A fast high-throughput method for the determination of acidity constants by capillary electrophoresis

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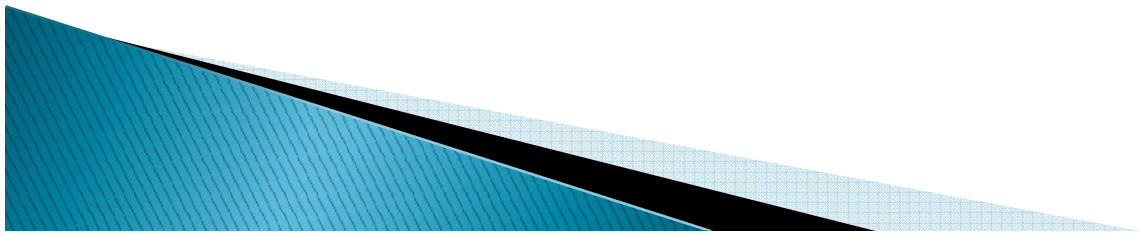
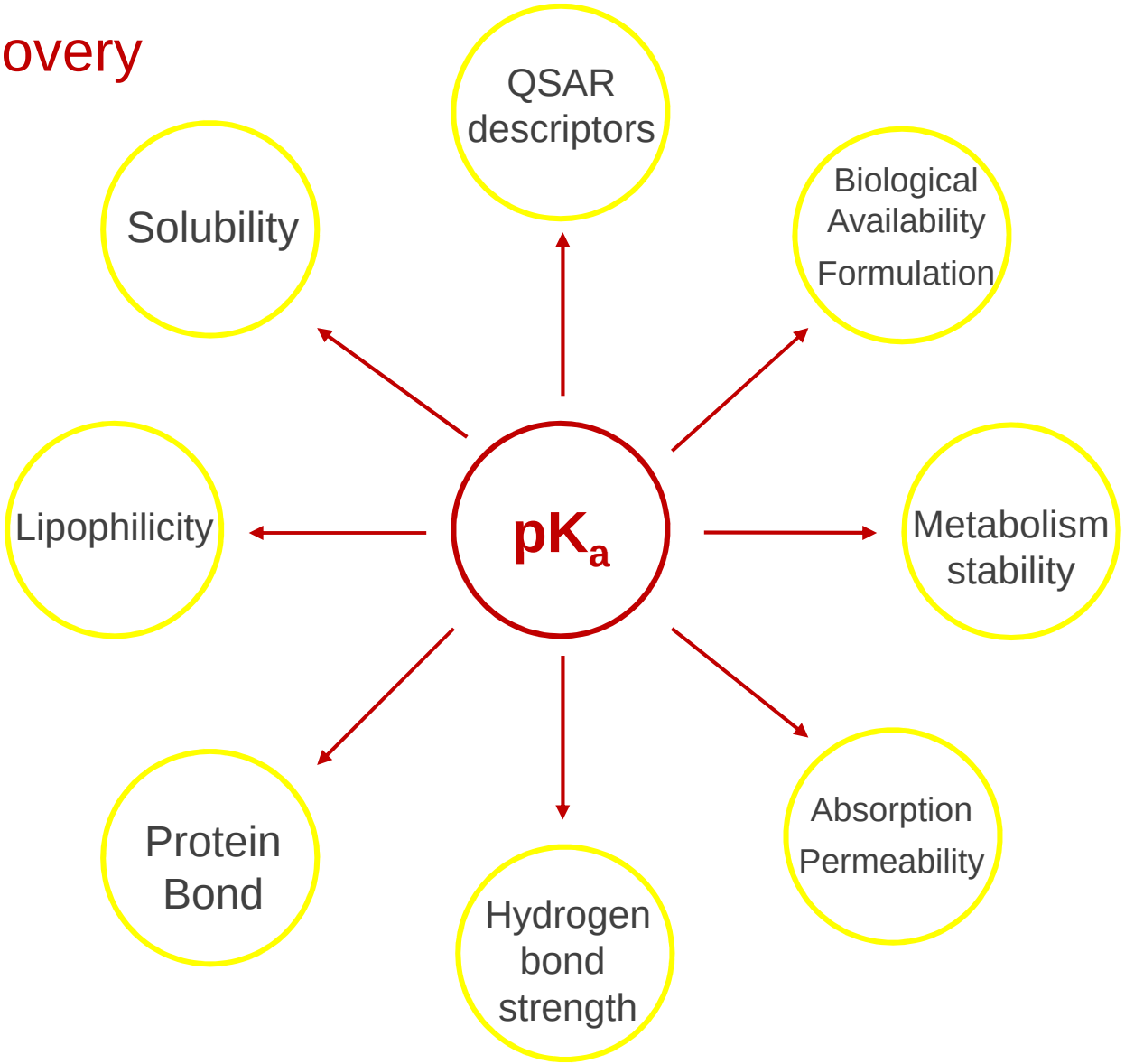
Interest of pK_a determination

- Buffering agents
- Organic synthesis
- Acid - base titration
- Analytical separations (HPLC, CE)
- Environmental distribution
- Drug discovery



Drug discovery



Kerns, E.H, Di L, Drug Discov Today, Technologies 1 (2004) 343

Methods for pK_a determination

Potentiometric titrations

☑ Accurate results

☒ Time consuming
☒ High purity
☒ High amount of sample

Spectrophotometric titrations

☑ Better sensitivity (compared to CE)

☒ Time consuming
☒ High purity
☒ Spectral differences between the neutral and the ionized form are required

CE

☑ Low amounts of sample and solvents
☑ Separation technique – no need of high purity
☑ Highly automated

☒ Time consuming

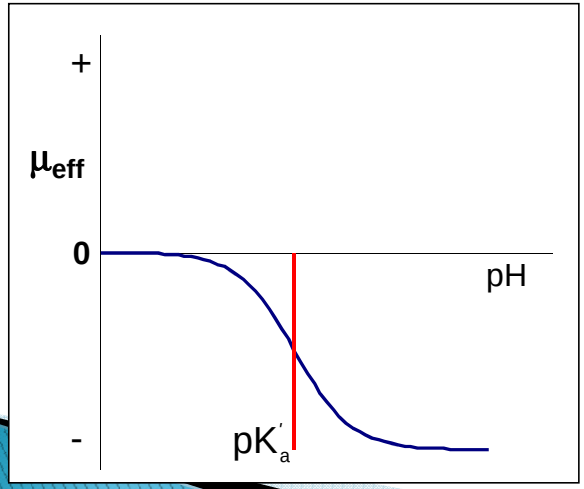
Determination of a pK_a by capillary electrophoresis

Monoprotic neutral acid

$$HA \rightleftharpoons H^+ + A^-$$

$$K_a' = \frac{a_{H^+}[A^-]}{[HA]} \qquad \mu_{eff} = \frac{[A^-]}{[HA]+[A^-]} \cdot \mu_{A^-}$$

$$\mu = \frac{\mu_{A^-}}{1+10^{pK_a'-pH}}$$



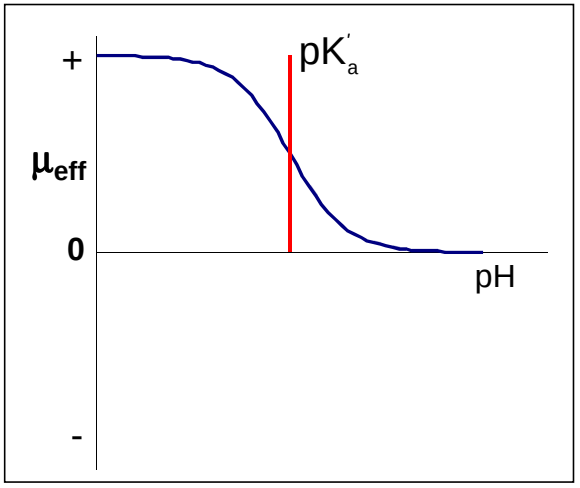
$$pK_a = pK_a' - \log \gamma_{A^-}$$

Monoprotic neutral base

$$BH^+ \rightleftharpoons H^+ + B$$

$$K_a' = \frac{a_{H^+}[B]}{[BH^+]} \qquad \mu_{eff} = \frac{[BH^+]}{[BH^+]+[B]} \cdot \mu_{BH^+}$$

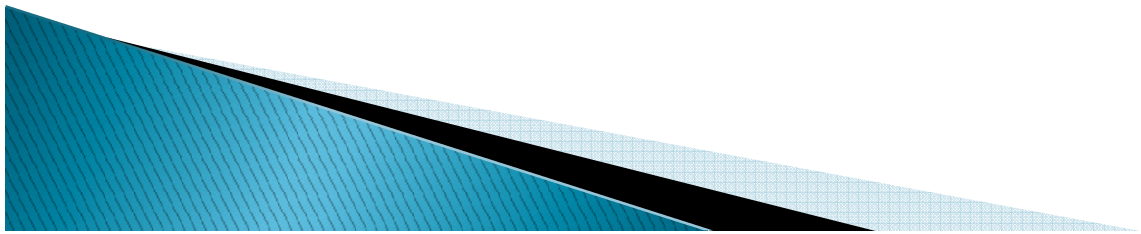
$$\mu = \frac{\mu_{BH^+}}{1+10^{pH-pK_a'}}$$



$$pK_a = pK_a' + \log \gamma_{BH^+}$$

Determination of acidity constants by CE: classical method

- Accurate pH measurement
- Temperature
- Ionic strength
- Adequate buffer solutions
  - Electrolysis
  - Stability
  - Specific interactions with analytes



Selected buffers

Buffer constituents	pK_a	Covered pH range
CH_3COOH/CH_3COO^-	4.76	3.70 – 5.80
MES/MES^-	6.15	5.00 – 7.20
$BisTrisH^+/BisTris$	6.48	5.50 – 7.50
$H_2PO_4^-/HPO_4^{2-}$	7.21	5.80 – 8.20
$HEPES/HEPES^-$	7.51	6.50 – 8.70
$TrisH^+/Tris$	8.08	7.00 – 9.00
$(EtO)_2NH_2^+/(EtO)_2NH$	8.88	8.00 – 10.10
$H_3BO_3/H_3BO_2^-$	9.50	8.00 – 10.60
NH_4^+/NH_3	9.25	8.20 – 10.20
$CHES/CHES^-$	9.50	8.40 – 10.10
$EtONH_3^+/EtONH_2$	9.50	8.50 – 10.80
$BuNH_3^+/BuNH_2$	10.66	9.20 – 11.10
$CAPS/CAPS^-$	10.40	9.40 – 11.60
HPO_4^{2-}/PO_4^{3-}	12.32	10.80 – 11.80

Selected buffers (I = 50 mM)

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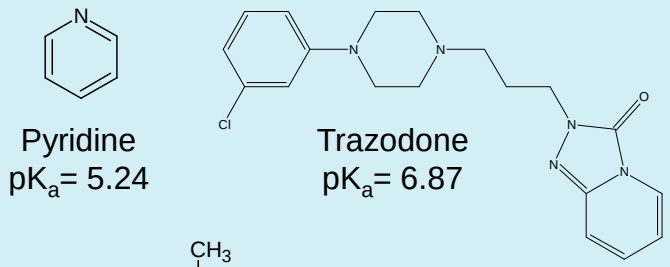
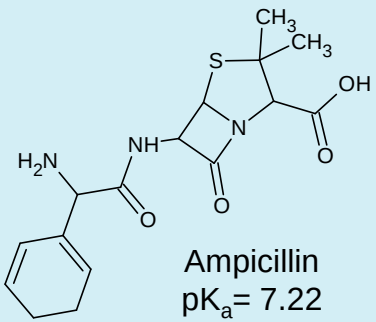
Test compounds

