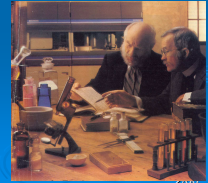


Izoelektrická fokusace

K. Šlais

Izoelektrická fokusace - IEF

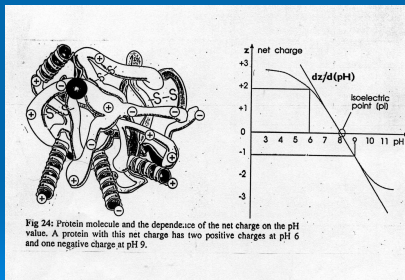
- elektromigrační separační analytická metoda využívající existence izoelektrického stavu amfolytů, kdy efektivní náboj je nulový.
- $pH = pI$
- Analyty - proteiny
- Separace - $\Delta pI < 0.01$
- Fokusace – zakoncentrování
- Charakterizace - pI



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Protein jako amfolyt

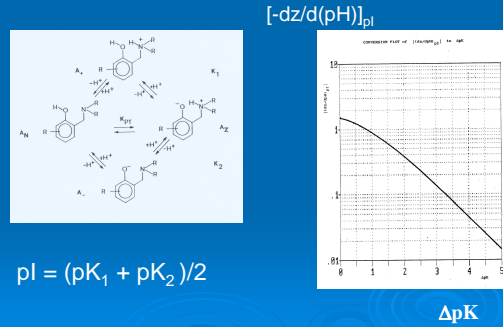


9/21/2009

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3/60

Biprotický amfolyt

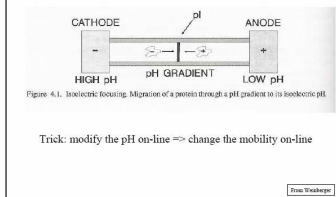


9/21/2009

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4/60

Isoelectric Focusing I

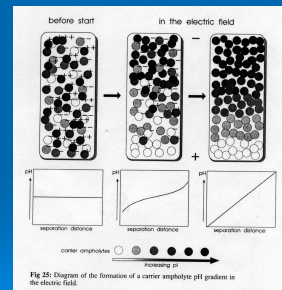


9/21/2009

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5/60

Vznik pH gradientu



9/21/2009

IEF

6/60

Druhy IEF

- Gelová IEF
 - S nosnými amfolyty
 - S imobilizovaným gradientem (IPG)
 - Dvourozměrná elektroforéza 2D = IEF + SDS PAGE
- Kapilární IEF
- Preparativní IEF
 - Free flow
 - Komorová (např. Rotofor)

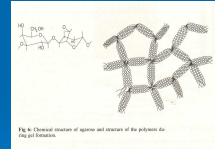
9/21/2009

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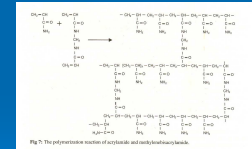
7/60

Struktura gelu

agarosa



polyakrylamid

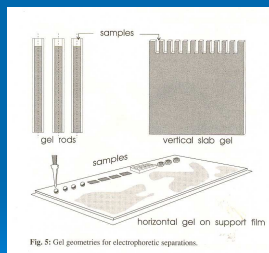


9/21/2009

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8/60

Varianty gelové IEF

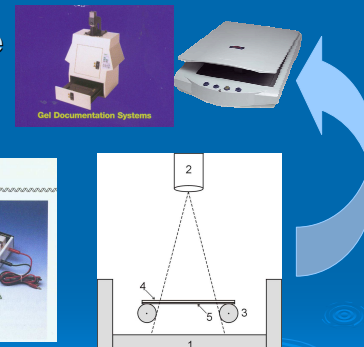


9/21/2009

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9/60

Instrumentace



(1) outer chamber, (2) CCD camera, (3) graphite electrodes, 50 mm distance between contact points, (4) glass plate, (5) polyacrylamide gel of size 125 x 54 x 0.4 mm.

