DEPARTMENT OF LIQUID PHASE SEPARATIONS

Ústav analytické chemie Akademie věd ČR Veveří 97, 611 42 BRNO

Isoelectric focusing

K. Šlais

Isoelectric focusing - IEF

- *Electromigration separation analytical method based on existence of isoelectric* state of ampholytes, where the effective charge is zero.
- pH = pI
- Analytes - proteins
- Separation - ΔpI < 0.01
- Focusing – concentratrion
- Characterization - pI

Protein as ampholyte

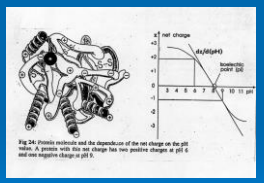
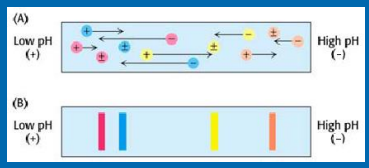
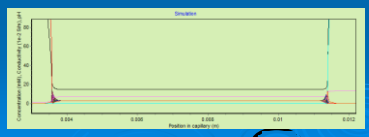


Table 5-2. **Isoelectric Points of Several Common Proteins**

Protein	pI
Pepsin	<1.0
Ovalbumin (hen)	4.6
Serum albumin (human)	4.9
Tropomyosin	5.1
Insulin (bovine)	5.4
Fibrinogen (human)	5.8
γ-Globulin (human)	6.6
Collagen	6.6
Myoglobin (horse)	7.0
Hemoglobin (human)	7.1
Ribonuclease A (bovine)	9.4
Cytochrome *c* (horse)	10.6
Histone (bovine)	10.8
Lysozyme (hen)	11.0
Salmine (salmon)	12.1



Simul 5



Kinds of IEF

- **Gel IEF**
  - **With carier ampholytes**
    - **With immobilised gradient (IPG)**
    - **Two dimmensional electrophoresis 2D = IEF+SDS PAGE**
- **Capillary IEF**
- **Preparative IEF**
  - **Free flow IEF**
  - **Chamber IEF**

Typical result of gel IEF

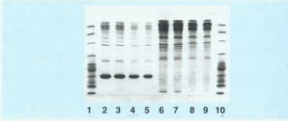
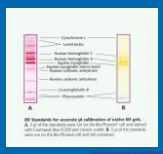
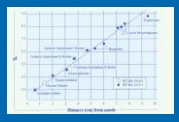


Fig. 2. Typical separation in the Model 111 Mini IEF Cell (5% polyacrylamide gel with 2% Bio-Lyte 3/10 ampholytes). Focusing was carried out in a stepped fashion (100 V for 15 minutes, 200 V for 15 minutes, 450 V for 1 hour). Samples are: lanes 1 & 10: Bio-Rad's IEF Standards; lanes 2-5: Dilutions of horseradish peroxidase; lanes 6-9: Dilutions of Japanese water moccasin snake venom.

pI standards - proteins



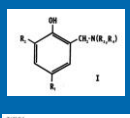


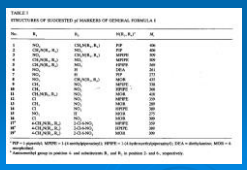
? stability
? purity
? price
? color
? solubility at pI

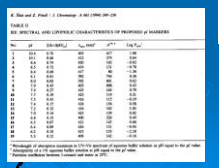
Low molecular mass colored pI markers

- Demands on pI markers
  - Scale of pI from ~ 2 up to 11, step ~ 0.5 pI
  - good ampholytes, -dz/dpH ~0.5 > 0.05, ΔpK ~2 < 4
  - water solubility at pH = pI, > 1 mg/ml
  - different colours λ_{max} > 400 $A_{1\%}$ > 100
  - Purity > 99 % ,
- Availability, price of pI marker
  - Stability - hydrolysis, oxidation, photodegradation, microorganisms

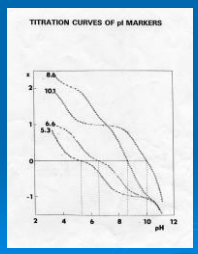
Aminomethylated nitrophenols

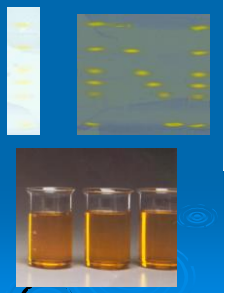




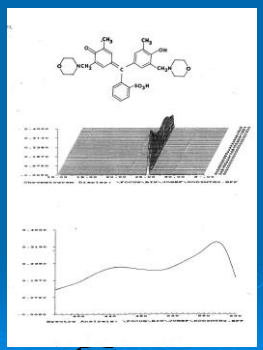


Yellow pI markers

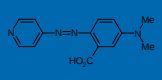




Example of colored pI marker



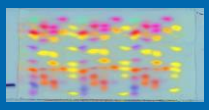
Spectrophotometric determination of pI



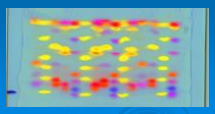
pK_1 = 3.50 ± 0.02
pI = 4.71 ± 0.02
pK_2 = 5.92 ± 0.02

Determination of pI by interpolation in gel IEF

Gradient pH

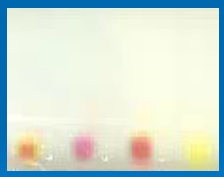


Mixture of 30 simple buffers

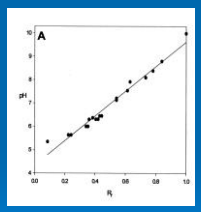


Biolyt 3 – 10

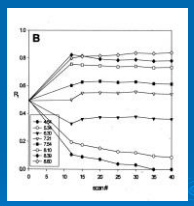
Dynamic of focusing in gel IEF



Dynamic of pH gradient Biolyt 3-10



Linear gradient pH 4 - 10



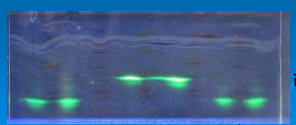
After ½ hour small changes in pH gradient

Dynamic of focusing in gel IEF - determination of focusing end

Development of fluorescent pI markers

Vis

fluorescence



ief446_INDEX.exe

yellow markers

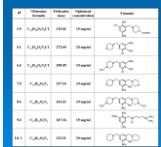
Pardubice 2005

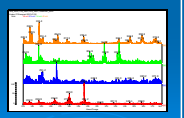
Mass spectrometric characterization of low-molecular-mass color pI markers and their use for direct determination of pI value of proteins

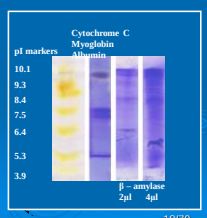
Mazanec, K, Slais, K., Chmelik, J.