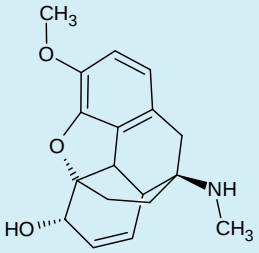


Pyridine
pK_a= 5.24

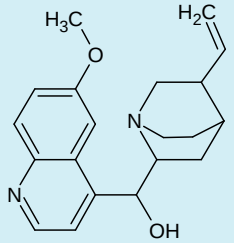
Trazodone
pK_a= 6.87



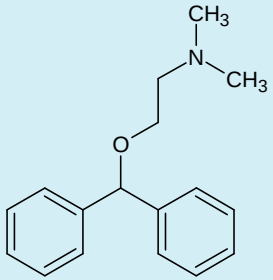
Ampicillin
pK_a= 7.22



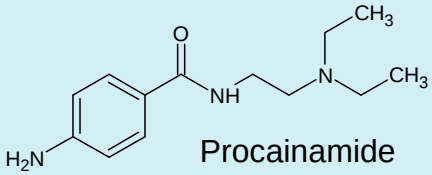
Codeine
pK_a= 8.24



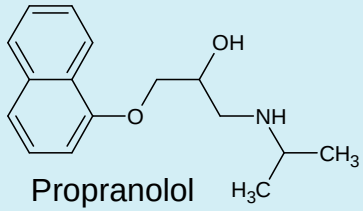
Quinine
pK_a= 8.48



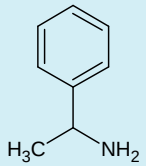
Diphenhydramine
pK_a= 9.17



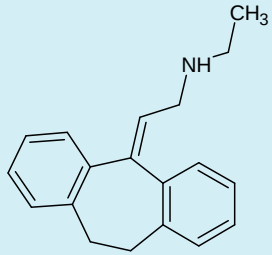
Procainamide
pK_a= 9.35



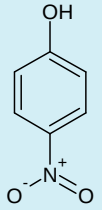
Propranolol
pK_a= 9.48



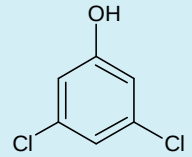
1-Aminoethylbenzene
pK_a= 9.49



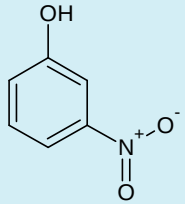
Nortriptyline
pK_a= 10.14



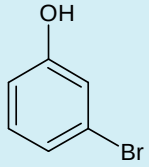
4-Nitrophenol
pK_a= 7.19



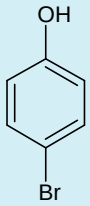
3,5-Dichlorophenol
pK_a= 8.21



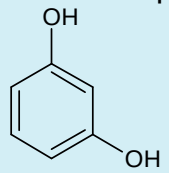
3-Nitrophenol
pK_a= 8.42



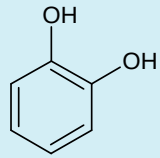
3-Bromophenol
pK_a= 9.01



4-Bromophenol
pK_a= 9.40

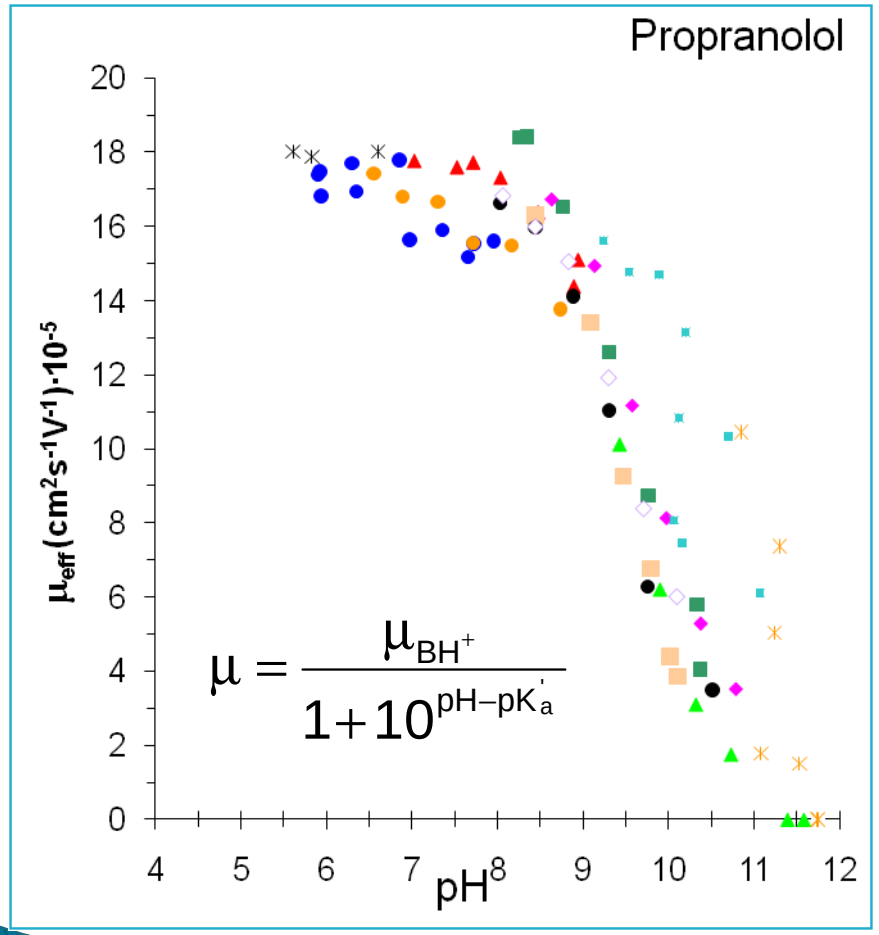


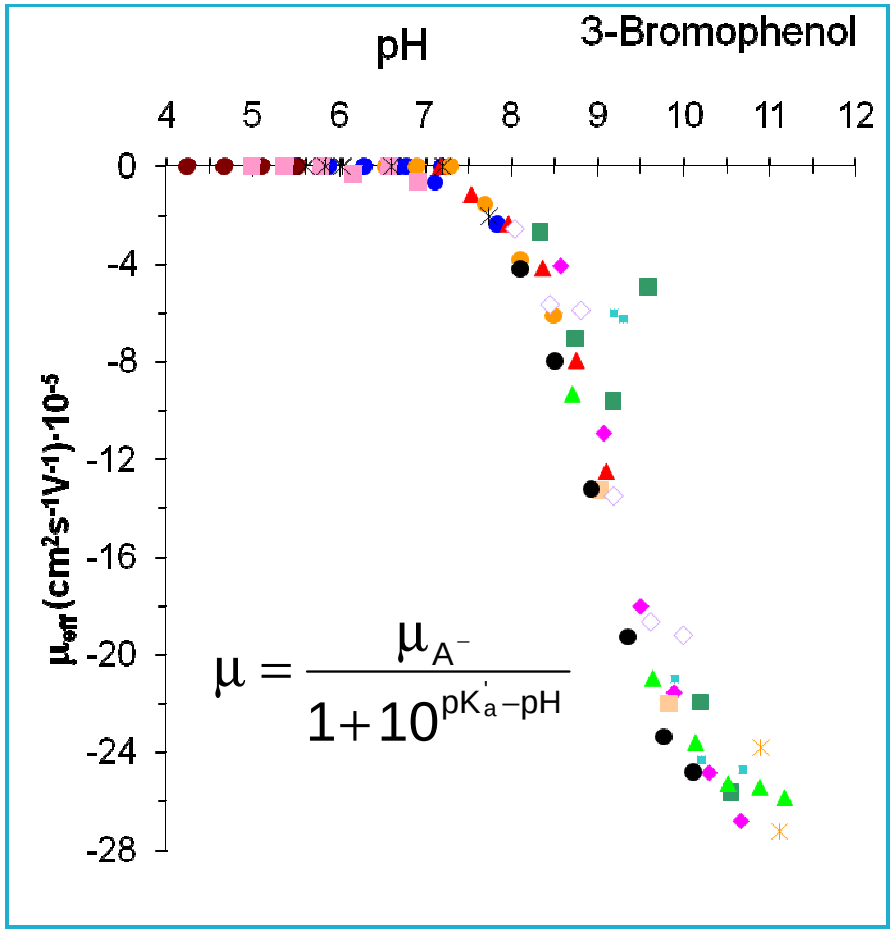
Resorcinol
pK_a= 9.50



Catechol
pK_a= 9.54

Mobility – pH curves





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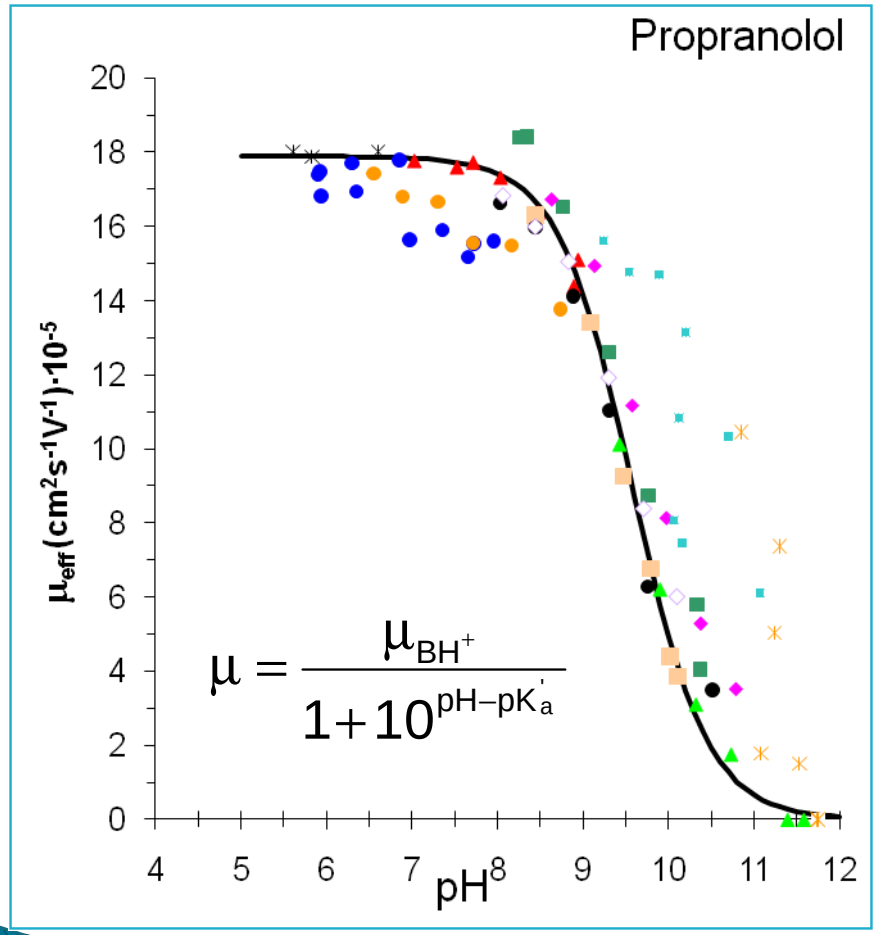