Vertical gel IEF



9/21/2009

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11/60

Typický výsledek gelové IEF



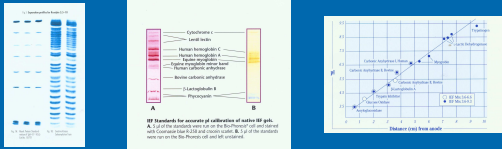
Fig. 2. Typical separation in the Model 111 Mini IEF Cell (5% polyacrylamide gel with 2% Bio-Lyte 3/10 ampholytes). Focusing was carried out in a stepped fashion (100 V for 15 minutes, 200 V for 15 minutes, 450 V for 1 hour). Samples are: lanes 1 & 10: Bio-Rad's IEF Standards; lanes 2-5: Dilutions of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

9/21/2009

IEF

12/60

Standardy pI - proteiny



nestabilní,
nečisté,
drahé,
málo barevné,
málo rozpustné při pI



Nízkomolekulární barevné pI markery



- Požadavky na pI markery
 - Škála pI od ~ 2 do 11, po ~ 0.5 pI
 - dobré amfolyty, -dz/dpH ~0.5 > 0.05, ΔpK ~2 < 4
 - rozpustnost ve vodě při pH = pI, > 1 mg/ml
 - různé barvy, λ_{max} > 400 nm, A_{1%} > 100
 - Čistota, > 99 %
- Dostupnost, cena markeru
- Stabilita - hydrolyza, oxidace, fotodegradace, mikroorganismy

Aminomethylované nitrofenoly

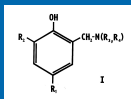


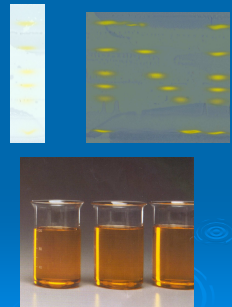
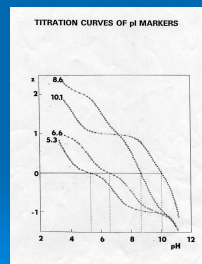
TABLE I
STRUCTURES OF REGISTERED pI MARKERS OF GENERAL FORMULA I

No.	R ₁	R ₂	IND. No.	pI
1	H	H	PP	4.60
2	H	H	PP	4.80
3	H	H	PP	5.00
4	H	H	PP	5.20
5	H	H	PP	5.40
6	H	H	PP	5.60
7	H	H	PP	5.80
8	H	H	PP	6.00
9	H	H	PP	6.20
10	H	H	PP	6.40
11	H	H	PP	6.60
12	H	H	PP	6.80
13	H	H	PP	7.00
14	H	H	PP	7.20
15	H	H	PP	7.40
16	H	H	PP	7.60
17	H	H	PP	7.80
18	H	H	PP	8.00
19	H	H	PP	8.20
20	H	H	PP	8.40
21	H	H	PP	8.60
22	H	H	PP	8.80
23	H	H	PP	9.00
24	H	H	PP	9.20
25	H	H	PP	9.40
26	H	H	PP	9.60
27	H	H	PP	9.80
28	H	H	PP	10.00
29	H	H	PP	10.20
30	H	H	PP	10.40
31	H	H	PP	10.60
32	H	H	PP	10.80
33	H	H	PP	11.00
34	H	H	PP	11.20
35	H	H	PP	11.40
36	H	H	PP	11.60
37	H	H	PP	11.80
38	H	H	PP	12.00
39	H	H	PP	12.20
40	H	H	PP	12.40
41	H	H	PP	12.60
42	H	H	PP	12.80
43	H	H	PP	13.00
44	H	H	PP	13.20
45	H	H	PP	13.40
46	H	H	PP	13.60
47	H	H	PP	13.80
48	H	H	PP	14.00
49	H	H	PP	14.20
50	H	H	PP	14.40
51	H	H	PP	14.60
52	H	H	PP	14.80
53	H	H	PP	15.00
54	H	H	PP	15.20
55	H	H	PP	15.40
56	H	H	PP	15.60
57	H	H	PP	15.80
58	H	H	PP	16.00
59	H	H	PP	16.20
60	H	H	PP	16.40
61	H	H	PP	16.60
62	H	H	PP	16.80
63	H	H	PP	17.00
64	H	H	PP	17.20
65	H	H	PP	17.40
66	H	H	PP	17.60
67	H	H	PP	17.80
68	H	H	PP	18.00
69	H	H	PP	18.20
70	H	H	PP	18.40
71	H	H	PP	18.60
72	H	H	PP	18.80
73	H	H	PP	19.00
74	H	H	PP	19.20
75	H	H	PP	19.40
76	H	H	PP	19.60
77	H	H	PP	19.80
78	H	H	PP	20.00
79	H	H	PP	20.20
80	H	H	PP	20.40
81	H	H	PP	20.60
82	H	H	PP	20.80
83	H	H	PP	21.00
84	H	H	PP	21.20
85	H	H	PP	21.40
86	H	H	PP	21.60
87	H	H	PP	21.80
88	H	H	PP	22.00
89	H	H	PP	22.20
90	H	H	PP	22.40
91	H	H	PP	22.60
92	H	H	PP	22.80
93	H	H	PP	23.00
94	H	H	PP	23.20
95	H	H	PP	23.40
96	H	H	PP	23.60
97	H	H	PP	23.80
98	H	H	PP	24.00
99	H	H	PP	24.20
100	H	H	PP	24.40

