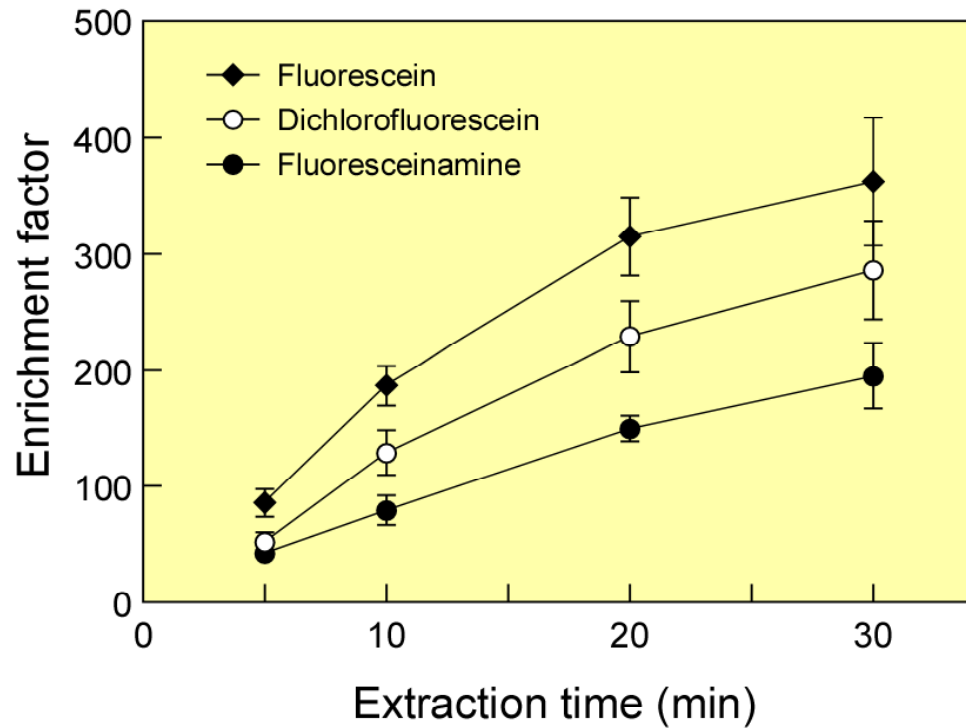
Enhancement of the organic phase stability on the capillary surface

# Hydrophobic Coating



# Extraction Time

## No stirring



### SDME conditions

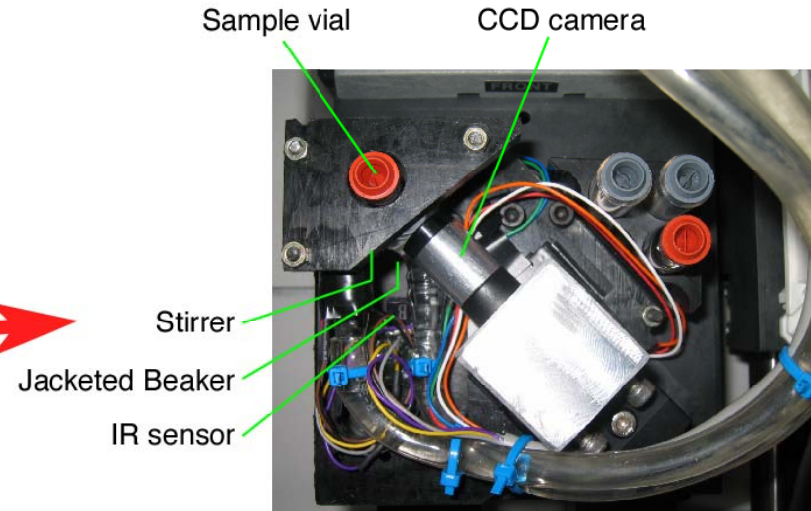
1  $\mu$ M sample in a glycine buffer (pH 1.5)