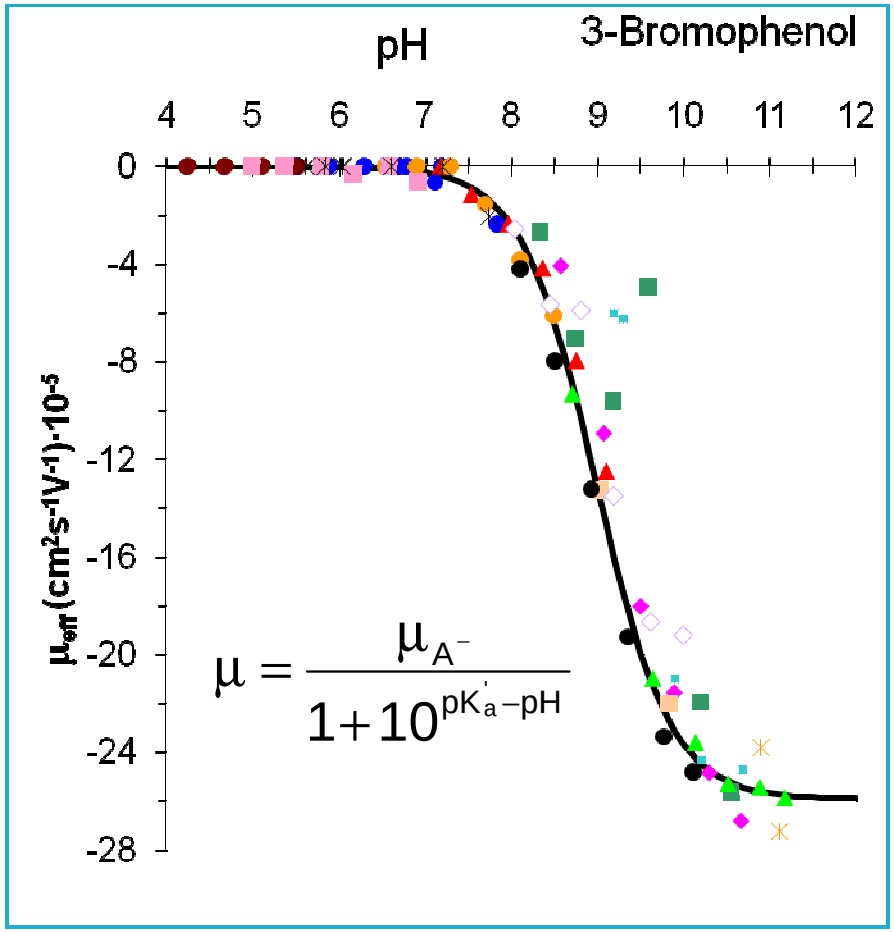

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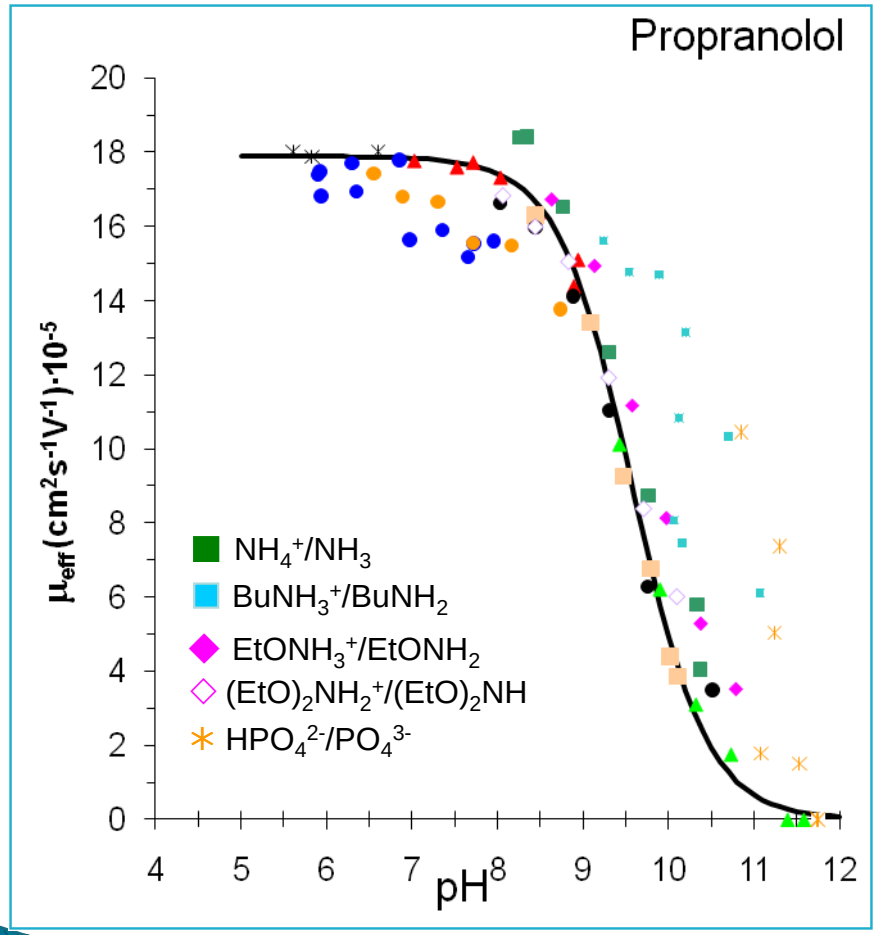
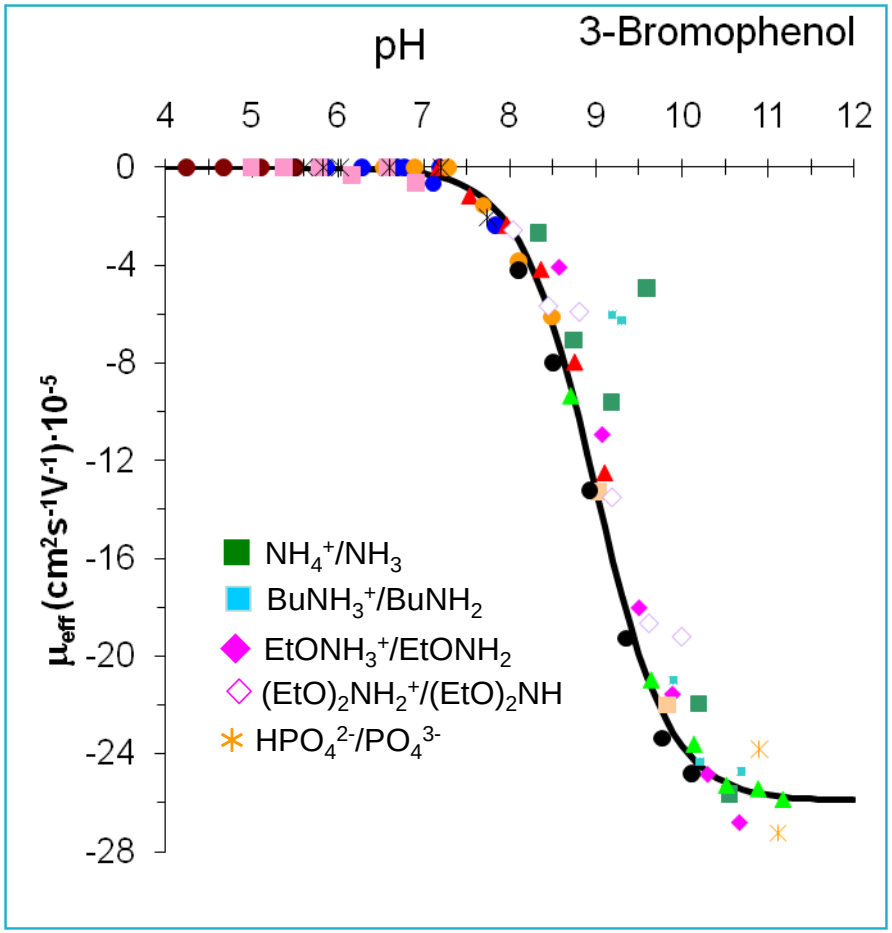
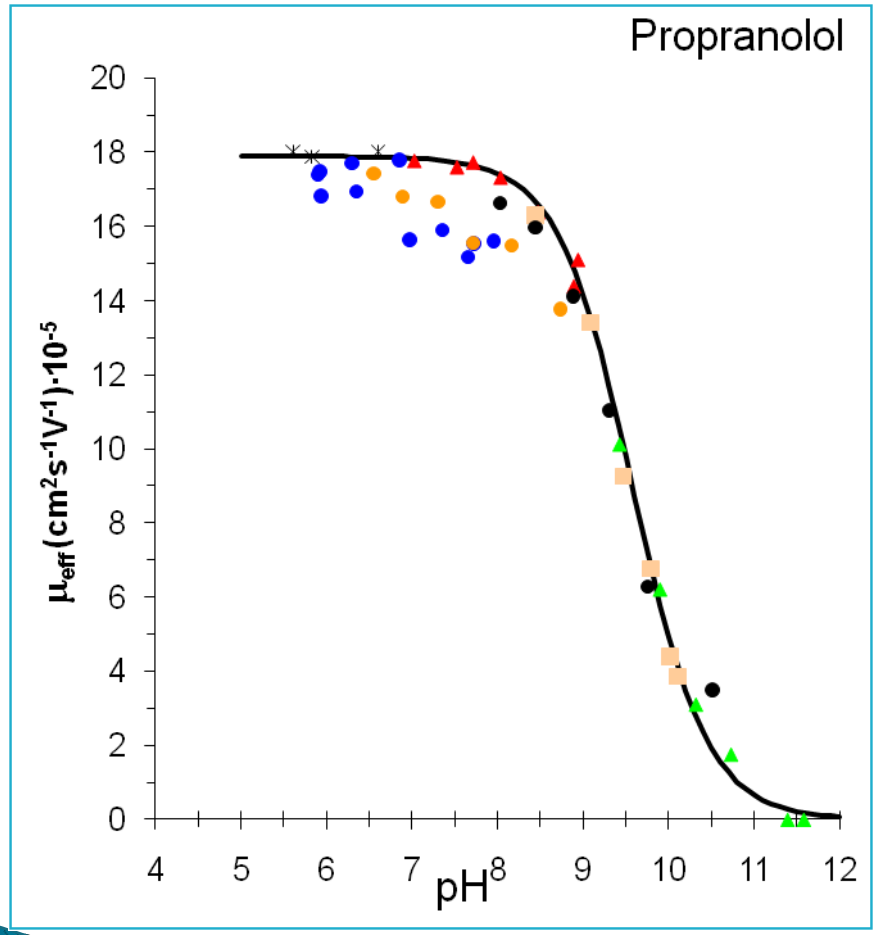
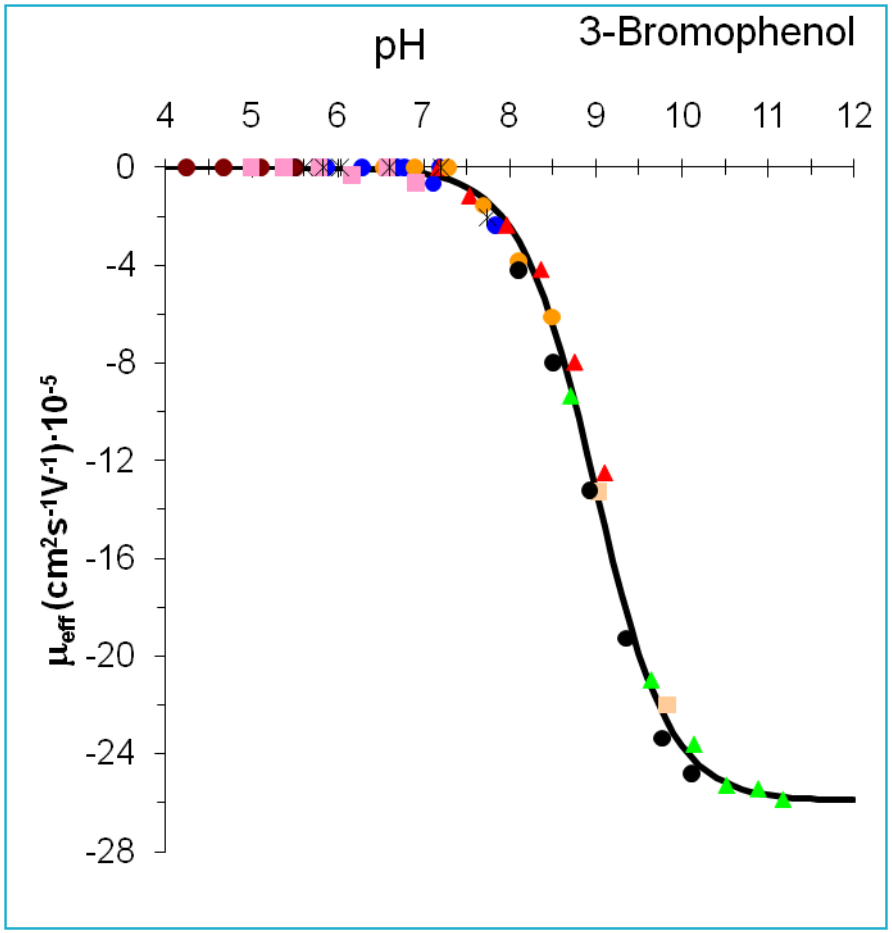