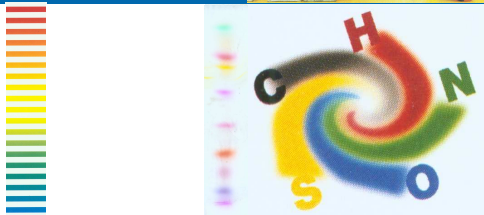
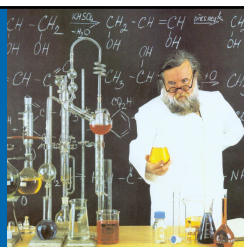
TABLE II
PHOTOMETRIC AND LUMINESCENT CHARACTERISTICS OF PROFILES OF MARKERS

No.	pI	λ _{max} (nm)	ε (l·mol ⁻¹ ·cm ⁻¹)	λ _{exc} (nm)	λ _{em} (nm)	Φ _{em}
1	4.60	410	1000	365	420	0.01
2	4.80	410	1000	365	420	0.01
3	5.00	410	1000	365	420	0.01
4	5.20	410	1000	365	420	0.01
5	5.40	410	1000	365	420	0.01
6	5.60	410	1000	365	420	0.01
7	5.80	410	1000	365	420	0.01
8	6.00	410	1000	365	420	0.01
9	6.20	410	1000	365	420	0.01
10	6.40	410	1000	365	420	0.01
11	6.60	410	1000	365	420	0.01
12	6.80	410	1000	365	420	0.01
13	7.00	410	1000	365	420	0.01
14	7.20	410	1000	365	420	0.01
15	7.40	410	1000	365	420	0.01
16	7.60	410	1000	365	420	0.01
17	7.80	410	1000	365	420	0.01
18	8.00	410	1000	365	420	0.01
19	8.20	410	1000	365	420	0.01
20	8.40	410	1000	365	420	0.01
21	8.60	410	1000	365	420	0.01
22	8.80	410	1000	365	420	0.01
23	9.00	410	1000	365	420	0.01
24	9.20	410	1000	365	420	0.01
25	9.40	410	1000	365	420	0.01
26	9.60	410	1000	365	420	0.01
27	9.80	410	1000	365	420	0.01
28	10.00	410	1000	365	420	0.01
29	10.20	410	1000	365	420	0.01
30	10.40	410	1000	365	420	0.01
31	10.60	410	1000	365	420	0.01
32	10.80	410	1000	365	420	0.01
33	11.00	410	1000	365	420	0.01
34	11.20	410	1000	365	420	0.01
35	11.40	410	1000	365	420	0.01
36	11.60	410	1000	365	420	0.01
37	11.80	410	1000	365	420	0.01
38	12.00	410	1000	365	420	0.01
39	12.20	410	1000	365	420	0.01
40	12.40	410	1000	365	420	0.01
41	12.60	410	1000	365	420	0.01
42	12.80	410	1000	365	420	0.01
43	13.00	410	1000	365	420	0.01
44	13.20	410	1000	365	420	0.01
45	13.40	410	1000	365	420	0.01
46	13.60	410	1000	365	420	0.01
47	13.80	410	1000	365	420	0.01
48	14.00	410	1000	365	420	0.01
49	14.20	410	1000	365	420	0.01
50	14.40	410	1000	365	420	0.01
51	14.60	410	1000	365	420	0.01
52	14.80	410	1000	365	420	0.01
53	15.00	410	1000	365	420	0.01
54	15.20	410	1000	365	420	0.01
55	15.40	410	1000	365	420	0.01
56	15.60	410	1000	365	420	0.01
57	15.80	410	1000	365	420	0.01
58	16.00	410	1000	365	420	0.01
59	16.20	410	1000	365	420	0.01
60	16.40	410	1000	365	420	0.01
61	16.60	410	1000	365	420	0.01
62	16.80	410	1000	365	420	0.01
63	17.00	410	1000	365	420	0.01
64	17.20	410	1000	365	420	0.01
65	17.40	410	1000	365	420	0.01
66	17.60	410	1000	365	420	0.01
67	17.80	410	1000	365	420	0.01
68	18.00	410	1000	365	420	0.01
69	18.20	410	1000	365	420	0.01
70	18.40	410	1000	365	420	0.01
71	18.60	410	1000	365	420	0.01
72	18.80	410	1000	365	420	0.01
73	19.00	410	1000	365	420	0.01
74	19.20	410	1000	365	420	0.01
75	19.40	410	1000	365	420	0.01
76	19.60	410	1000	365	420	0.01
77	19.80	410	1000	365	420	0.01
78	20.00	410	1000	365	420	0.01
79	20.20	410	1000	365	420	0.01
80	20.40	410	1000	365	420	0.01
81	20.60	410	1000	365	420	0.01
82	20.80	410	1000	365	420	0.01
83	21.00	410	1000	365	420	0.01
84	21.20	410	1000	365	420	0.01
85	21.40	410	1000	365	420	0.01
86	21.60	410	1000	365	420	0.01
87	21.80	410	1000	365	420	0.01
88	22.00	410	1000	365	420	0.01
89	22.20	410	1000	365	420	0.01
90	22.40	410	1000	365	420	0.01
91	22.60	410	1000	365	420	0.01
92	22.80	410	1000	365	420	0.01
93	23.00	410	1000	365	420	0.01
94	23.20	410	1000	365	420	0.01
95	23.40	410	1000	365	420	0.01
96	23.60	410	1000	365	420	0.01
97	23.80	410	1000	365	420	0.01
98	24.00	410	1000	365	420	0.01
99	24.20	410	1000	365	420	0.01
100	24.40	410	1000	365	420	0.01