Organic phase: octanol, 6 psi  $\times$  5 s injection

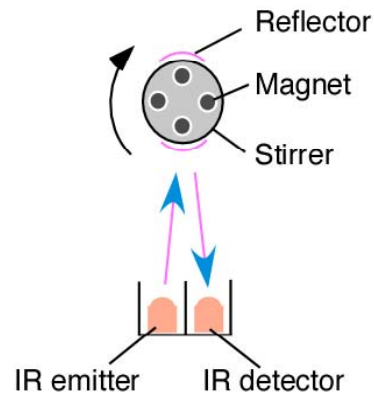
Drop formation: 1 psi  $\times$  36 s

Extraction: no stirring, 25°C

# Microstirrer Retrofit to a CE Instrument

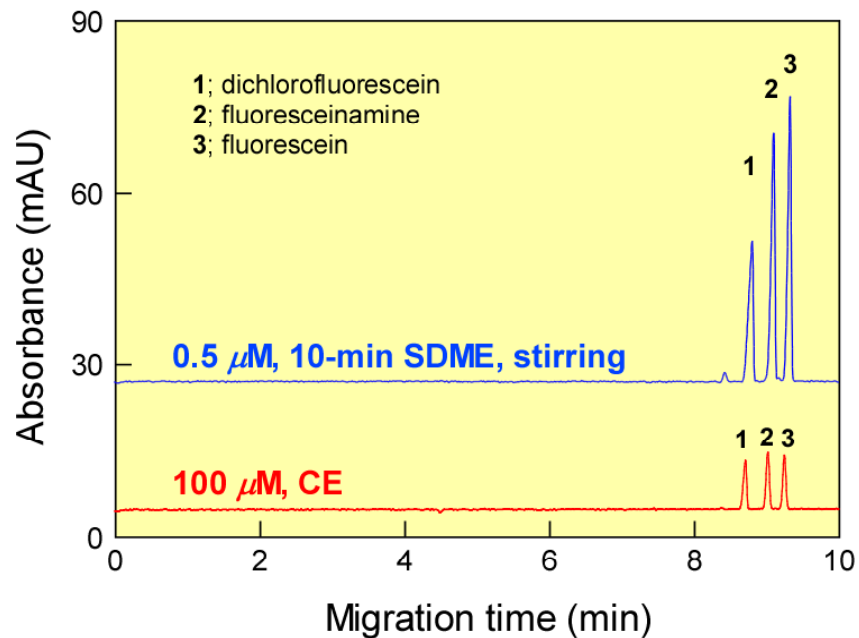
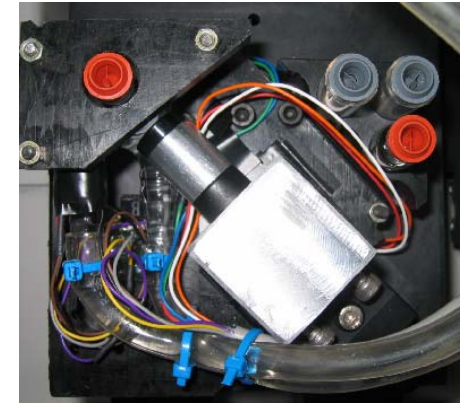


Microstirrer using a coin motor



Tachometer (IR type)

# In-line SDME with a Commercial Instrument With a Microstirrer



## CE

100  $\mu\text{M}$  sample in a borate buffer (pH 9.2)

## SDME

0.5  $\mu\text{M}$  sample in a glycine buffer (pH 1.5)