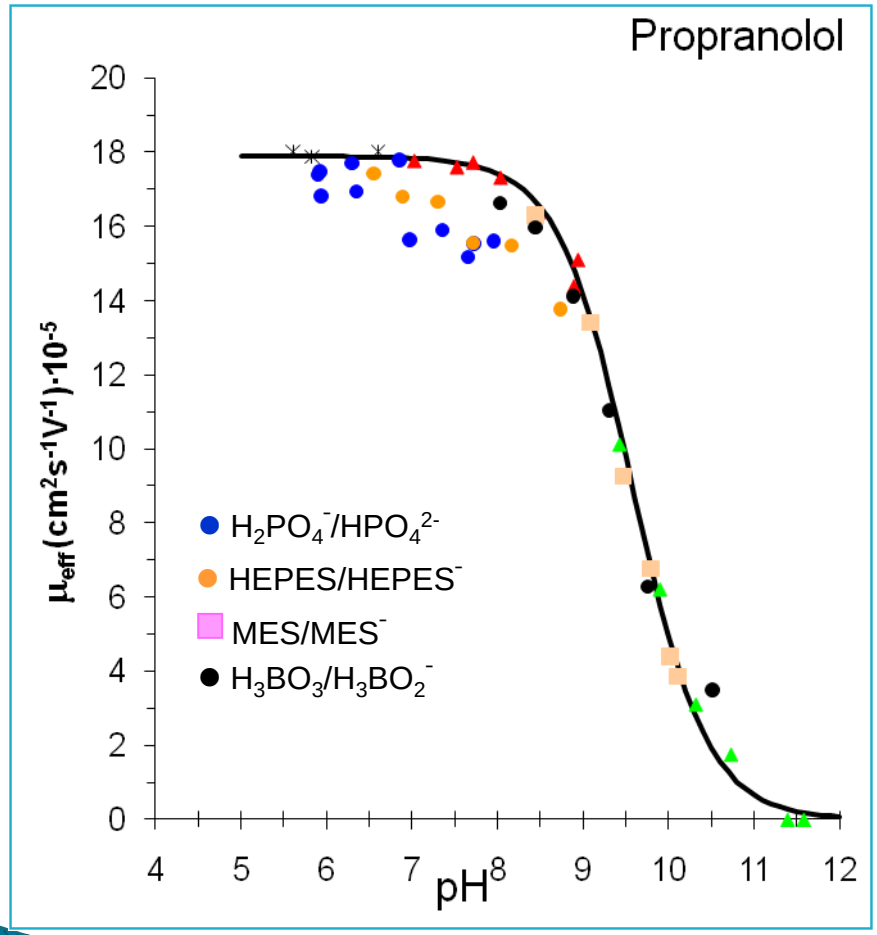


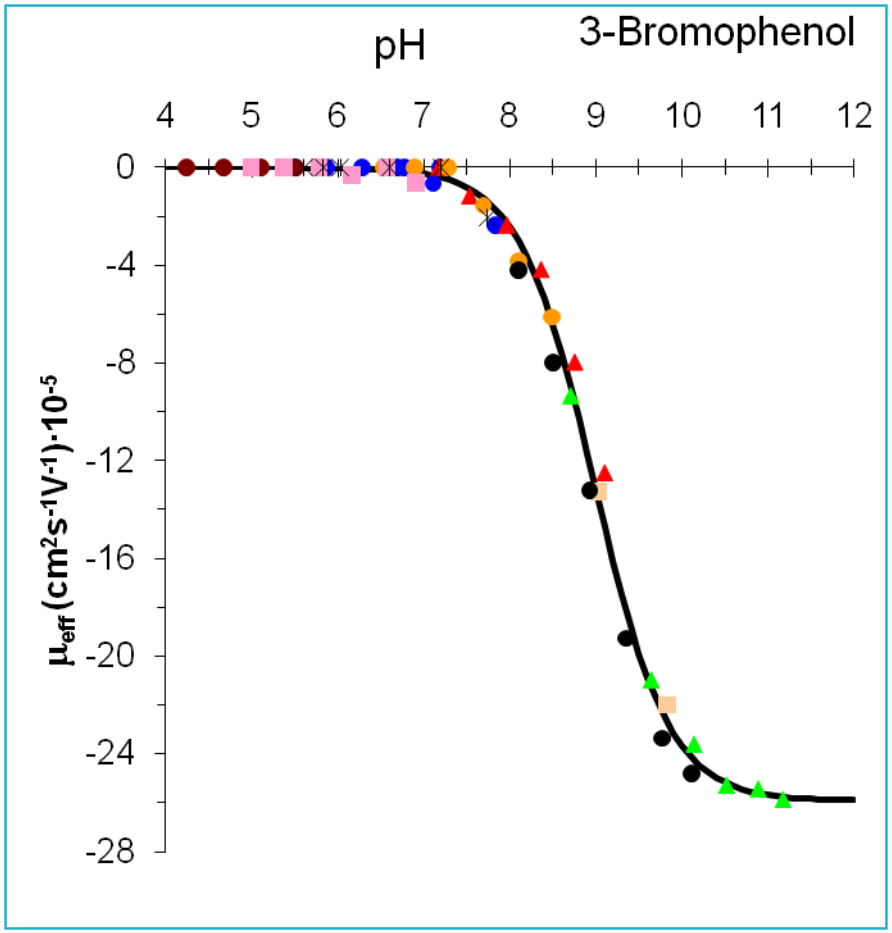
Phosphate buffer: HPO_4^{2-}/PO_4^{3-}

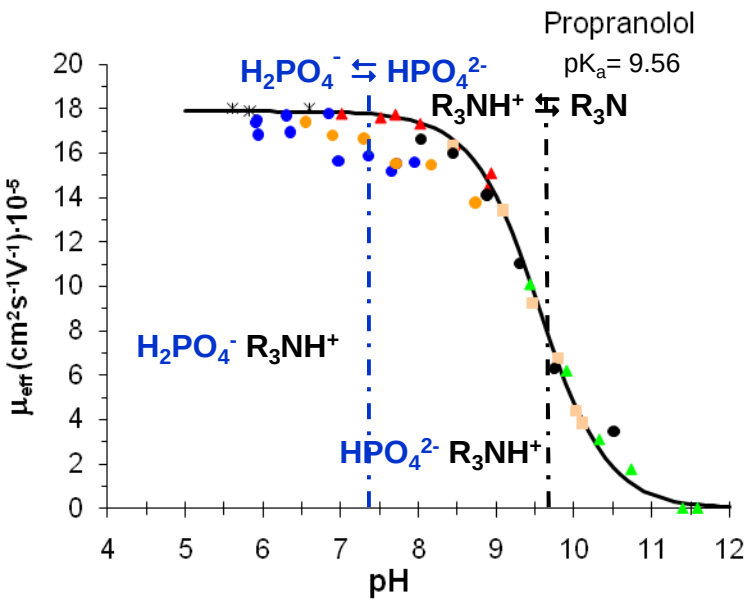


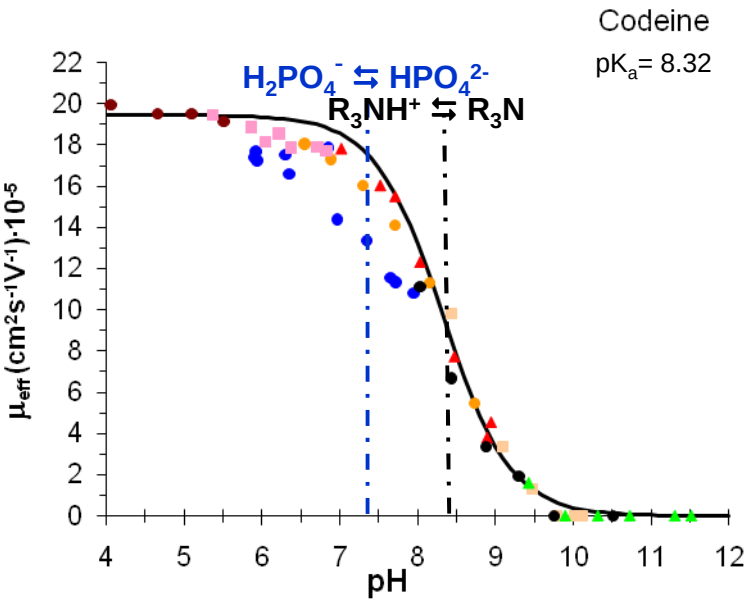


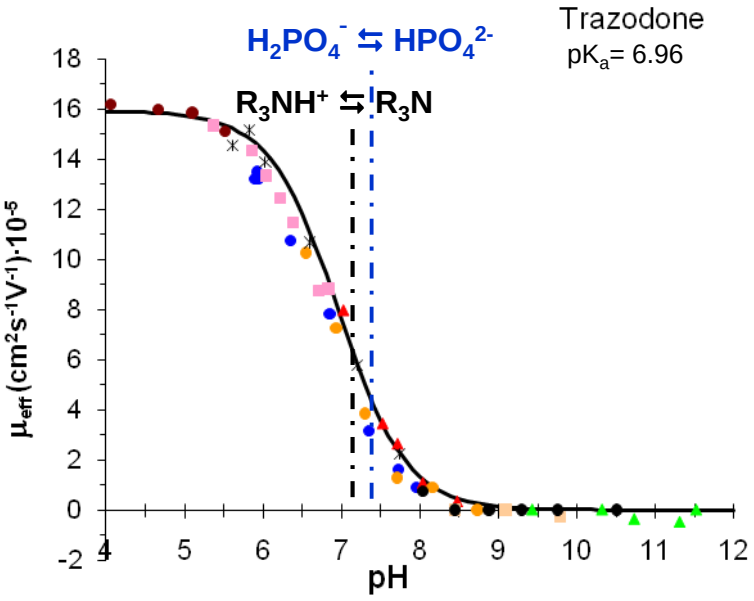
Phosphate buffer: $H_2PO_4^-/HPO_4^{2-}$