J. Mass Spectrom. 41 **2006** 1570-1577

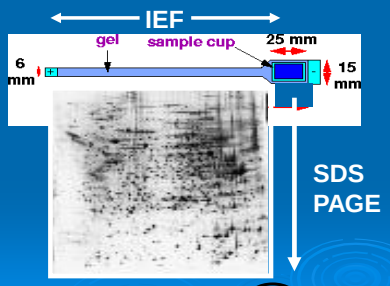
Mass spectra of nitro-substituted pI markers



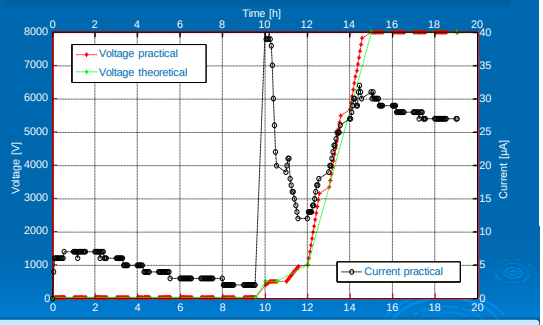




2D Gel electrophoresis

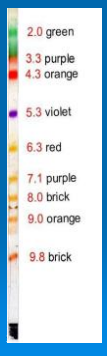


Voltage and current record in IEF on IPG strip



Credit:
Deng, X., Hahne, T., Schröder, S., Redweik, S., Nebija, D., Schmidt, H., Janssen, O., Lachmann, B., Wätzig, H., (2012). The challenge to quantify proteins with charge trains due to isoforms or conformers. *Electrophoresis*, *33*(2), 263–9. doi:10.1002/elps.201100321

Test of color pI markers - LM ladder



Hanspeter Schickle,

ETC Elektrophorese-Technik GmbH, Kirchentellinsfurt, Germany

Strips rehydrated 2 hours under Kerosene

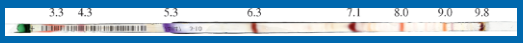
run native 7 hours with Nitrogen

Amersham Multiphor.

Courtesy of Dr. Hanspeter Schickle

IEF of mixture of chosen pI markers in the first dimension strip of 2D gel electrophoresis

in Clinical Proteomics. From Diagnosis to Therapy. J. Van Eyk and M.J. Dunn (Eds.),
Chapter 2, Protein Separation by Two-Dimensional Electrophoresis
Pamela M. Donoghue, Miroslava Stastna, Michael J. Dunn, p 13,
2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim



Immobiline Dry Strip (Amersham Biosciences) pH 3–10, 18 cm.
Apparatus: Protean IEF Cell (BioRad).
Sample: 10 ml of pI markers mixture diluted with 340ml of IEF buffer (8M urea, 2M thiourea, 4% CHAPS, 1% DTT, 0.01% bromophenol blue, 1.5% (v/v) hydroxyethyl disulfide, 0.2% (v/v) IPG buffer pH 3–10).
The acidic end is on the left and the basic end on the right side of the strip.
The pI values of individual pI markers are marked in the picture

2D - typical result – silver staining

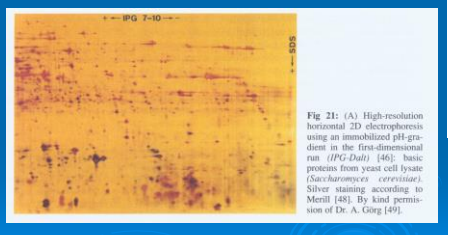
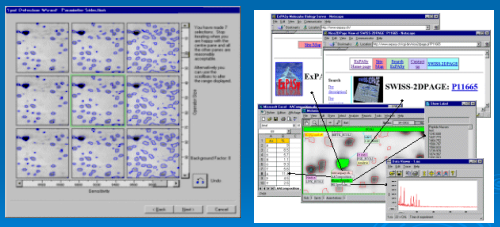
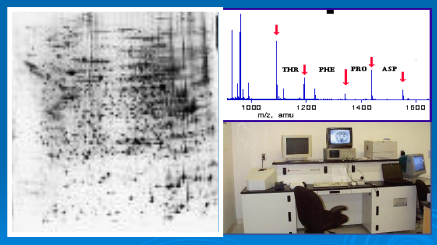


Fig 21: (A) High-resolution horizontal 2D electrophoresis using an immobilized pH-gradient in the first-dimensional run (*IPG-Dalt*) [46]: basic proteins from yeast cell lysate (*Saccharomyces cerevisiae*). Silver staining according to Merill [48]. By kind permission of Dr. A. Görg [49].