Organic phase: octanol, 6 psi  $\times$  5 s injection

Drop formation: 1 psi  $\times$  36 s

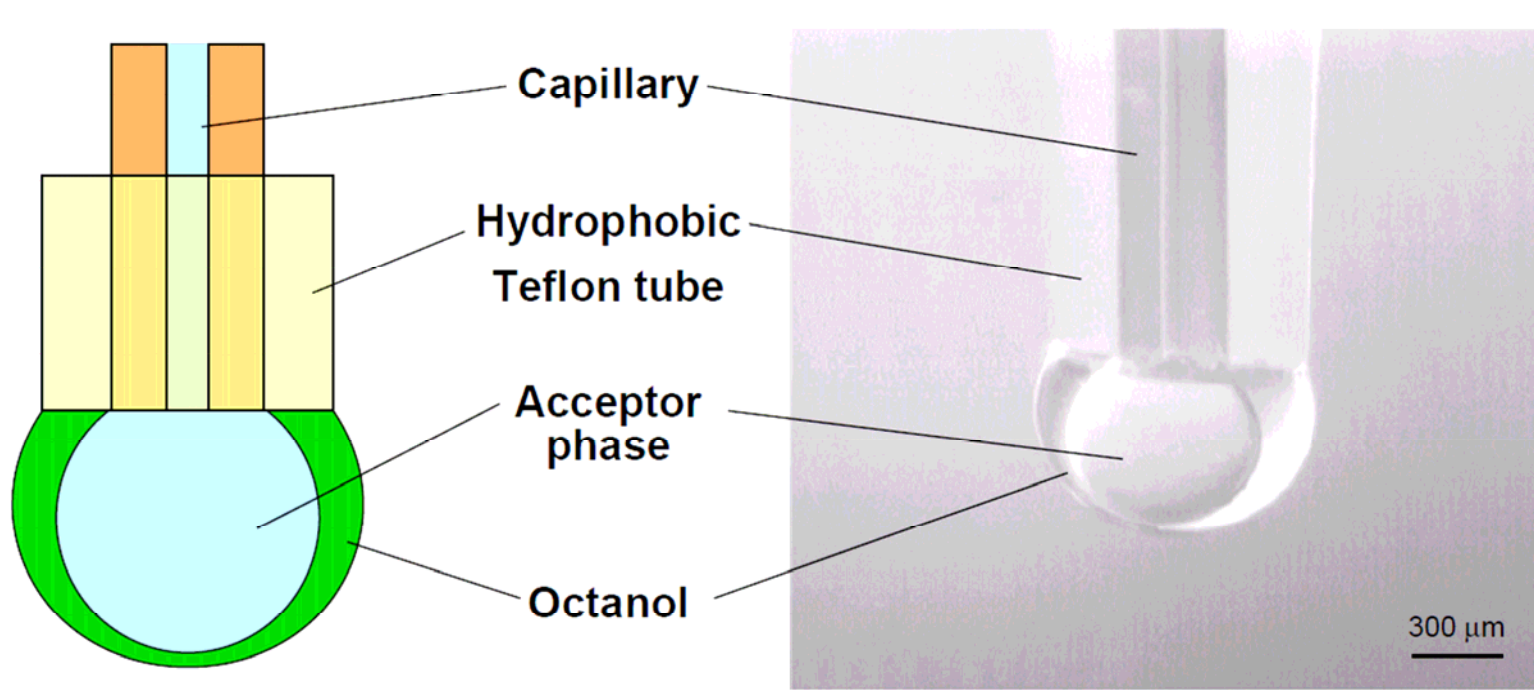
Extraction: 10 min, stirring, 25°C

**EF = 2000 (fluorescein)**

10-min SDME, stirring

# SDME + CE + SWEEPING

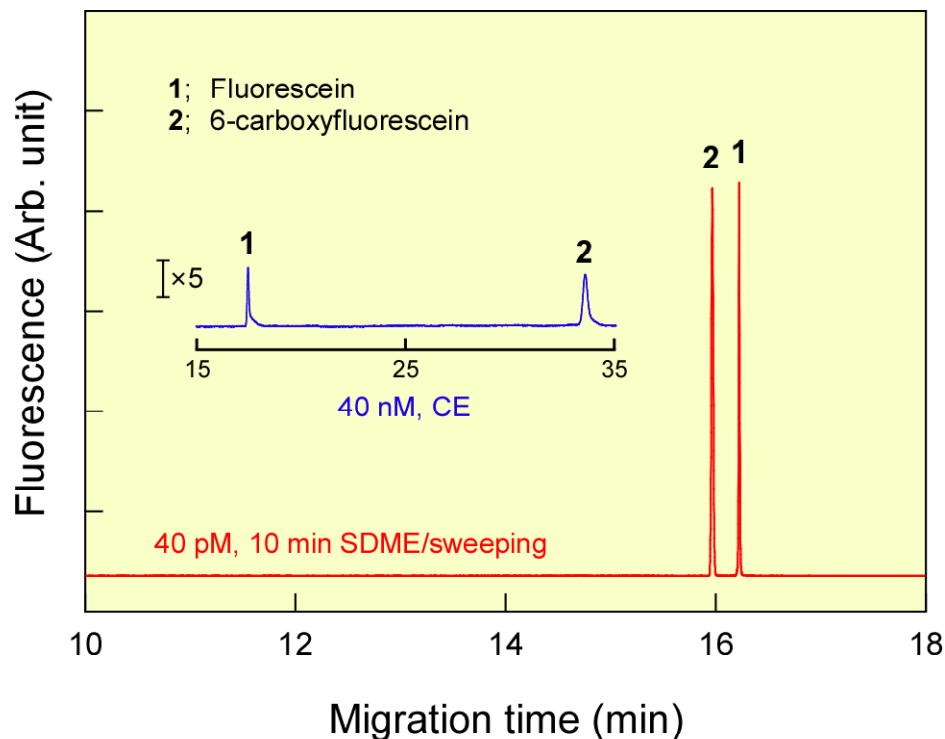
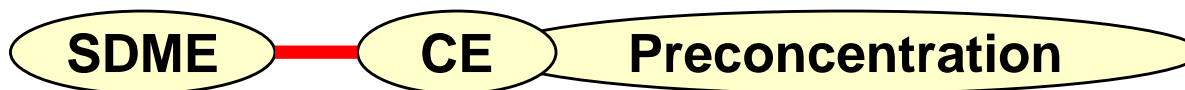
## Large Volume SDME with a Teflon Sleeve



Organic phase stabilization on the capillary surface

Large volume of the acceptor phase

# In-line Large Volume SDME/Sweeping



## Enrichment factors

	SDME	Sweeping	SDME/sweeping
FL	34	670	28 000
6-FAM	110	430	32 000

## CE

25  $\mu$ m ID, 60/50 cm fused silica capillary  
45 mM phosphate buffer (pH 9.0)  
15 kV, LIF at 520 nm

## SDME

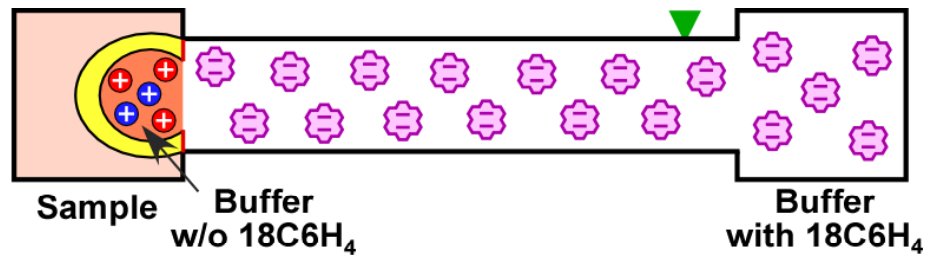
Donor phase: 2 mL, 40 pM in 0.1 M HCl  
Organic phase: Octanol  
Acceptor phase: 45 mM phosphate (pH 9.0)  
Extraction: 10 min, no stirring, 25  $^{\circ}$ C

## Sweeping

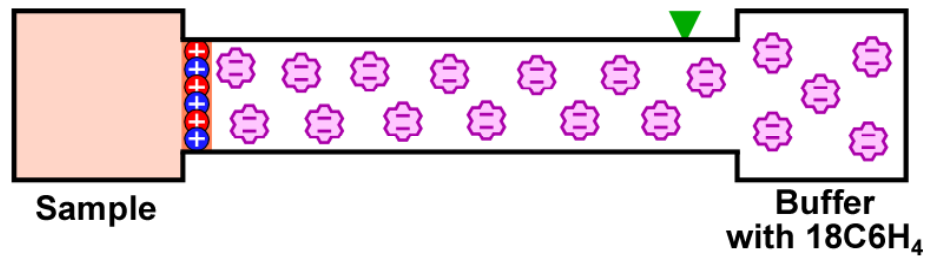
Buffer: 40 mM phosphate, 20 mM DTAB  
15% acetonitrile (pH 9.0)  
Voltage: -15 kV  
Sample plug: 6 cm (30 nL)