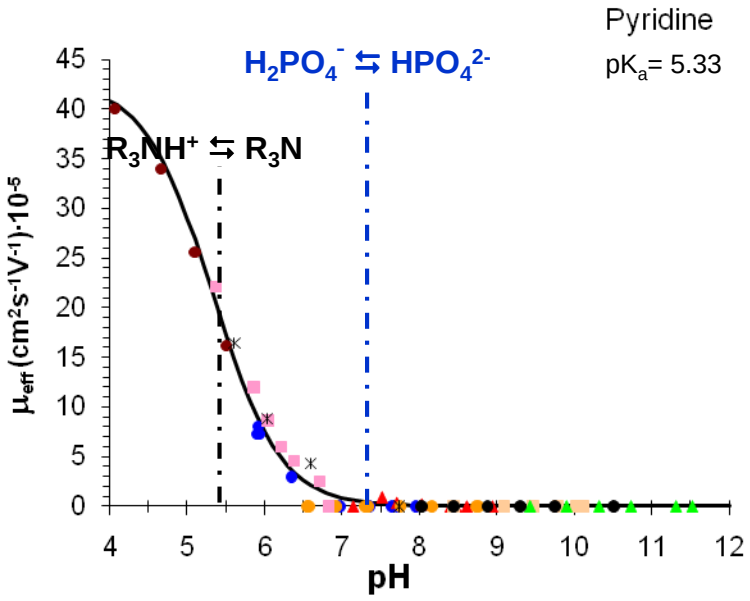






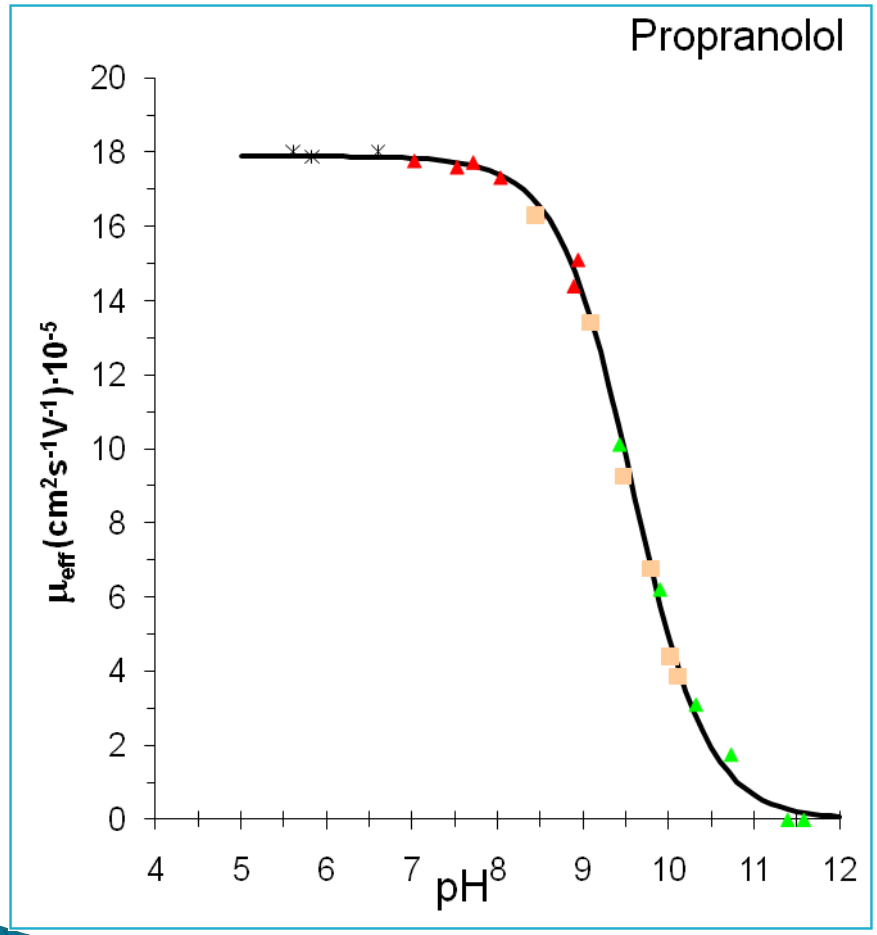


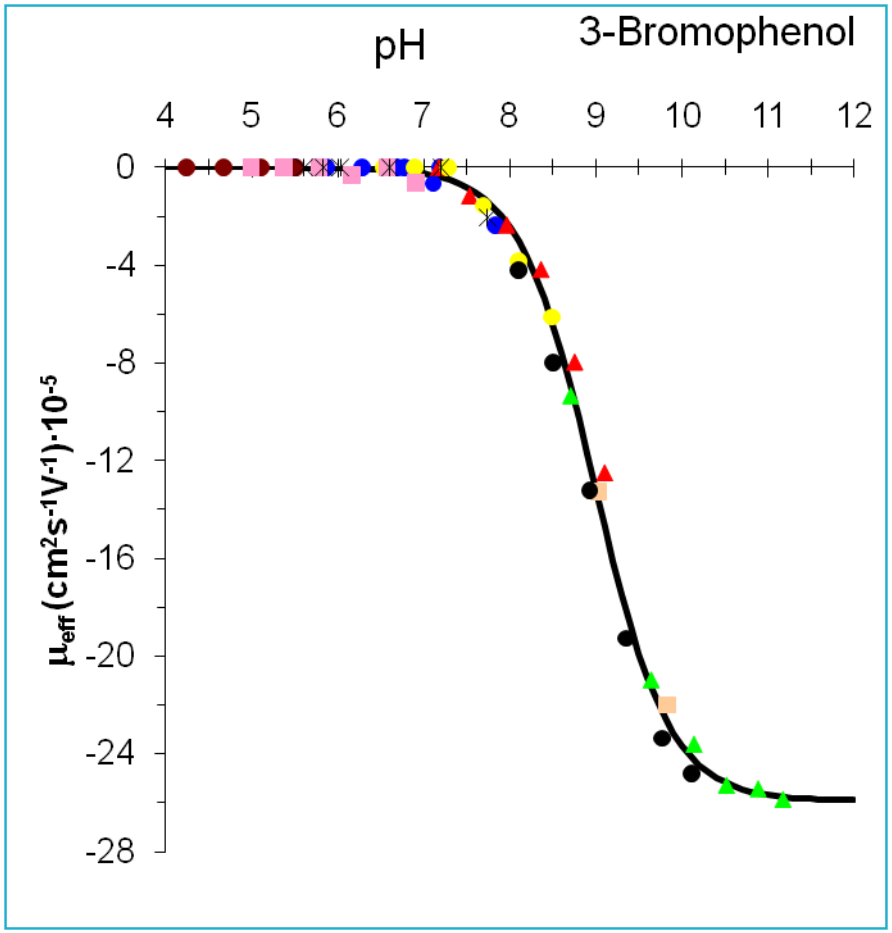




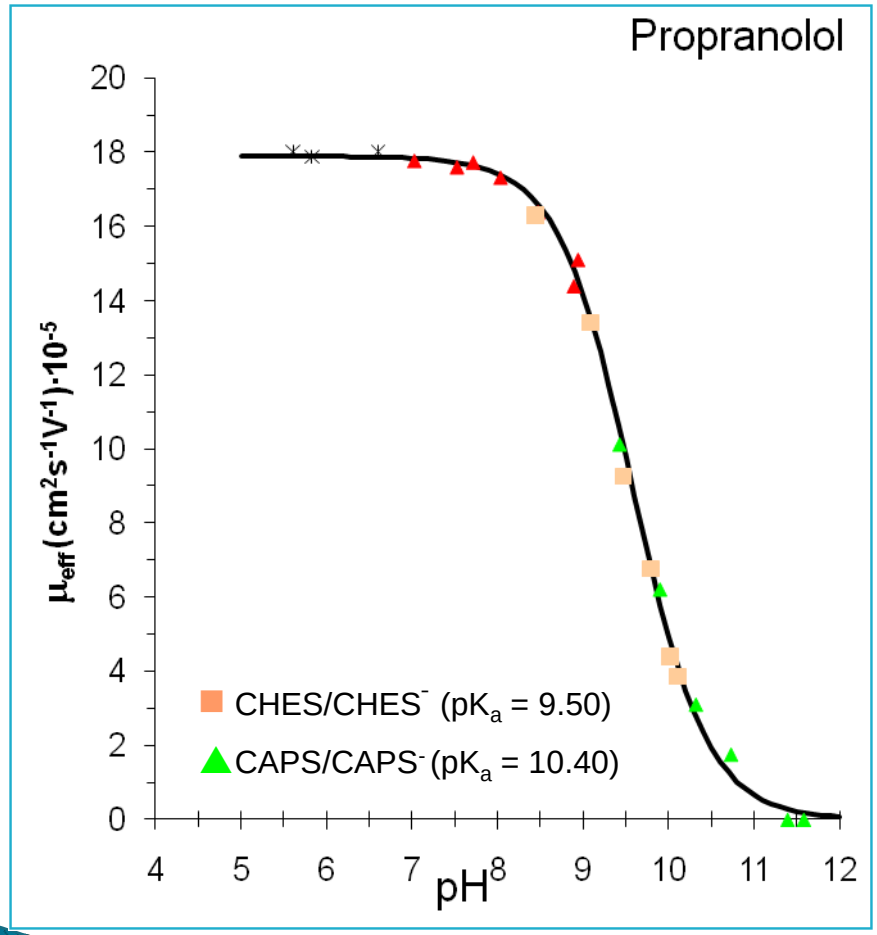
Pastore, P., Badocco, D., Zanon, F., *Electrochim. Acta* 2006, *51*, 5394-5401.
Wilson, H. R., Williams, R. J. P., *J. Chem. Soc. Faraday Trans. 1* 1987, *83*, 1885-1892.