2D Gel electrophoresis - Software

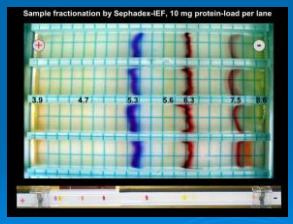


Protein identification by 2D gel electrophoresis -MS



IEF in Granulated Sephadex Gels

Methods in Molecular Biology, vol. 424: Volume 1: Sample Preparation and Pre-Fractionation, Edited by: A. Posch ,
Chapter 22, **Sample Prefractionation in Granulated Sephadex IEF Gels**
Angelika Görg, Carsten Lück, and Walter Weiss, p 277,
Humana Press Inc., 2007, Totowa, NJ



Use of coloured **pI - markers** to determine the slope of the pH gradient and the position where to ‚cut' and remove the individual Sephadex fractions in order to fit to the corresponding narrow pH range IPGs

Courtesy of Carsten Lück

IEF in Sephadex gels and IPG strips

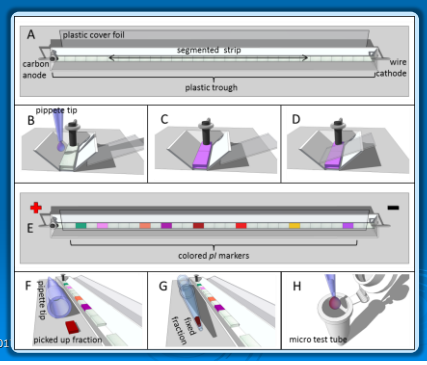
Hodný Z., Přidalová J.,

Institute of Experimental Medicine AV ČR, v.v.i., Prague

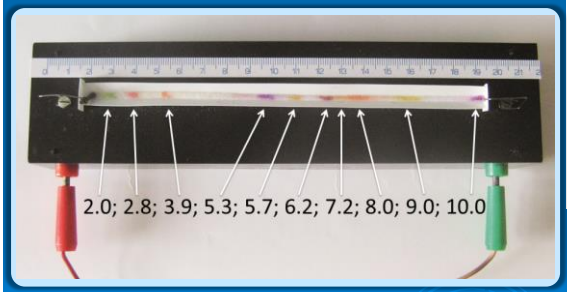
Courtesy of Z. Hodný

pI markers - LM ladder
Home made strip,
linear gradient pH 4-10,
11cm,
1 min 30V,
50 min 30V -> 3500V,
2 hours 3500V.
Courtesy of J. Přidalová

Micropreparative sIEF in nonwoven strip



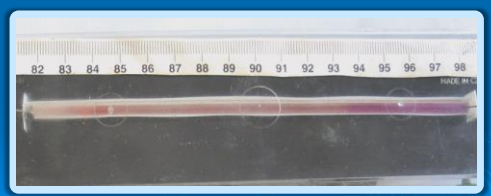
sIEF with carrier buffers and colored pI markers



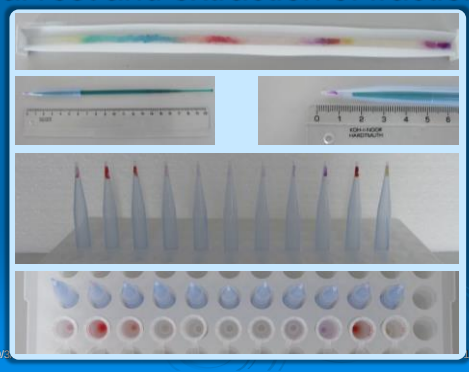
focused mixture:
0,15 ml stock of 12 carreir buffers
0,05 ml stock pf colored pI markers
0,15 ml ethylen glycol; 0,05 ml butanolu 0,1 ml water

Animation of separation course

- Time of separation cca 12 hours
  - Evenimg - Sampling and power switch on
  - Next morning – fraction harvest

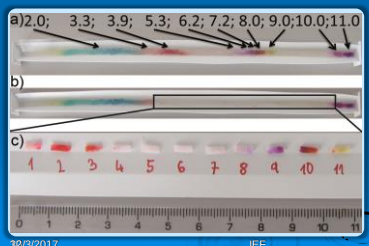


Harvest and extraction of fractions



Whey fractionation

- Aim – pure caseinomacropeptide
  - sample – 1% (m/V) solution of raw dry whey
  - Additional spacers – IMAC (imidazol-1-yl-acetic acid) and Tris (tris(hydroxymethyl)aminometane)



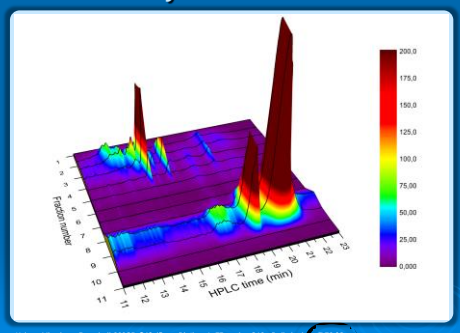
program of power source:

100 V – 200 V 4 h.
200 V – 1000 V 4 h.
1000 V – 3000 V 4 h.
3000 V → harvest

vzorek:
0,375 ml 1% (w/v) whey;
0,05 ml stock colored *pI* markers;
0,1 ml ethylen glycol;
0,05 ml butanol;
0,025 ml 0,1 mol·l^{-1} IMAC;
0,1 ml of 0,1 mol·l^{-1} Tris;

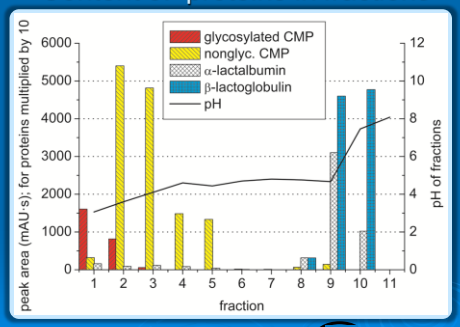
extraction: 6mm strip segment in to 100 µl water

HPLC analysis of sIEF fractions



Kolona Microbore Poroshell 300SB-C18 (5 µm částice, 1×75 mm) + C18 předkolonka, při 70 °C
Průtok 20 µl·min-1
Fáze A/B: voda/ACN s 0.1% (v/v) TFA lineární gradient od 5 do 80 % ACN (30 min)
dávkovaný objem: 4 µl