10-min SDME, **no stirring**

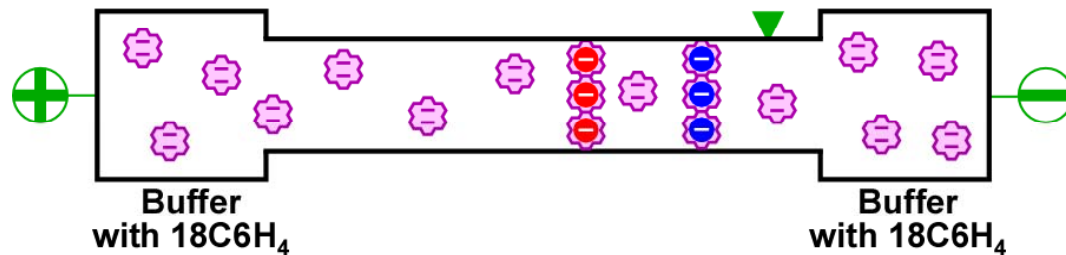
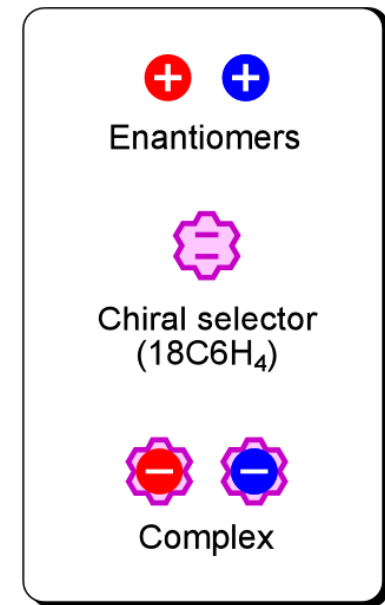
# SDME + Chiral CE



1. SDME

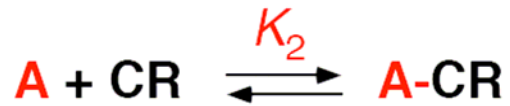
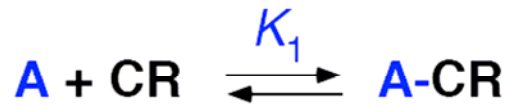
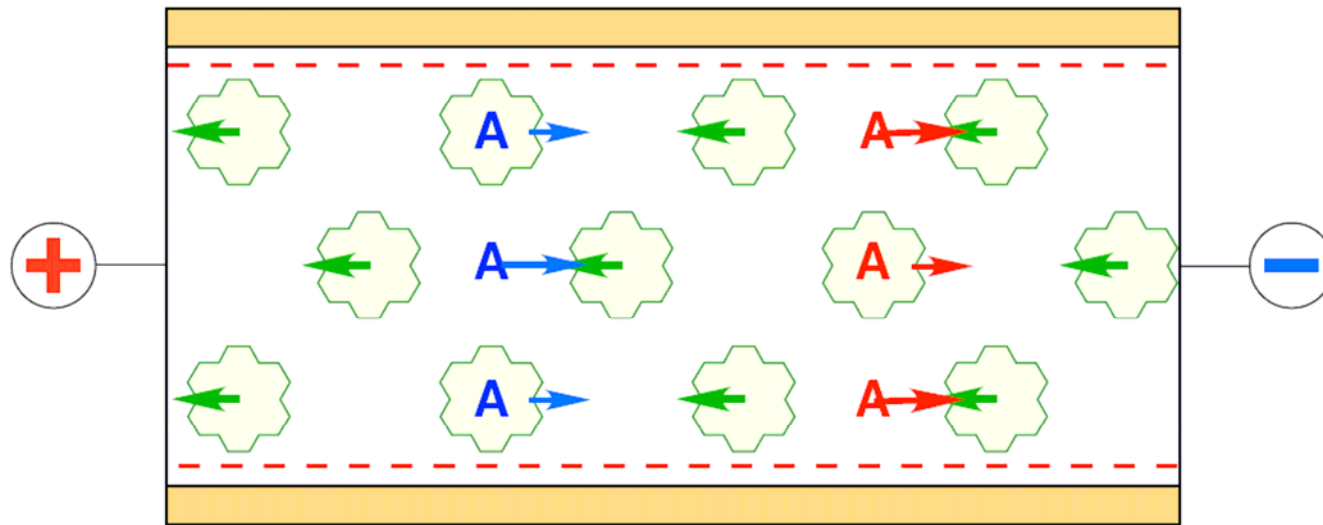


2. Injection



3. Enantioseparation

# Chiral Capillary Electrophoresis



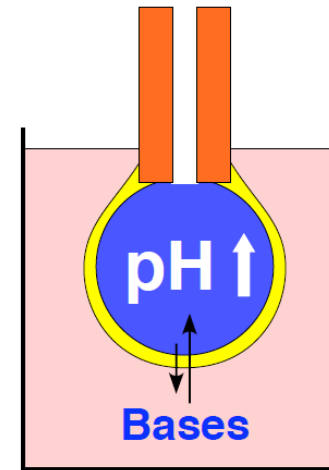
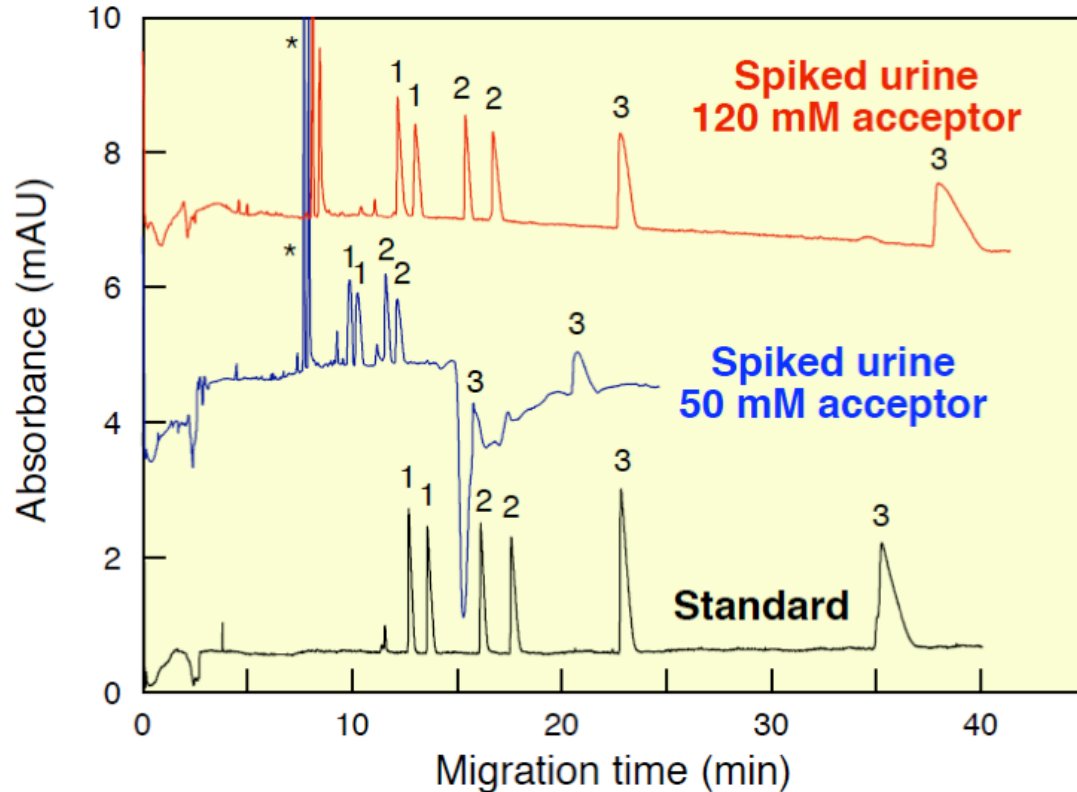
$$K_1 > K_2$$