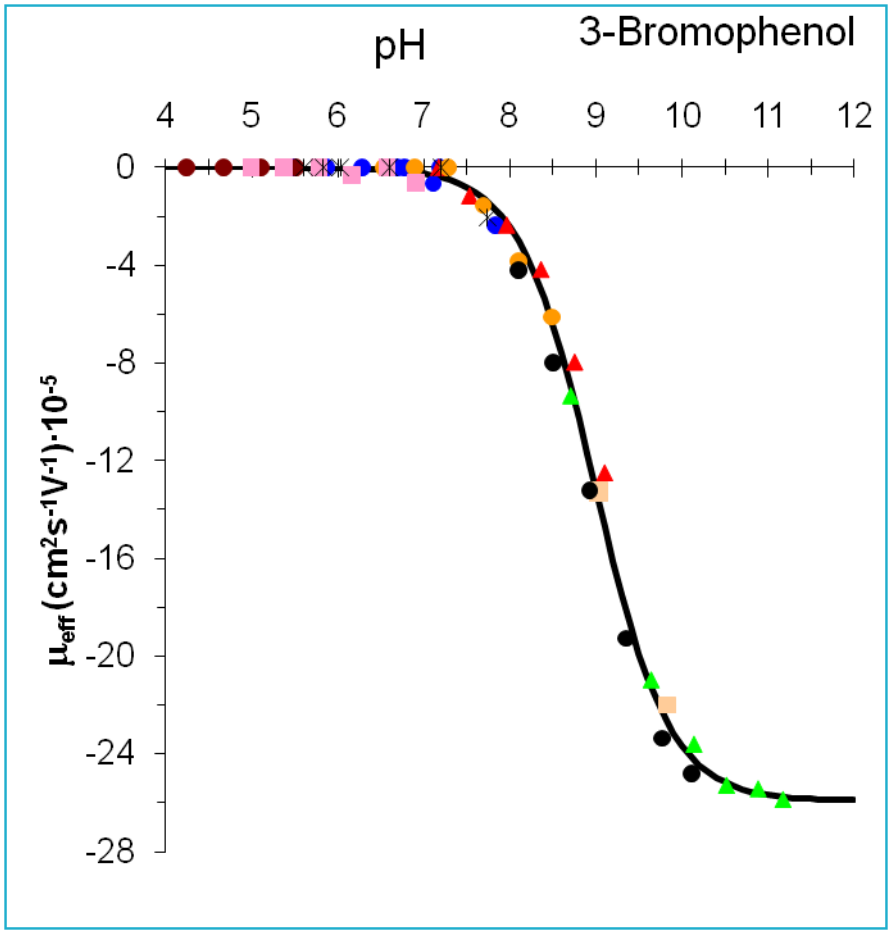
Mobility – pH curves



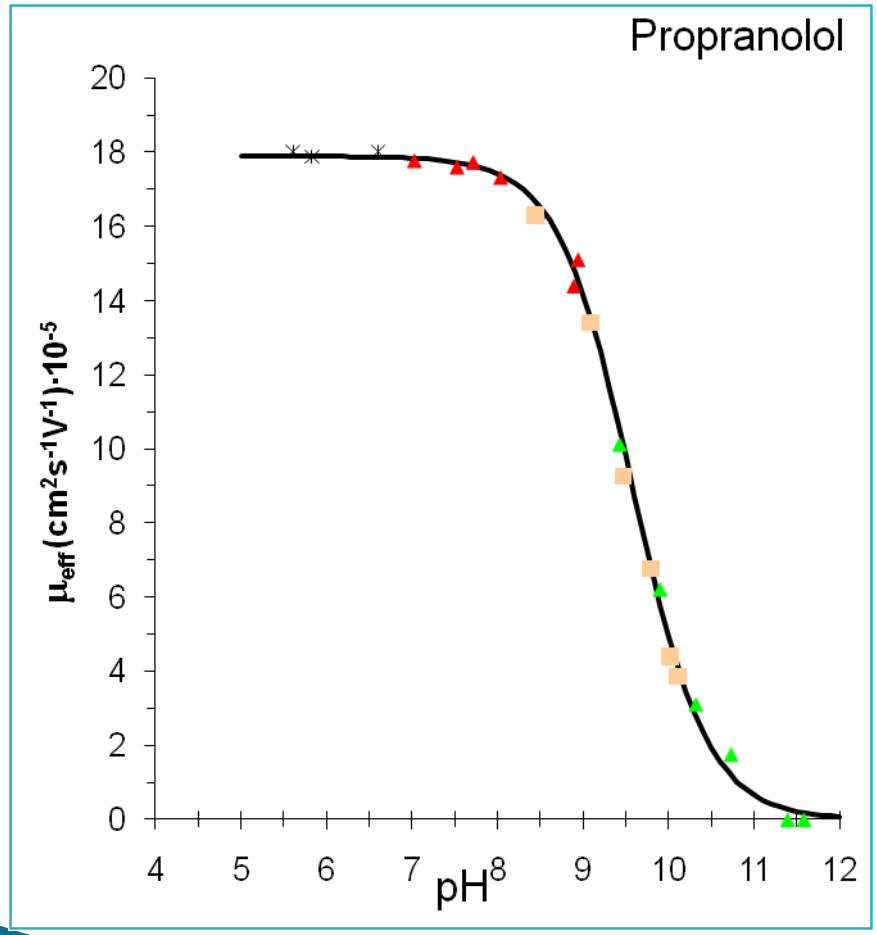


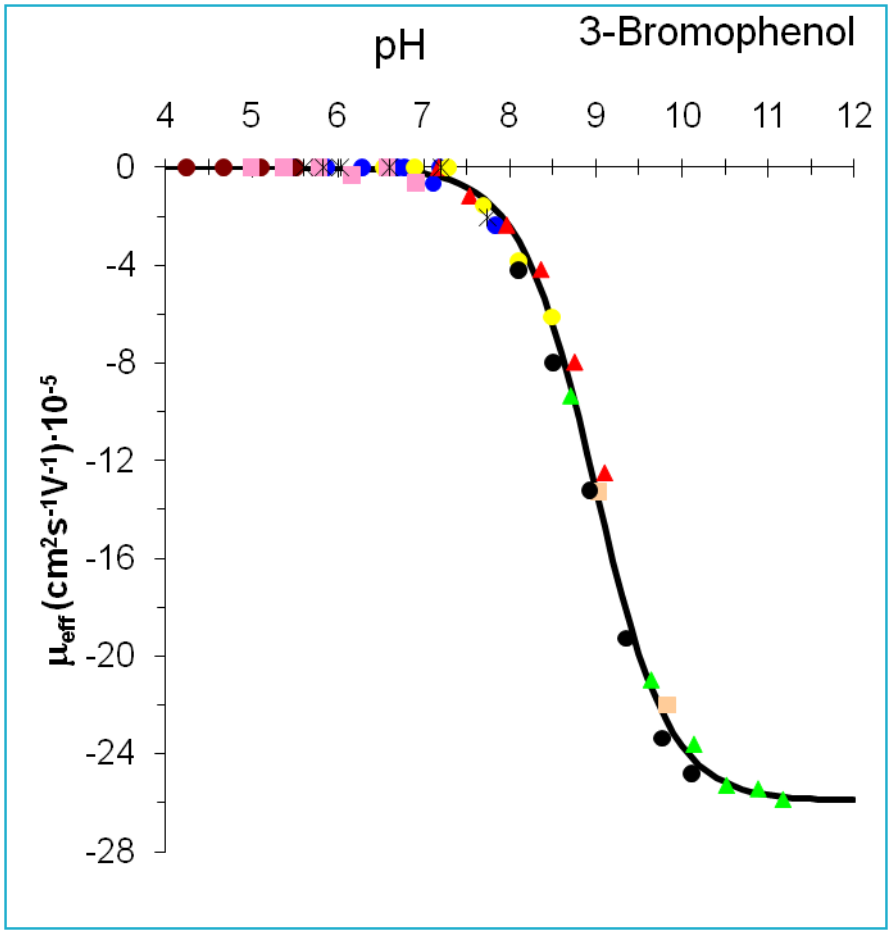
Zwitterionic buffers





Mobility – pH curves





Recommended buffers

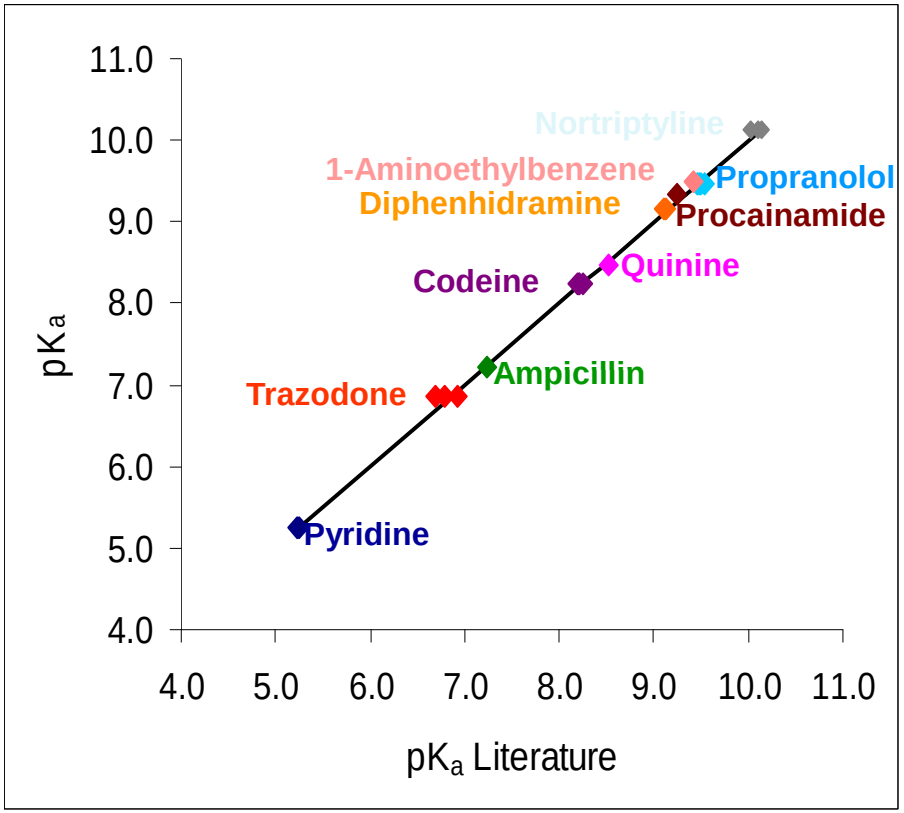
Amines: CH_3COOH/CH_3COO^-, $BisTrisH^+$/BisTris, $TrisH^+$/Tris, $CHES/CHES^-$, and $CAPS/CAPS^-$

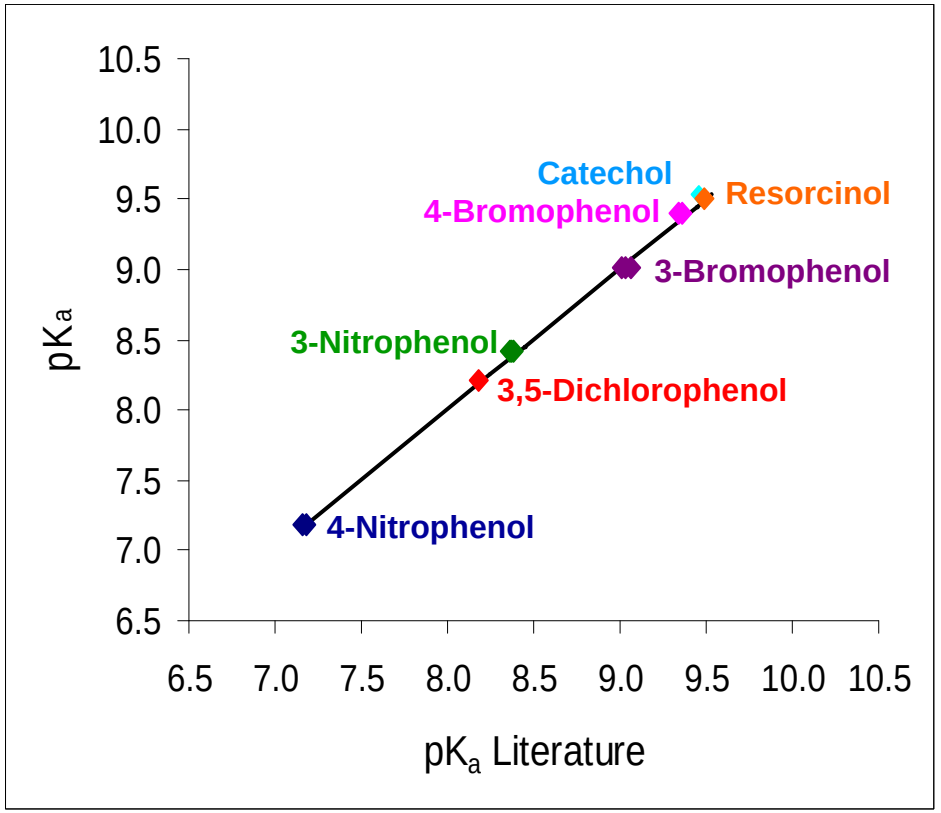
- → All buffers that show interactions have been rejected
- → Some of the rejected buffers can be used depending on their pK_a and the pK_a of the analyte.