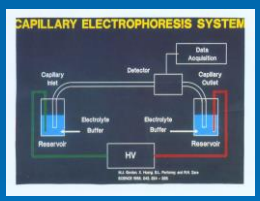
Content of proteins in fractions

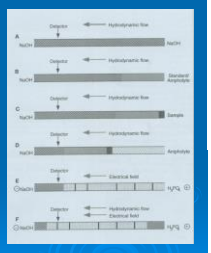


Výtěžek:

glykosylovaný CMP	44 %	neglykosylovaný CMP	80 %
α-laktalbumin	77 %	β-laktoglobulin	101 %

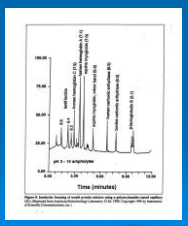
Capillary IEF



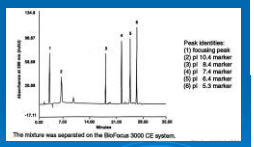


Capillary IEF of standards

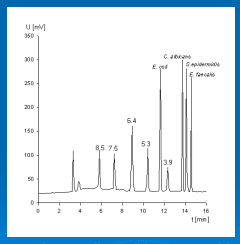
proteins



pI markers

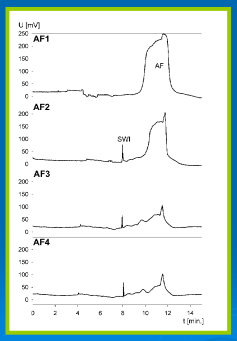


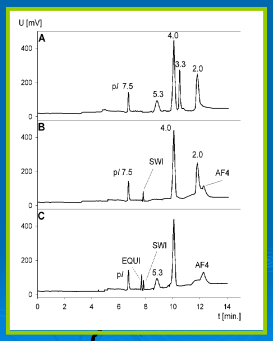
CIEF of microorganisms



Sample: *E. coli, C. albicans, S. epidermidis, E. faecalis* in physiological saline solution, 4×10^6 cell ml^{-1}.

CIEF viruses with UV detection



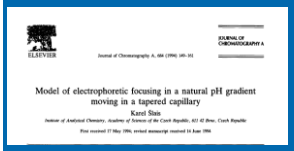


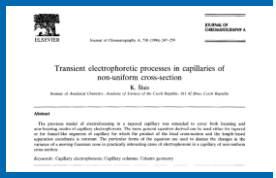
Patogenes of different sources

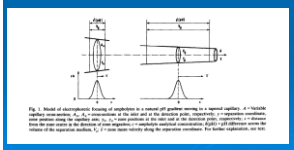
Table 1. The strains of the plant pathogens included in this study, comparison of their isoelectric points, pI, and RSDs from three measurement of the migration times, t, for each from the strains.

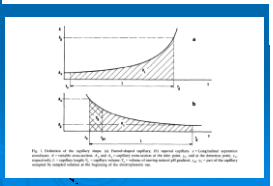
Abbreviation in Figs.	Strain	pI
C. michiganensis	*Clavibacter michiganensis* subsp. *michiganensis* CCM 1635	4.6
	Clavibacter michiganensis subsp. *michiganensis* VURV C254	4.6
	Clavibacter michiganensis subsp. *michiganensis* VURV 2/4/99	4.7
	Clavibacter michiganensis subsp. *michiganensis* VURV 5090	4.6
	Clavibacter michiganensis subsp. *michiganensis* VURV 5059	4.7
	Clavibacter michiganensis subsp. *michiganensis* VURV 7008	4.7
	Clavibacter michiganensis subsp. *michiganensis* VURV 7018	4.6
	Clavibacter michiganensis subsp. *michiganensis* VURV 7030	4.7
	C. michiganensis	pI = 4.7, RSD = 1.9 %
X. vesicatoria	*Xanthomonas vesicatoria* CCM 2101	4.0
	Xanthomonas vesicatoria CCM 2102	4.1
	Xanthomonas vesicatoria VURV P-1-1	4.0
	Xanthomonas vesicatoria VURV P-6-1	4.1
	Xanthomonas vesicatoria LMG 2804	4.1
	Xanthomonas vesicatoria LMG 667	4.1
	X. vesicatoria	pI = 4.1, RSD = 0.7 %
P. syringae	*Pseudomonas syringae pv. tomato* CFBP 5422	4.0
	Pseudomonas syringae pv. tomato CFBP 2212	4.0
	Pseudomonas syringae pv. tomato IVIA 1733.3	4.0
	P. syringae	pI = 4.0, RSD =1.9 %
P. corrugata	*Pseudomonas corrugata* CFBP 4901	2.4
	Pseudomonas corrugata CFBP 5465	2.4
	Pseudomonas corrugata CFBP 6663	2.4
	Pseudomonas corrugata IVIA 614.5.3	2.4
	P. corrugata	pI = 2.4, RSD = 0.9 %

Tapered capillary in cIEF

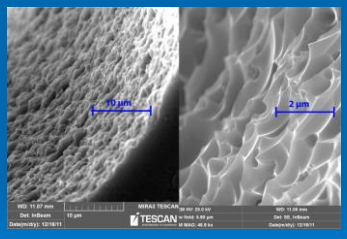






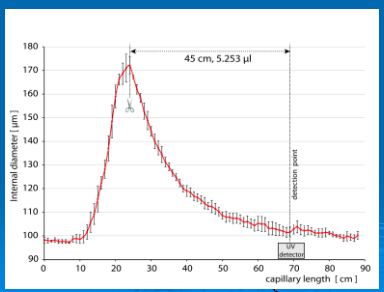


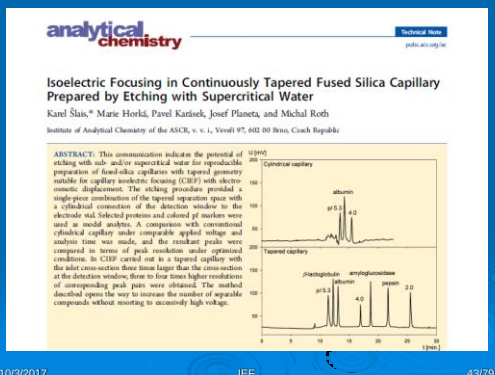
Supercritical water in preparation of tapered fused silica capillaries



Effect of treatment of 100 µm i.d. fused silica capillary with supercritical water in semi-dynamic mode. Experimental conditions: 400 °C, 32 MPa, 20 replacements of supercritical water.