$$\mu_A = \mu_A > \mu_{A-CR} = \mu_{A-CR}$$



$$\overline{\mu}_A > \overline{\mu}_A$$

# Direct SDME/Chiral CE of Human Urine



Acceptor phase  
buffer capacity  
**50 mM → 120 mM**

**950-Fold Enrichment**  
10-min SDME, stirring

## **SDME/CE**

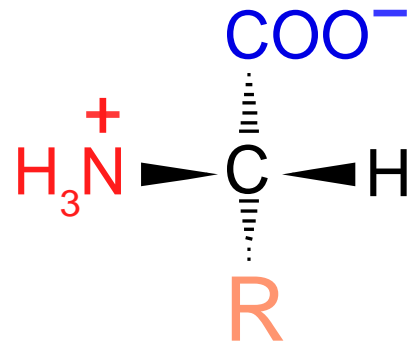
**Donor:** 500 nM AM, 200 nM Cl-AM, 600 nM AD in 0.5 M NaOH (pH 13.7)

**Human urine:** 4.5 mL of spiked urine + 0.5 mL of 5 M NaOH

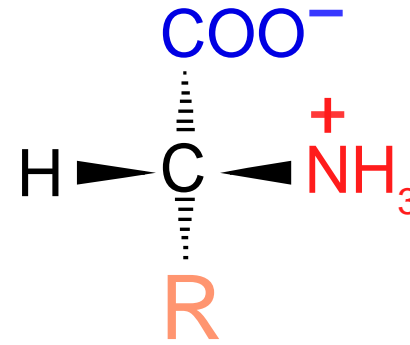
**Extraction:** 10-min with stirring, 25°C

**Run buffer:** **50 mM** Bis-Tris/CA with 0.8 mM 18C6H<sub>4</sub> (pH 4.0)

# Chiral Analysis of Amino Acids



L-amino acids



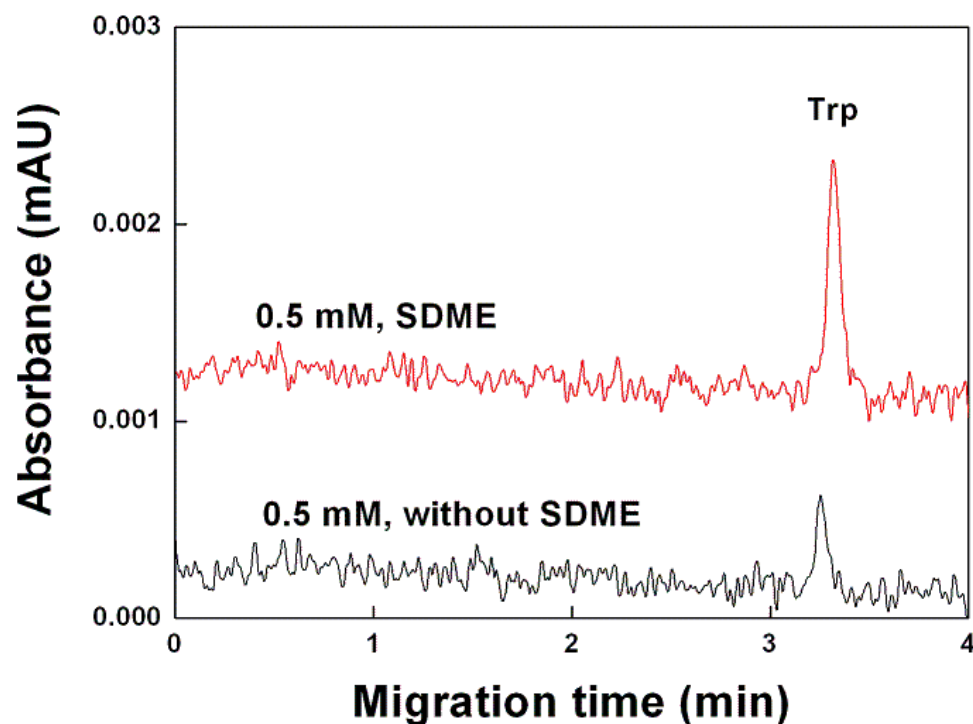
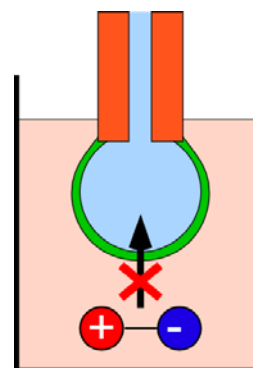
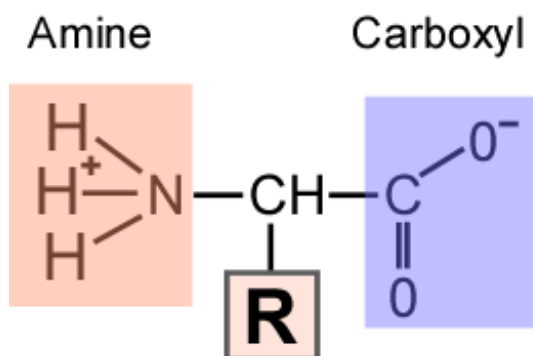
D-amino acids