Phenols: CH_3COOH/CH_3COO^-, $BisTrisH^+$/BisTris, MES/MES^-, $H_2PO_4^-/HPO_4^{2-}$, $HEPES/HEPES^-$, $TrisH^+$/Tris, $H_3BO_3/H_2BO_3^-$, $CHES/CHES^-$, and $CAPS/CAPS^-$

- → $H_3BO_3/H_2BO_3^-$ excluded when shows specific interactions

pK_a values determined with the recommended buffers





Determination of acidity constants: the internal standard method

Advantages over the classical method

- No need of pH measurement
- Specific interactions can be corrected
- Temperature variations can be corrected
- Save of time

Requirements: an internal standard (pK_a similarity)

$$pK_{a,IS} = pK_{a,AN} \pm 1$$

The internal standard method: theoretical basis

Monoprotic neutral acid:

$$\mu_{eff} = \frac{\mu_{A^-}}{1+10^{pK_a^{'}-pH}} \Rightarrow pK_a^{'} = pH + \log\frac{\mu_{eff}-\mu_{A^-}}{\mu_{eff}} = pH + \log Q$$

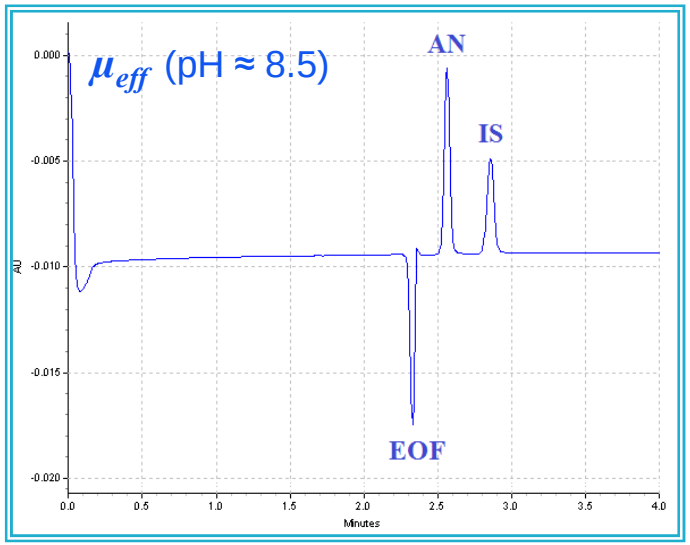
$$pK_{a,AN}^{'} = pH + \log Q_{AN} \qquad pK_{a,IS}^{'} = pH + \log Q_{IS}$$

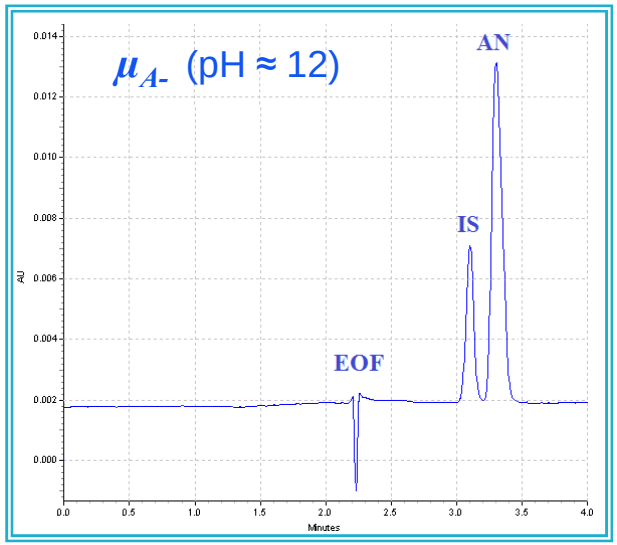
$$pK_{a,AN}^{'} = pK_{a,IS}^{'} + \log Q_{AN} - \log Q_{IS}$$

Monoprotic neutral base:

$$pK_{a,AN}^{'} = pK_{a,IS}^{'} - \log Q_{AN} + \log Q_{IS}$$

The internal standard method: example 3-chlorophenol





Internal Standard: methylparaben

	$t_{M,EOF}$	$t_{M,IS}$	$t_{M,AN}$
pH=8.5	2.329	2.858	2.563
pH=12	2.238	3.104	3.304

	IS	AN
μ_{eff}	-79.8	-39.4
μ_{A^-}	-125.2	-144.7

$Q_{AN} = 2.678$

$Q_{IS} = 0.569$

$pK_{a,IS} = 8.36$

$pK_{a,AN} = 9.03$

The internal standard method: internal standards

Acidic internal standards

Internal Standard	N	pK_a	Internal Standard	N	pK_a
2-Chlorobenzoic acid	4	2.85 ± 0.02	2,4,6-Tribromophenol	5	6.03 ± 0.08
2,6-Dibromo-4-nitrophenol	6	3.32 ± 0.03	4-Nitrophenol	5	7.10 ± 0.05
4-Nitrobenzoic acid	5	3.38 ± 0.01	Vanillin	6	7.37 ± 0.06
2,6-Dinitrophenol	7	3.69 ± 0.01	4-Hydroxybenzaldehiyde	6	7.62 ± 0.04
3-Bromobenzoic acid	8	3.79 ± 0.02	Phenobarbital	6	7.54 ± 0.04
2,4-Dinitrophenol	7	4.12 ± 0.03	3,5-Dichlorophenol	7	8.19 ± 0.04
Benzoic acid	7	4.21 ± 0.03	Methylparaben	7	8.36 ± 0.03
Ibuprofen	6	4.48 ± 0.02	2-Chlorophenol	7	8.51 ± 0.04
Nicotinic acid	7	4.84 ± 0.03	3-Chlorophenol	6	9.04 ± 0.01
Warfarin	6	5.16 ± 0.04	4-Bromophenol	5	9.28 ± 0.01
2,5-Dinitrophenol	4	5.28 ± 0.05	Paracetamol	4	9.58 ± 0.02
Sulfacetamide	4	5.40 ± 0.05	Phenol	3	9.89 ± 0.01

The internal standard method: internal standards

Acidic internal standards

Compost	N	$pK_{a\,(lit)}$	$pK_{a\,R1}$	$pK_{a\,R2}$	$pK_{a\,R3}$	$pK_{a\,R4}$	$pK_{a\,(exp)}$
2-Chlorobenzoic acid	4	2.91	2.90	2.88	2.86	2.85	2.85 ± 0.02
2,6-Dibromo-4-nitrophenol	6	3.38	3.34	3.34	3.32	3.32	3.32 ± 0.03
4-Nitrobenzoic acid	5	3.42	3.43	3.40	3.39	3.38	3.38 ± 0.01
2,6-Dinitrophenol	7	3.71	3.73	3.70	3.70	3.69	3.69 ± 0.01
3-Bromobenzoic acid	8	3.74	3.79	3.80	3.79	3.79	3.79 ± 0.02
2,4-Dinitrophenol	7	4.10	4.13	4.13	4.13	4.12	4.12 ± 0.03
Benzoic acid	7	4.21	4.19	4.20	4.21	4.21	4.21 ± 0.03
Ibuprofen	6	4.40	4.44	4.48	4.48	4.48	4.48 ± 0.02
Nicotinic acid	7	4.82	4.80	4.81	4.83	4.83	4.84 ± 0.03
Warfarin	6	5.15	5.10	5.11	5.13	5.14	5.16 ± 0.04
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮

The internal standard method: internal standards

Acidic internal standards

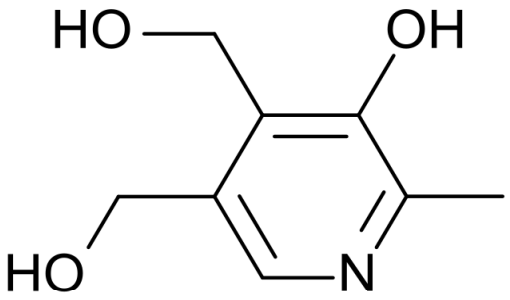
Internal Standard	N	pK_a	Internal Standard	N	pK_a
2-Chlorobenzoic acid	4	2.85 ± 0.02	2,4,6-Tribromophenol	5	6.03 ± 0.08
2,6-Dibromo-4-nitrophenol	6	3.32 ± 0.03	4-Nitrophenol	5	7.10 ± 0.05
4-Nitrobenzoic acid	5	3.38 ± 0.01	Vanillin	6	7.37 ± 0.06
2,6-Dinitrophenol	7	3.69 ± 0.01	4-Hydroxybenzaldehiyde	6	7.62 ± 0.04
3-Bromobenzoic acid	8	3.79 ± 0.02	Phenobarbital	6	7.54 ± 0.04
2,4-Dinitrophenol	7	4.12 ± 0.03	3,5-Dichlorophenol	7	8.19 ± 0.04
Benzoic acid	7	4.21 ± 0.03	Methylparaben	7	8.36 ± 0.03
Ibuprofen	6	4.48 ± 0.02	2-Chlorophenol	7	8.51 ± 0.04
Nicotinic acid	7	4.84 ± 0.03	3-Chlorophenol	6	9.04 ± 0.01
Warfarin	6	5.16 ± 0.04	4-Bromophenol	5	9.28 ± 0.01
2,5-Dinitrophenol	4	5.28 ± 0.05	Paracetamol	4	9.58 ± 0.02
Sulfacetamide	4	5.40 ± 0.05	Phenol	3	9.89 ± 0.01

The internal standard method: internal standards

Basic internal standards

Internal Standard	N	pK_a	Internal Standard	N	pK_a
Aniline	3	4.62 ± 0.01	Clonidine	5	8.09 ± 0.05
Quinoline	3	4.92 ± 0.01	Bupivacaine	5	8.15 ± 0.03
4-*tert*-Butylaniline	4	4.92 ± 0.01	Quinine	3	8.40 ± 0.05
N,N-Dimethyl-*N*-phenylamine	4	5.19 ± 0.05	1-Phenylpiperazine	4	8.19 ± 0.02
Pyridine	6	5.29 ± 0.03	*N,N*-Dimethyl-*N*-benzylamine	7	8.97 ± 0.05
Acridine	4	5.56 ± 0.06	Diphenhydramine	4	9.05 ± 0.04
4-*tert*-Butylpyridine	4	6.04 ± 0.06	Imipramine	3	9.33 ± 0.03
Papaverine	4	6.41 ± 0.06	Procainamide	4	9.24 ± 0.04
2,4-Lutidine	4	6.81 ± 0.04	Propranolol	5	9.46 ± 0.02
Trazodone	4	6.82 ± 0.03	1-Aminoethylbenzene	3	9.51 ± 0.02
Pilocarpine	5	7.08 ± 0.04	Ephedrine	4	9.71 ± 0.02
2,4,6-Trimethylpyridine	5	7.50 ± 0.02	Nortriptyline	3	10.07 ± 0.01
Lidocaine	5	7.91 ± 0.04			

The internal standard method: example pyridoxine



$$\mu_{eff} = \frac{\mu_{H_nA^z} + \sum_{i=1}^{n} 10^{ipH - \sum_{j=1}^{i} pK'_{aj}} \mu_{H_{n-i}A^{z-i}}}{1 + \sum_{i=1}^{n} 10^{ipH - \sum_{j=1}^{i} pK'_{aj}}}$$

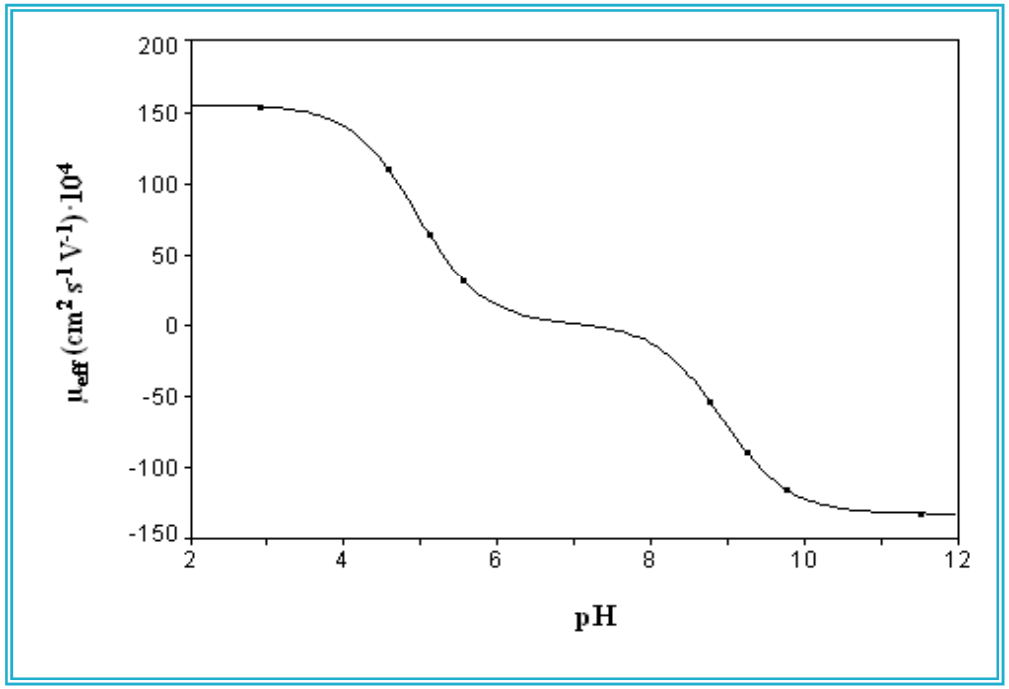
$$\mu_{eff} = \frac{\mu_{H_2A^+} + 10^{pH - pK'_{a1}} \mu_{HA} + 10^{2pH - pK'_{a1} - pK'_{a2}} \mu_{A^-}}{1 + 10^{pH - pK'_{a1}} + 10^{2pH - pK'_{a1} - pK'_{a2}}}$$

$$pK'_{a,IS} = pH + \log Q_{IS}$$

Internal Standard	$pK_{a,IS}$	pH_{theo}	μ_{IS}	pH_{cal}	μ_{AN}
Quinoline	4.92	3.0	207.8	-	152.4
		4.5	149.9	4.59	108.7
		5.0	90.8	5.12	63.5
		5.5	45.8	5.55	30.8
3-Clorophenol	9.04	11.5	-168.3	-	-133.1
		10.0	-146.8	9.79	-116.7
		9.5	-111.8	9.25	-89.8
		9.0	-66.4	8.77	-54.5