Dependence the local internal diameter of etched fused silica capillary on the capillary length.
The cutout of the segment used as the tapered capillary in cIEF and the detection window are indicated.




analytical chemistry

Technical Note

pubs.acs.org/ac

Isoelectric Focusing in Continuously Tapered Fused Silica Capillary Prepared by Etching with Supercritical Water

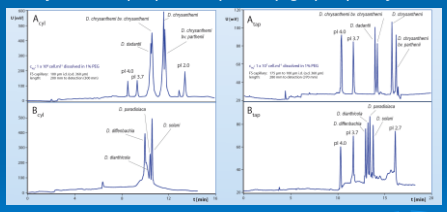
Karel Šlais,* Marie Horká, Pavel Karásek, Josef Planeta, and Michal Roth

Institute of Analytical Chemistry of the ASCR, v. v. i., Veveří 97, 602 00 Brno, Czech Republic

ABSTRACT: This communication indicates the potential of etching with sub- and/or supercritical water for reproducible preparation of fused-silica capillaries with tapered geometry suitable for capillary isoelectric focusing (CIEF) with electro-osmotic displacement. The etching procedure provided a single-piece combination of the tapered separation space with a cylindrical connection of the detection window to the electrode vial. Selected proteins and colored pI markers were used as model analytes. A comparison with conventional cylindrical capillary under comparable applied voltage and analysis time was made, and the resultant peaks were compared in terms of peak resolution under optimized conditions. In CIEF carried out in a tapered capillary with the inlet cross-section three times larger than the cross-section at the detection window, three to four times higher resolutions of corresponding peak pairs were obtained. The method described opens the way to increase the number of separable compounds without resorting to excessively high voltage.

Resolution of several *Dickeya* bacterium species with similar isoelectric points by capillary isoelectric focusing employing

cylindrical (left) and tapered (right) capillary.



Combination of Capillary Isoelectric Focusing in a Tapered Capillary with MALDI-TOF MS for Rapid and Reliable Identification of Dickeya Species from Plant Samples
Horka, M; Salplachta, J; Karasek, P; Kubesova, A; Horky, J; Matouskova, H; Slais, K ; Roth, M
ANALYTICAL CHEMISTRY Volume: 85 Pages: 6806-6812 JUL 16 2013

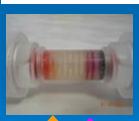
Preparative Liquid phase IEF

BIO-RAD

Rotofor

MicroRotofor

Total 2.5 ml sample
Ten 0.25 ml fractions
2 hours run time



Preparative autofocusing of peptides + pI markers

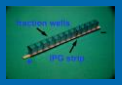
Tomas, R; Yan, LS; Krenkova, J; Foret, F. ELECTROPHORESIS, 28 (13): 2283-2290 2007

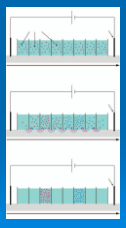
ZOOM® IEF Fractionator (Invitrogen)

- Focusing chambers are separated by polyacrylamide discs with immobilized pH
- Proteins have to pass through discs toward their pI
- Chamber volume 650 µl
- Sample preparation: dissolve, denaturation, alkylation

OFF - GEL electrophoresis

Agilent Technologies





P.E. Michel, F. Reymond, I. L. Arnaud, J. Josser, H. H. Girault, J. S. Rossier, *Electrophoresis* 2003, *24*, 3–11

Agilent 3100 OFFGEL Fractionator

pI-based fractionation of proteins and peptides with liquid-phase recovery. introduced *May 30, 2006,* Co-developed with DiagnoSwiss S.A.

Use of pI-dye markers as on-line trackers for the focusing of peptides during electrophoresis on the OFFGEL fractionator device (OGE). 22 hours runtime

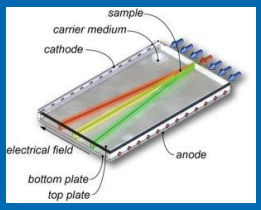
Courtesy of M. Heller DKF, University of Bern, Switzerland

Use of pI-dye markers as on-line trackers for the focusing of peptides during electrophoresis on the OFFGEL fractionator device (OGE).

- **pI-marker dyes** were added at 10 ug (dark orange, pI 3.9; violet, pI 5.2; red, pI 6.2; bright orange, pI 8.0) or 30 ug (yellow, pI 10.1), respectively.
- Peptide/dye solution was distributed into the 13 wells of the OGE.
- IPG strips pH 3-10 from BioRad re-hydrated in OGE buffer were used.
- Focusing was done by setting a maximal potential (1250 or 1500 V) and a current limit of 50 uA.

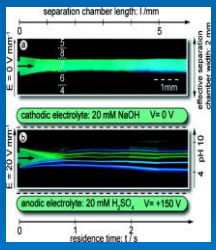
Courtesy of M. Heller, DKF, University of Bern, Switzerland

Free-Flow Electrophoresis



The miniaturization of FFE implies several advantages especially considering sample volume and separation speed. In contrast to the tens of milliliters of sample consumed by conventional large scale FFE devices, microfluidic FFE systems require only tens of nanoliters up to hundreds of microliters of sample. This is especially interesting in clinical analysis where often only low sample volumes are available. Furthermore, instead of residence times of up to tens of minutes, microfluidic FFE (μ-FFE) devices separate within several seconds.

Microfluidic high-resolution free-flow isoelectric focusing



FFIEF of seven fluorescent IEF markers

Voltage = 150 V, current = 50 mA

Markers (pI 4, 5.1, 6.2, 7.2, 8.1, 9, and 10.3) are fully separated within less than 2 s.