Žluté pI markery



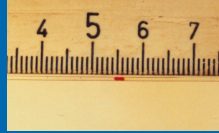
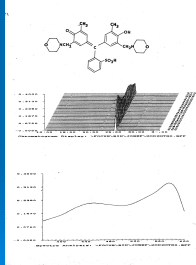
? Barevné pI markery ?



Chemie → nízkomolekulární barevné pI markery



Příklad barevného pI markeru

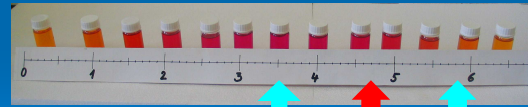
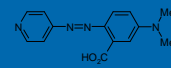


9/21/2009

IEF

19/60

Spektrofotometrické určení pI



$pK_1 = 3.50 \pm 0.02$

$pI = 4.71 \pm 0.02$

$pK_2 = 5.92 \pm 0.02$

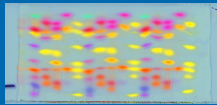
9/21/2009

IEF

20/60

Určení pI interpolací v gelové IEF

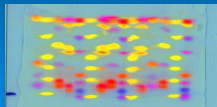
Gradient pH



Směs 30 jednoduchých pufrů



Biolyt 3 - 10

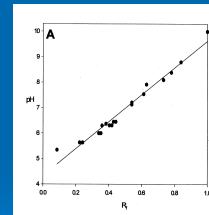


9/21/2009

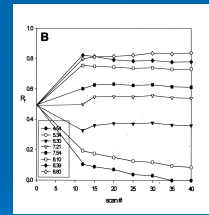
IEF

21/60

Dynamika pH gradientu Biolyt 3-10



Lineární gradient pH 4 - 10



Po 1/2 hod malé změny pH gradientu

9/21/2009

IEF

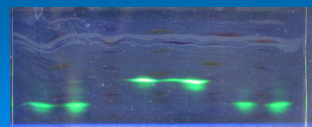
22/60

Vývoj fluorescenčních pI markerů

Vis



fluorescence



9/21/2009

IEF

23/60

colored markers

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% (w/v) hydroxyethyl disulfide, 0.2% (w/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