## Analysis of D-amino acids

- ◆ Clinical diagnostics
- ◆ Estimation of age at death for forensic remains

*Large excess of L-amino acids in real samples*

# SDME of Amino Acids without Derivatization



## SDME

Donor phase: 100 mM phosphate buffer (pH 5.9)

Organic phase: octanol

Acceptor phase: 80 mM borate buffer (pH 9.3)

Extraction: 10 min, 25°C

## Separation

25  $\mu\text{m}$  ID, 60/50 cm fused silica capillary

80 mM borate buffer (pH 9.3)

Pressure 10 psi, absorbance at 280 nm

**EF  $\leq$  1.6**

10-min SDME, no stirring

# SDME of Amino Acids with FITC Derivatization

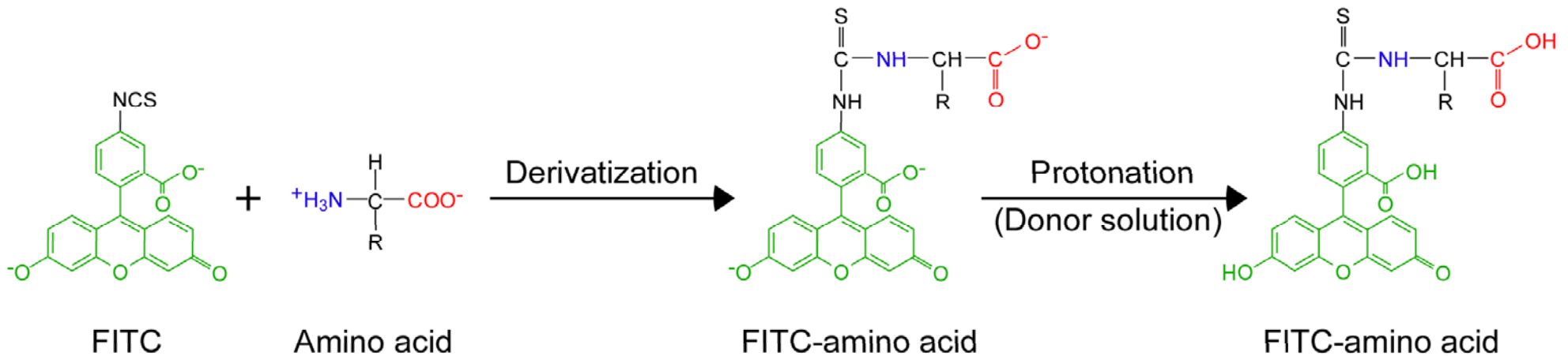
## FITC derivatization

Extraction efficiency

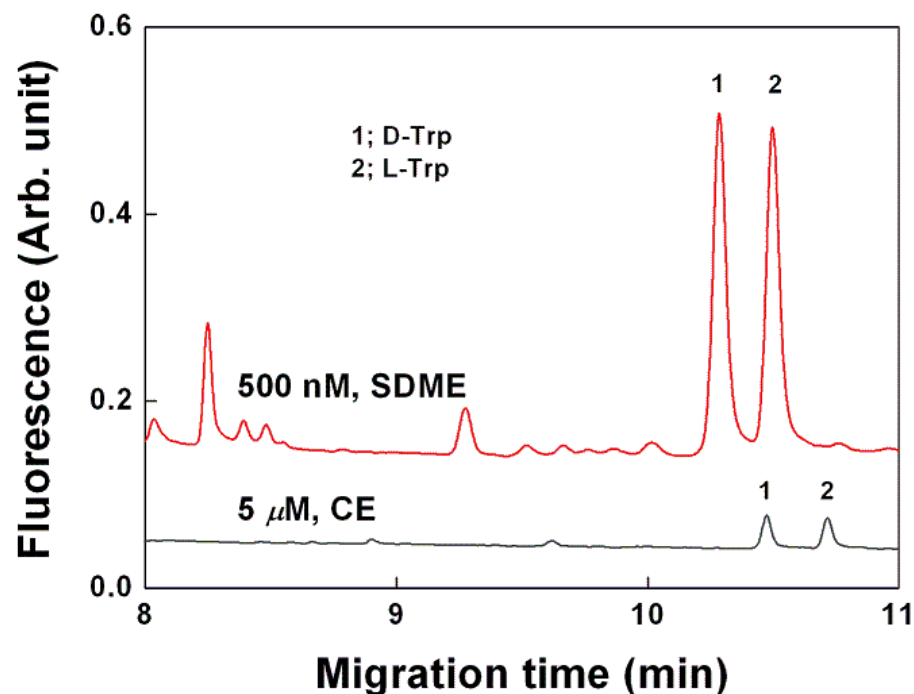
Detection sensitivity

Zwitterions  $\rightarrow$  carboxylic acid

Laser-induced fluorescence detection



# SDME of Amino Acids with FITC Derivatization



## SDME

Donor phase: 500 nM sample in 0.1 mM HCl

Organic phase: Octanol

Acceptor phase: 80 mM borate buffer (pH 9.3)