The sample flow rate was 0.4 mL/min (v = 2 mm/s).

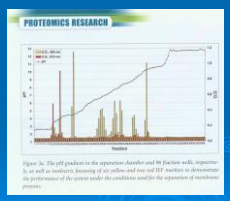
The apparent kinks in the fluorescent tracer paths are caused by merging multiple photographs.

Kohlheyer, D., Eijkel, J. C. T., Schlautmann, S., van den Berg, A., Schasfoort, R. B. M., *Anal. Chem.* 2007, *79*, 8190–8198.

Preparative Free Flow Electrophoresis

FFE Service GmbH
Dr. Gerhard Weber
D-85551 Kirchheim
Germany

WEBER, Gerhard; Margeritenweg 23 85551 Kirchheim (DE).
WO/2002/050524, 07.12.2001,



Divergent Flow IEF (DF IEF)

Basic ideas

- Fluidics - continuous widening of the flat channel while the liquid flows from channel inputs toward the outputs which generates a divergent flow

and, at the same time

- IEF - small transversal voltage drop at the channel input and high transversal voltage drop at the channel output.

Šlais K. Electrophoresis 29 **2008** 2451-2457

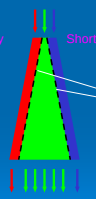
Divergent flow IEF

Fluidics – divergent flow

Carriers & analytes

anolyte catholyte

High flow velocity Short separation path



Membranes

Divergent flow IEF

Fluidics – divergent flow

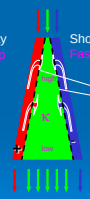
IEF – electricity control by electrolyte cnductivity κ

Carriers & analytes

anolyte catholyte

High flow velocity
Low voltage drop

Short separation path
Fast separation



Membranes

High voltage drop

Efficient focusing

Separated fractions

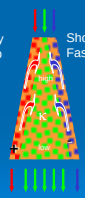
Divergent flow IEF

Carriers & analytes

anolyte catholyte

High flow velocity
Low voltage drop

Short separation path
Fast separation



High voltage drop

Efficient focusing

Separated fractions

Fluidics – divergent flow

IEF – electricity control
by electrolyte cnductivity κ

Simple device :

Membranes eliminated
by porous layer bed

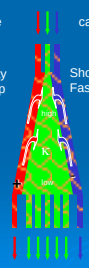
Divergent flow IEF

Carriers & analytes

anolyte catholyte

High flow velocity
Low voltage drop

Short separation path
Fast separation



High voltage drop

Efficient focusing

Separated fractions

Fluidics – divergent flow

IEF – electricity control
by electrolyte cnductivity κ

Simple device :

Membranes eliminated
by porous layer bed

Separation area,
flow inputs and outputs
made from
non-woven fabric

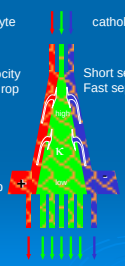
Divergent flow IEF

Carriers & analytes

anolyte catholyte

High flow velocity
Low voltage drop

Short separation path
Fast separation



High voltage drop

Efficient focusing

Separated fractions

Fluidics – divergent flow

IEF – electricity control
by electrolyte cnductivity κ

Simple device:

Membranes eliminated
by porous layer bed

Separation area,
flow inputs and outputs
made from
non-woven fabric

Electrode contacts made from
non-woven fabric

Flow generated by
hydrostatics

Divergent flow IEF

Šlais K. Electrophoresis 29 **2008** 2451-2457

The polypropylene nonwoven web 0.1 mm thick lies on white polyvinylchloride flexible sheet

input strips dipped in Petri dishes containing:
above left – anolyte
above middle - solution of carriers and pI markers
above right – catholyte

middle left - carbon rod anode
middle right – carbon rod cathode

output strips - bottom - microplate

Streamlines of red pI markers from left -
pI = 3.3, 4.7, 6.2, 7.6, 11.0

Flow due to hydrostatics and capillary elevation

Constant power load 1 W

No cooling

Divergent flow IEF Continuos flow IEF in rectangular space

separation space material, length 15 cm and output width 8 cm
power load 1 W
solutions
output flow rate 6 mL/h = 0.9 cm/min
are the same in both configurations

Dynamics of divergent flow IEF

Šlais K. Electrophoresis 29 **2008** 2451-2457

1 W constant power load

switched off at 11 hod 30 min

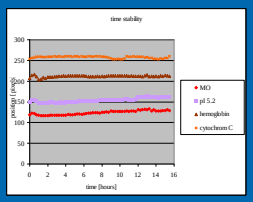
switched on at 11 hod 40 min

Flow inputs:
Anolyte: 0.05 M H_3PO_4 , 5.2 mS/cm,1 mL/h
Catholyte: 0.05 M NaOH, 11mS/cm, 1 mL/h
Carriers and pI markers: 0.75 mS/cm, 4 mL/h,

Holdup volume: 1 ml
Separation area: 71 cm^2

Streamlines of red pI markers from left
pI = 3.3, 4.7, 6.2, 7.6, 11.0

Performance stability of SI DF IEF



Streamlines from left :
orange – marker pI 2.6; lavender - marker pI 5.2; brown – hemoglobin, 0.5 mg/ml; brick – cytochrom C, 0.5 mg/ml; flow 0.18 mL/min

Streamlines fluctuation 3.96 %, 3.94 %, 1.26 % and 1.88 %, respectively.