9/21/2009

IEF


24/60

7 cm IPG strip pH 3-10 NL



pI 3.3 - červená	1 µg
pI 4.3 - oranžová	3 µg
pI 5.3 - levandulová	3 µg
pI 5.7 - žlutá	5 µg
pI 6.2 - červená	3 µg
pI 7.6 - červená	3 µg
pI 9.0 - žlutá	5 µg

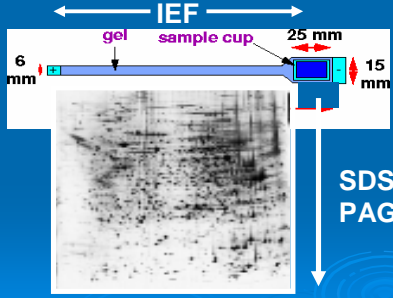
7 cm IPG strip pH 4-7



pI 4.3 - oranžová	3 µg
pI 5.3 - levandulová	3 µg
pI 5.7 - žlutá	5 µg
pI 6.2 - červená	3 µg

9/21/2009 IEF 25/60

2D Gel electrophoresis



IEF gel sample cup 25 mm 15 mm 6 mm

SDS PAGE

9/21/2009 IEF 26/60

2D - typical result – silver staining

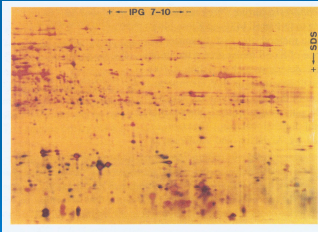


Fig 21: (A) High-resolution horizontal 2D electrophoresis using an immobilized pH-gradient in the first-dimensional run (IPG-Daily) [46]; basic proteins from yeast cell lysate (*Saccharomyces cerevisiae*). Silver staining according to Merrill [48]. By kind permission of Dr. A. Görg [49].

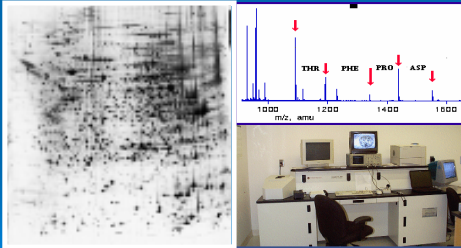
9/21/2009 IEF 27/60

2D Gel electrophoresis - Software



9/21/2009 IEF 28/60

Protein identification by 2D gel electrophoresis -MS

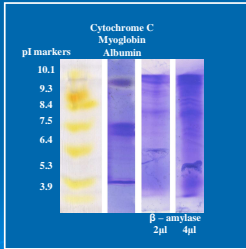


THOR, PHE, PRO, ASP

9/21/2009 IEF 29/60

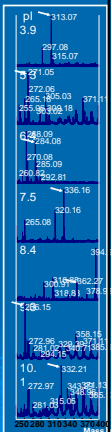
LOW-MOLECULAR MASS pI MARKERS IN COMBINATION OF GEL IEF WITH MALDI-TOF MS

pI	Molecular Weight	Molecular Mass	Significant Substrates	Structure
10.1	14,000	14,000	14 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>
9.3	12,000	12,000	12 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>
8.4	10,000	10,000	10 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>
7.5	8,000	8,000	8 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>
6.4	6,000	6,000	6 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>
5.3	4,000	4,000	4 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>
3.9	2,000	2,000	2 kDa	<chem>C1=CC=C(C=C1)C(=O)N</chem>



Cytochrome C
Myoglobin
Albumin

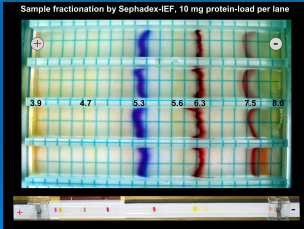
β - amylase
2µl 4µl



9/21/2009 IEF

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

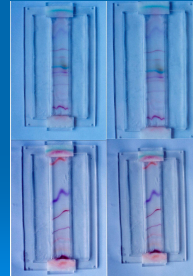
9/21/2009

IEF

33/60

IEF in Sephadex gels and IPG strips

Hodný Z., Pridalová J., Institute of Experimental Medicine AV ČR, v.v.i., Prague



Courtesy of Z. Hodný



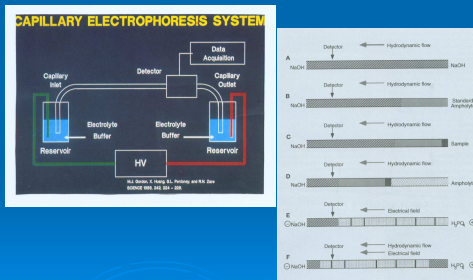
pI markers - LM ladder
Home made strip,
linear gradient pH 4-10,
11cm,
1 min 30V,
50 min 30V -> 3500V,
2 hours 3500V,
Courtesy of J. Pridalová