The internal standard method: example pyridoxine

$$\mu_{eff} = \frac{\mu_{H_2A^+} + 10^{pH-pK'_{a1}}\mu_{HA} + 10^{2pH-pK'_{a1}-pK'_{a2}}\mu_{A^-}}{1 + 10^{pH-pK'_{a1}} + 10^{2pH-pK'_{a1}-pK'_{a2}}}$$

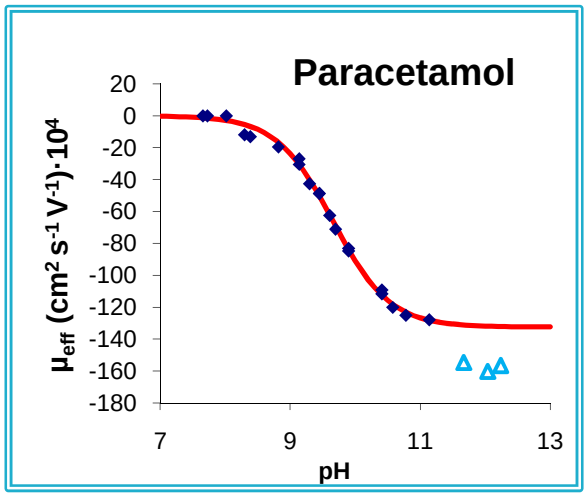


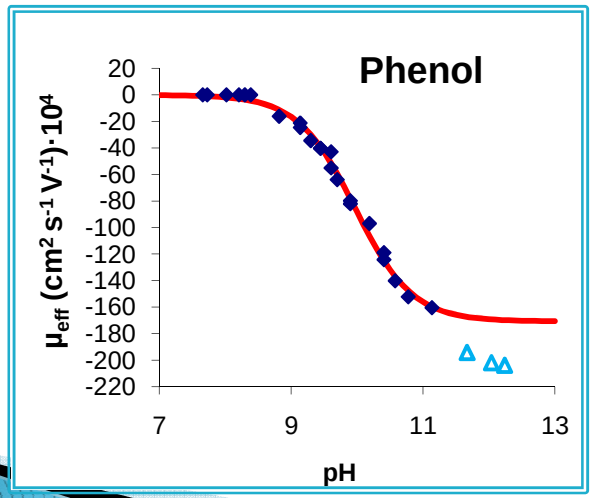
Parameter		s
r^2	0,99999	-
$\mu_{H_2A^+}$	154.09	0.50
μ_{A^-}	-133.27	0.43
pK'_{a1}	4.96	0.01
pK'_{a2}	8.93	0.01

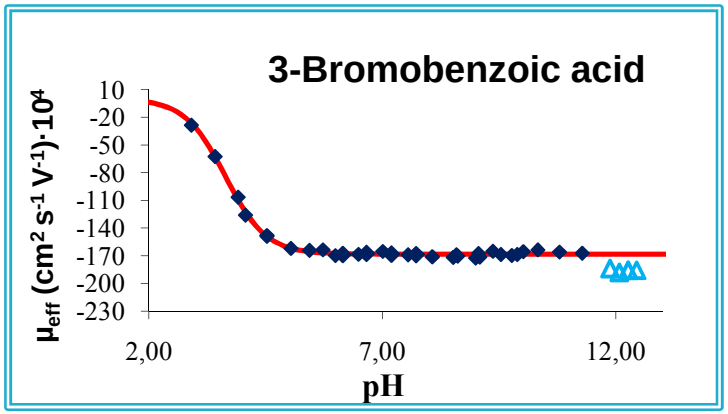
I correction

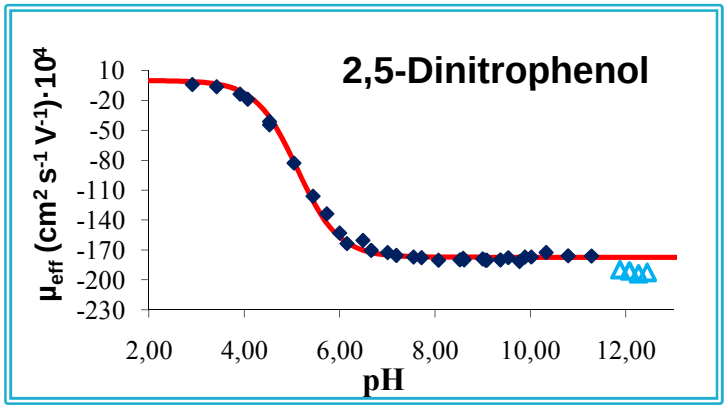
$pK_{a1} = 4.88 \pm 0.01$
$pK_{a2} = 9.02 \pm 0.01$

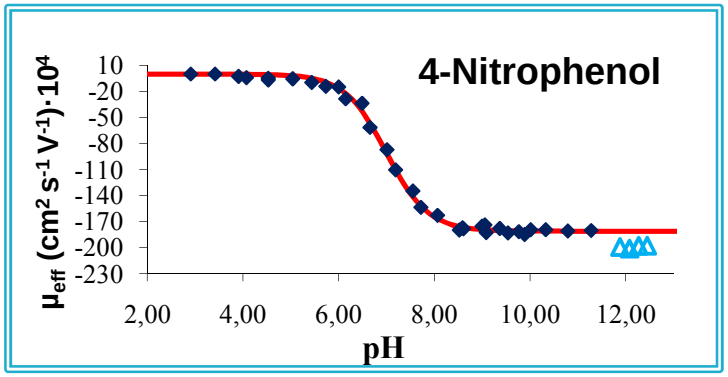
The internal standard method: correction of specific interactions



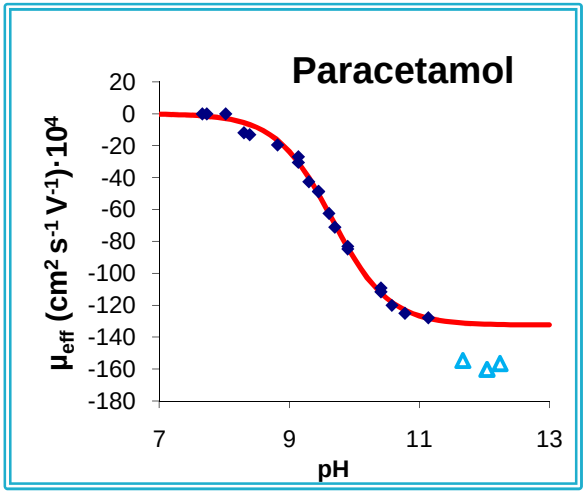




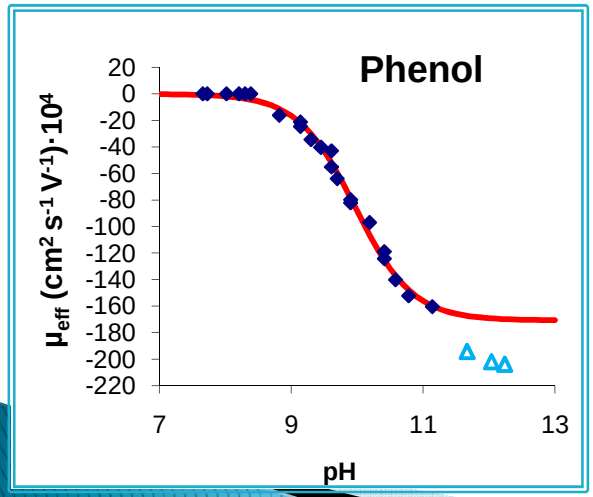




The internal standard method: correction of specific interactions



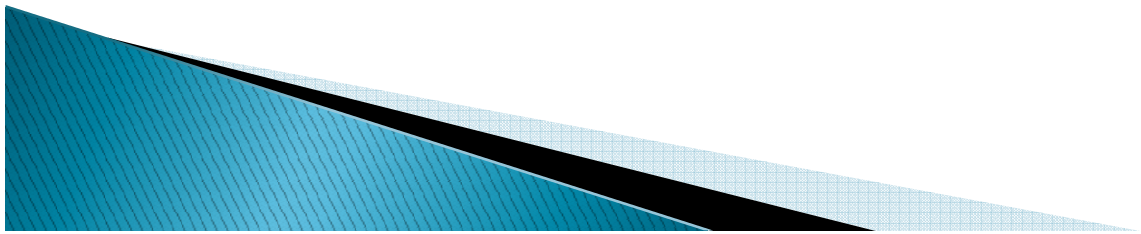
	Classical method	IS method
With NaOH	9.71 ± 0.04	9.58 ± 0.02
Without NaOH	9.57 ± 0.02	9.61 ± 0.01



	Classical method	IS method
With NaOH	10.03 0.03	9.89 0.01
Without NaOH	9.89 0.03	9.89 0.01

The internal standard method: temperature

Pyridine (AN) + Aniline (IS)									
T (°C)	$\mu_{BH+,\ AN}$	$\mu_{BH+,\ IS}$	$\mu_{eff,\ AN}$	$\mu_{eff,\ IS}$	Q_{AN}	Q_{IS}	$pK_{a,IS\ lit}$	$pK_{a,AN\ calc}$	$pK_{a,AN\ calc} - pK_{a,AN\ lit}$
20	207.3	155.2	167.7	77.9	0.24	0.99	4.70	5.32	0.04
25	227.0	171.6	178.0	77.5	0.27	1.22	4.60	5.25	0.02
30	248.0	188.5	188.0	76.1	0.32	1.48	4.51	5.18	-0.01
35	268.8	205.1	196.5	73.9	0.37	1.78	4.42	5.10	-0.02
40	289.2	221.0	203.3	71.5	0.42	2.09	4.34	5.03	-0.04
45	308.2	237.5	209.6	68.4	0.47	2.47	4.27	4.99	-0.01
50	331.4	253.4	216.9	65.0	0.53	2.90	4.19	4.93	-0.01



The internal standard method: temperature correction

T (ºC)	$pK_{a,AN\ calc}$	$pK_{a,AN\ calc} - pK_{a,AN\ lit}$
20	3.80	0.01
25	3.78	-0.01
30	3.78	-0.01
35	3.78	-0.01
40	3.78	-0.01
45	3.78	-0.01
50	3.77	-0.02

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

AN: 3-Bromobenzoic acid

pK_a (25 ºC) = 3.79

ΔH = 0.17

Similar enthalpies

IS: Benzoic acid

pK_a (25 ºC) = 4.21

ΔH = 0.12

T (ºC)	$pK_{a,AN\ calc}$	$pK_{a,AN\ calc} - pK_{a,AN\ lit}$
20	4.23	0.11
25	4.15	0.03
30	4.12	0.00
35	4.10	-0.02
40	4.07	-0.05
45	4.05	-0.07
50	4.02	-0.10

AN: 2,4-Dinitrophenol

pK_a (25 ºC) = 4.12

ΔH = 4.11

Different enthalpies

IS: Benzoic acid

pK_a (25 ºC) = 4.21

ΔH = 0.12

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