Extraction: 10 min, no stirring, 25°C

## CE

25 μm ID, 60/50 cm fused silica capillary

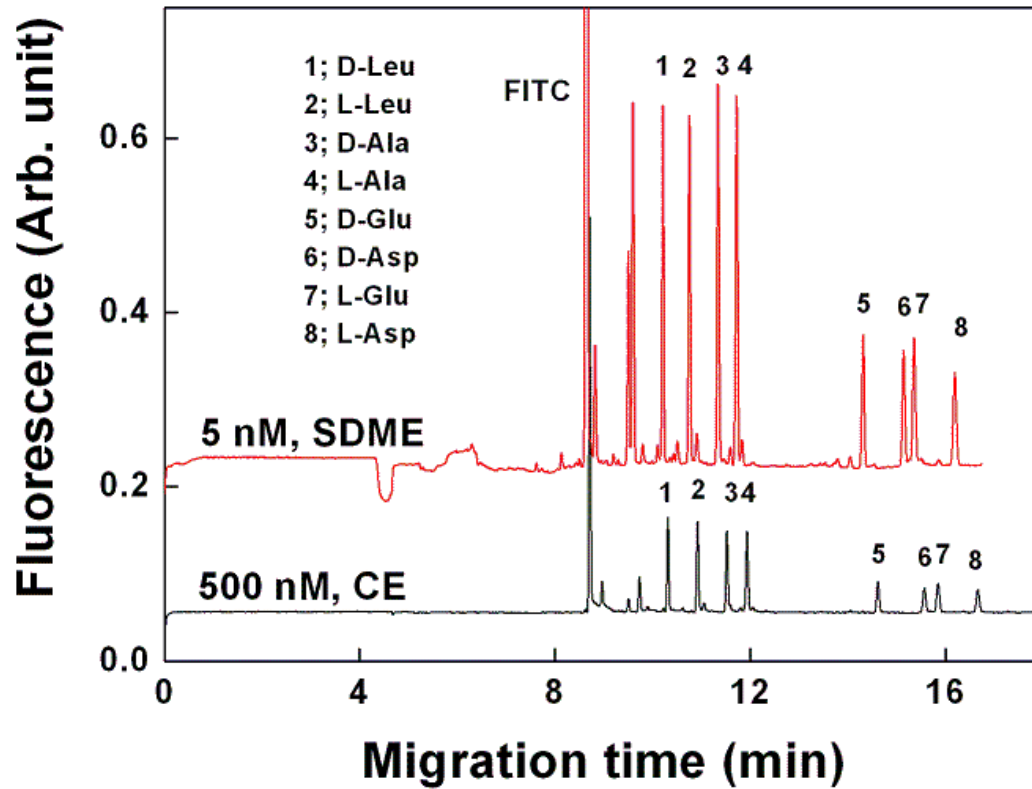
Run buffer: 80 mM borate with 12 mM β-CD, and  
18 mM taurodeoxycholate (pH 9.3)

25 kV, LIF detection

**EF = 125**

10-min SDME, no stirring

# Chiral analysis of Amino Acids with SDME



## SDME

Donor phase: 5 nM sample in 0.1 mM HCl

Organic phase: Octanol

Acceptor phase: 80 mM borate buffer (pH 9.3)

Extraction: 10 min, no stirring, 25°C

## CE

25  $\mu$ m ID, 60/50 cm fused silica capillary

Run buffer: 80 mM borate with 12 mM  $\beta$ -CD, and  
18 mM taurodeoxycholate (pH 9.3)