Šťastná M., Šlais K., Electrophoresis, accepted -00293

Preparative DF IEF of bear

Mazanec K., Bobalova J., Slais K. Anal Bioanal Chem 2009, 393, 1769-1778

DF IEF of barley extract, malt and bear

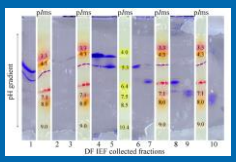
Continuously sampled pI markers:
orange – marker pI 2.5
pink - marker pI 11

1 drop of pI markers mixture

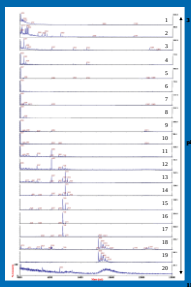
Input- Raw barley extract + buffers + markers pI 2.5 and 11
flow rate - 0.23 ml/ min,
conductivity- 1.0 mS/cm
Input electrodes 4 mA, 20 V
Output electrodes: 6 mA, 800 V

Mazanec K., Bobalova J., Slais K.
Anal Bioanal Chem 2009, 393, 1769-1778

Combined scan of IEF gel fractions from DF IEF of bear



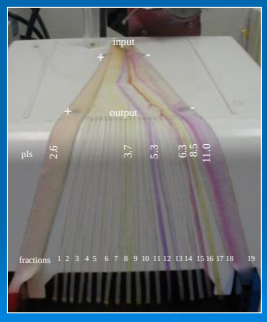
Colored pI markers scanned immediately after gel IEF
Proteins scanned after Commassie staining

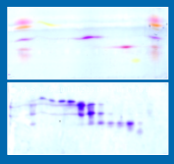


MALDI-MS spectra of 20 DF IEF fraction of proteins from raw malt extract.

Mazanec K., Bobalova J., Slais K.
Anal Bioanal Chem 2009, 393, 1769-1778

Preparative divergent flow IEF without carrier ampholytes for separation of complex biological samples





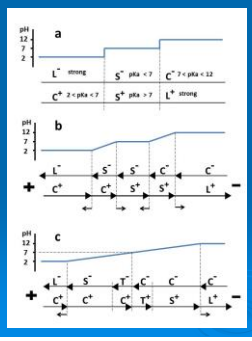
Separation of proteins in individual yeast lysate DF IEF fractions by polyacrylamide gel IEF.

DF IEF **without carrier ampholytes** with yeast lysate sample and colored pI markers.

Desalting, preconcentration, preseparation

M. Stastna, K. Slais, Electrophoresis,31, 2010,433-439

Electrolyte system for fast preparative focusing in wide pH range based on bidirectional isotachophoresis (BITP)



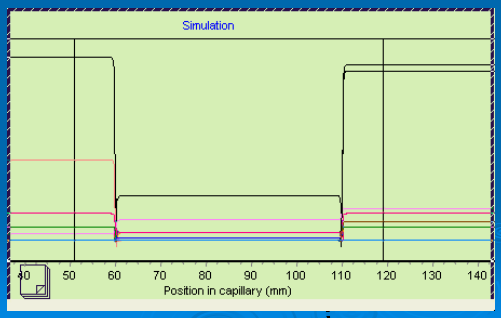
L^- leading anion of strong acid
L^+ - leading cation of strong base,
C^- - anionic counter ions,
C^+ - cationic counter ions,
S^- - anionic spacers,
S^+ - cationic spacers,
T^- - the fastest C^- in LB in anionic ITP part and
T^+ - the fastest C^+ in LA in cationic ITP part.

The composition of LB, LA and spacer electrolytes used for simulation and in the experiment verification

catholyte	pK	$\mu \times 10^{-9}$ [$m^2 V^{-1} s^{-1}$]	M_r	simulated conc [mM]	conc [mgL^{-1}]
lysine	10.79	-26.4	146.19	7	1023.3
ACA	10.80	-28.8	131.20	10	1312.0
GABA	10.56	-29.0	103.10	10	1031.0
β-alanine	10.24	-30.8	89.10	10	891.0
glycine	9.78	-37.4	75.00	7	525.0
asparagine	9.02	-31.6	130.00	5	650.0
TAPS	8.30	-25.0	243.28	5	1216.4
TAPSO	7.70	-26.0	259.28	5	1296.4
NaOH	13.70	+51.9	40.00	60	2400.0
analyte					
glutamic acid	2.16, 4.32	+27.4, +27.6	147.13	5	735.7
β-alanine	3.55	+38.5	89.10	10	891.0
GABA	4.03	+28.8	103.10	10	1031.0
ACA	4.37	+29.8	131.20	10	1312.0
creatinine	4.83, 9.20	+37.0, 37.2	113.10	5	565.5
HPN	5.29	+30.0	123.20	5	616.0
BisTris	6.40	+26.0	209.20	5	1046.0
HEMorf	6.80	+30.2	131.20	5	656.0
H_2O_2	-	-82.9	86.00	25	2450.0

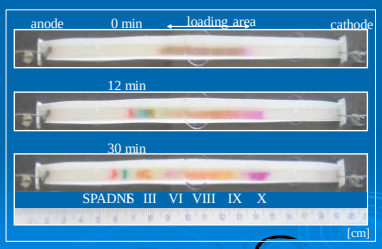
	pK	$\mu \times 10^{-9}$ [$m^2 V^{-1} s^{-1}$]	M_r	simulated conc [mM]	conc [mgL^{-1}]
spacers					
MOPS	7.20	-26.9	209.30	3	627.9
ACES	6.70	-31.3	182.20	3	546.6
MES	6.09	-28.0	213.25	3	639.8
picolinic acid	5.30	-29.6	123.11	3	369.3
ammonium acetate	4.76	-42.7,-39.4	77.09	3	231.3
glycolic acid	3.89	-42.4	118.10	3	354.3
phosphoric acid	2.16,7.21,12.67	344,61.4,71.5	192.12	3	576.4
imidazol	7.15	+52.0	68.10	3	204.3
EtMorf	7.70	+30.0	115.20	3	345.6
Tris	8.08	+29.5	121.10	3	363.3
morpholine	8.35	+41.3	87.00	3	261.0
aemediol	8.70	+33.5	105.10	3	315.3
aminomethylpropanol	9.60	+30.0	89.14	3	267.4
ethylaminoethanol	10.00	+30.0	89.14	3	267.4
piperidin	11.12	+39.8	85.10	3	255.3

Computer simulation of dynamics in newly suggested electrolyte system based on bidirectional ITP



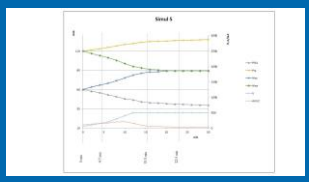
The animation of the experiment with colored indicators subjected to BITP electrofocusing in newly suggested electrolyte system and carried out on nonwoven strip in V-shape trough during 30 min.