25 kV, LIF detection

**EF = 270 – 460**

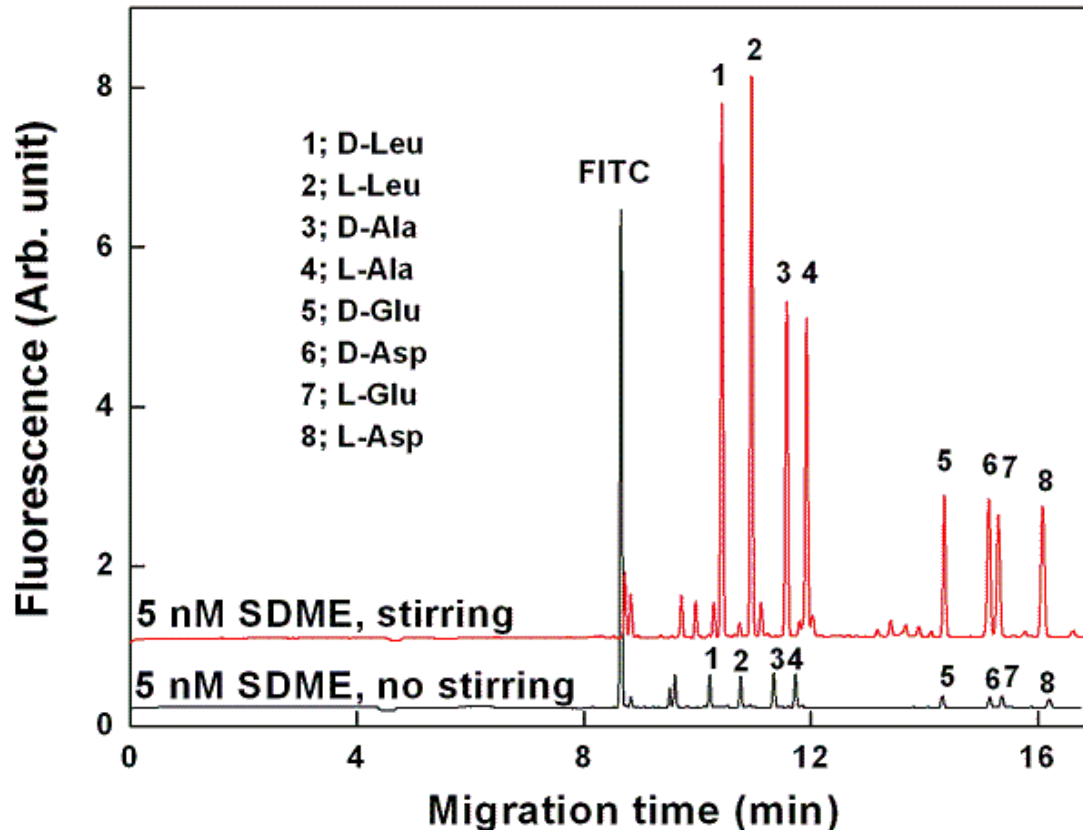
10-min SDME, no stirring

# Analytical Performance

Without stirring

Analyte	Enrichment factor	LOD (S/N = 3)	Resolution
D-Leu	310	0.05 nM	8.2
L-Leu	270	0.05 nM	
D-Ala	340	0.05 nM	4.9
L-Ala	330	0.05 nM	
D-Glu	420	0.1 nM	10
L-Glu	430	0.1 nM	
D-Asp	440	0.1 nM	9.3
L-Asp	460	0.1 nM	

# SDME with **Stirring** for Amino Acids



## SDME

Donor phase: 5 nM sample in 0.1 mM HCl

Organic phase: Octanol

Acceptor phase: 80 mM borate buffer (pH 9.3)

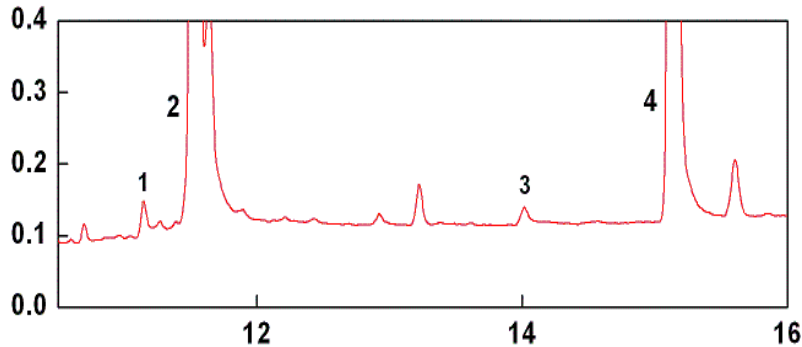
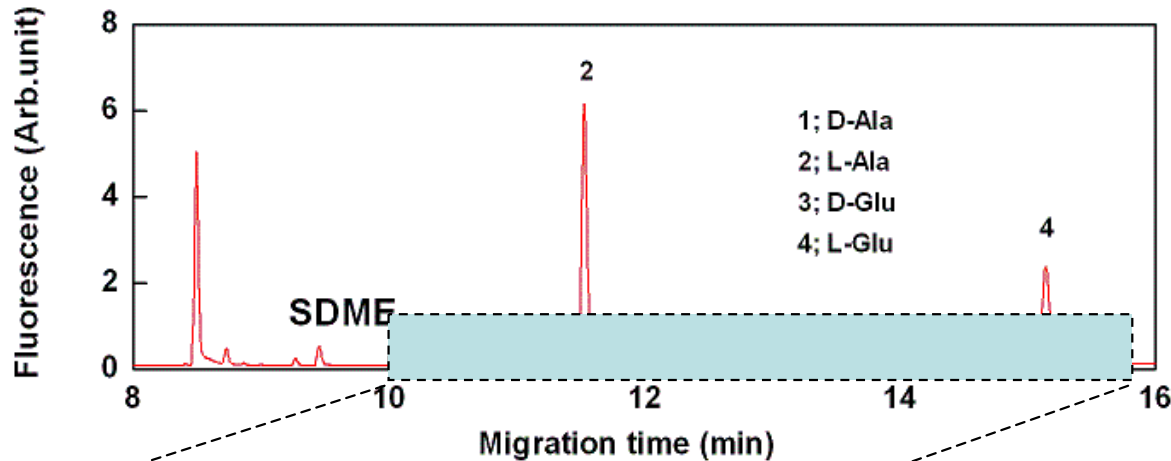
Extraction: 10 min, stirring, 25°C

Run buffer: 80 mM borate, 12 mM  $\beta$ -CD and  
18 mM taurodeoxycholate (pH 9.3)

**EF = 3000 – 6800**

10-min SDME, **stirring**

# Analysis of D-Amino Acids in an Enantiomeric Excess Mixture



## SDME

Donor phase: 80 nM L-Ala, L-Glu

0.5 nM D-Ala, D-Glu in 0.1 mM HCl

Organic phase: Octanol

Acceptor phase: 80 mM borate buffer (pH 9.3)

Extraction: 10 min, no stirring, 25°C

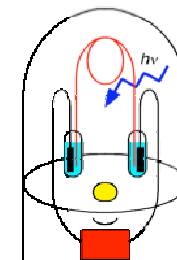
Run buffer: 80 mM borate, 12 mM  $\beta$ -CD and  
18 mM taurodeoxycholate (pH 9.3)

**SDME in a 98.8% enantiomeric excess mixture**

# Conclusions

- ◆ SDME of zwitterions using **FITC derivatization**  
LIF detection
- ◆ **Sensitive chiral detection** of amino acids with in-line SDME  
EFs without stirring: 270 – 460  
EFs with stirring: 3000 – 6800
- ◆ Analysis of **D-amino acids** in 98.8% enantiomeric excess mixture

# Acknowledgement



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Jin-Soo Kim

Jihye Kim

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