The examples of representative images displaying bidirectional ITP electrofocusing process in nonwoven strip in V-shape trough with colored pH indicators taken at 0, 12 and 30 minutes.

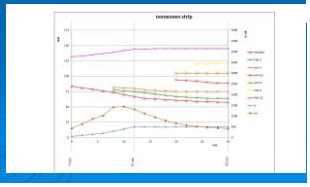


The electrofocusing dynamics shown as dependence of zone position on analysis time

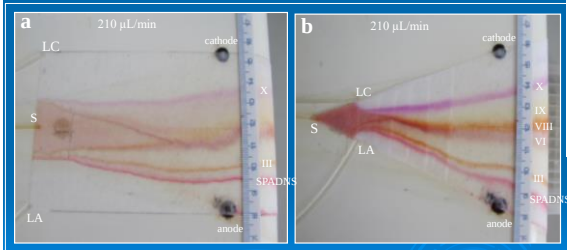
simulation



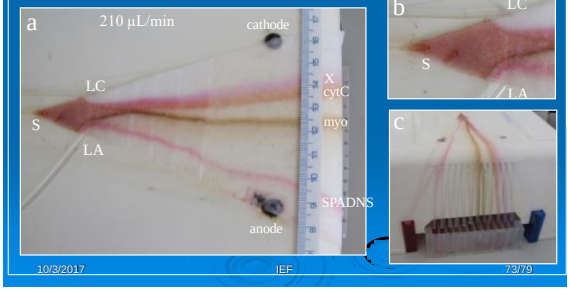
experiment carried out on linear nonwoven strip in the V-shape trough



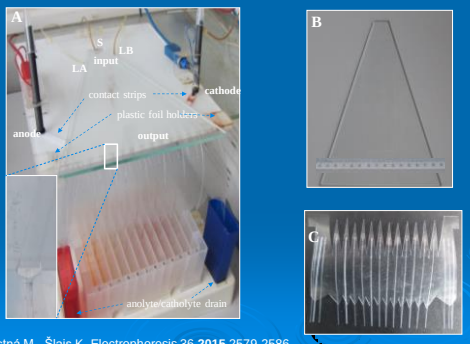
The images of bidirectional ITP electrofocusing with continuous flow in rectangular (a) and trapezoidal (b) separation beds under the same experimental conditions



The example of bidirectional ITP separation and electrofocusing in continuous flow of cytochrome C (cytC) and myoglobin (myo)

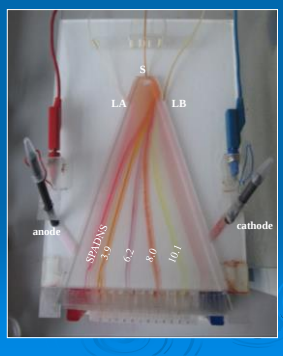


Continuous fast focusing in trapezoidal void channel based on bidirectional isotachophoresis in wide pH range.

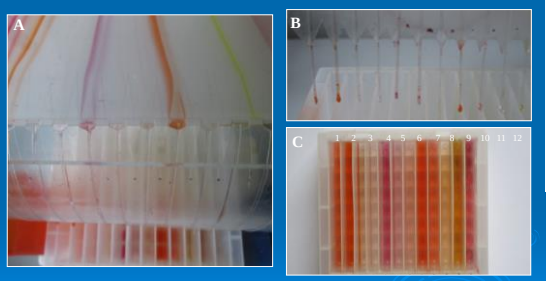


Šťastná M., Šlais K. Electrophoresis 36 **2015** 2579-2586

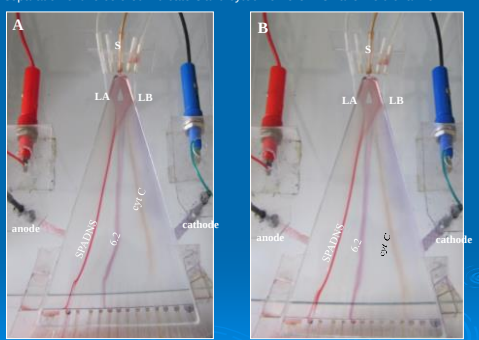
The separation of colored indicators in instrumentation with a larger void closed channel.



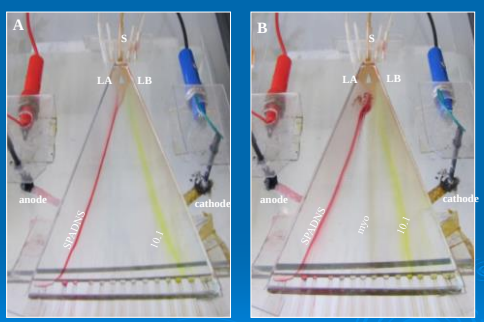
The details of the instrumentation output with collected fractions in twelve well plate.



The separation of two colored indicators and cytochrome C in smaller void channel.



The separation of two colored indicators and myoglobin in smaller void channel.



Conclusions

Divergent flow isoelectric focusing (DF IEF)

- combines
  - speed and low demand of electricity typical for micro fluidic channels
  - sample loadability and separation efficiency of preparative devices
- has a potential
  - for further shape and material optimization
  - for scaling up and down

- Single input design (SI DF IEF) simplifies miniature devices
- Carriers based on mixtures of simple buffers are cheap and advantageous for further processing of collected fractions
- Stability of streamlines is promising for the designs with more collected fractions
- Thickness of separation layer can conveniently be adjusted by sandwiching of non woven fabric or glas plate distance