9/21/2009

IEF

32/60

Kapilární IEF



9/21/2009

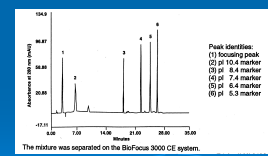
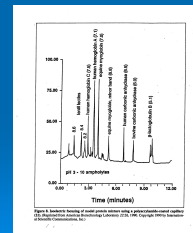
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33/60

Kapilární IEF standardů

proteiny

pI markery



Peak Identific:
(1) focusing peak
(2) pI 10.4 marker
(3) pI 8.6 marker
(4) pI 7.4 marker
(5) pI 6.3 marker
(6) pI 5.2 marker

The mixture was separated on the BioFocus 3000 CE system.

9/21/2009

IEF

34/60

Kapilární IEF s DAD detekcí

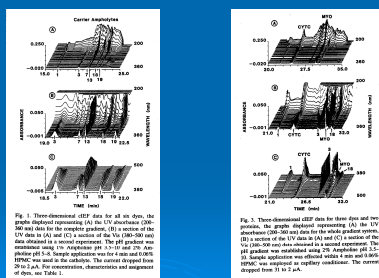


Fig. 1. Three-dimensional DAD data for all on-line, on-gel and off-gel samples. (A) The 100-1000 nm DAD data for the focusing peak, (B) a mixture of the 100-1000 nm DAD data for the focusing peak and the 100-1000 nm DAD data for the focusing peak, (C) a mixture of the 100-1000 nm DAD data for the focusing peak and the 100-1000 nm DAD data for the focusing peak. The pI markers are indicated by the arrows. The pI markers are indicated by the arrows. The pI markers are indicated by the arrows. The pI markers are indicated by the arrows.

9/21/2009

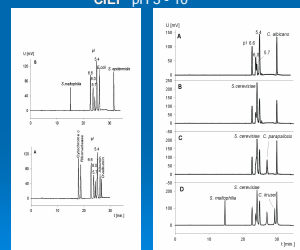
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35/60

Capillary isoelectric focusing and fluorometric detection of proteins and microorganisms dynamically modified by poly(ethylene glycol)

pyrenebutanoate
Horká, M., Růžička, F., Horký, J., Holá, V., Šlais, K.

Anal. Chem., 78 2006 8438-8444
CIEF pH 3 - 10



9/21/2009

IEF

36/60

Mikroorganismy (MO) = bakterie, kvasinky, viry, paraziti

Normální flóra každého jedince = bakterie, event. plísně, ale **nikdy** viry.

😊 Mikroorganismy, význam = výrobní prostředek v biotechnologických koloběh lártek, symbiotické organismy

☹️ Patogenní mikroorganismus = živé biologické agens schopné vyvolat masové infekční onemocnění nebo otravu lidí, zvířat či rostlin



9/21/2009



16.stol. - mor



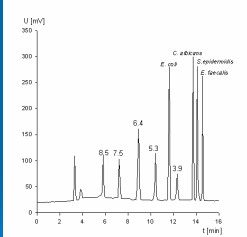
Robert Koch (1843-1908)




Mycobacterium tuberculosis


IEF 37/60

CIEF mikroorganismů



U [mV]
t [min]

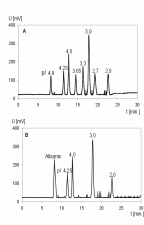




Sample: *E. coli*, *C. albicans*, *S. epidermidis*, *E. faecalis* in physiological saline solution, 4×10^8 cell ml⁻¹.

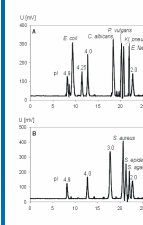
9/21/2009 IEF 38/60

280 nm, pH gradient 2 - 5



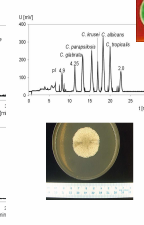
1

E. coli



2

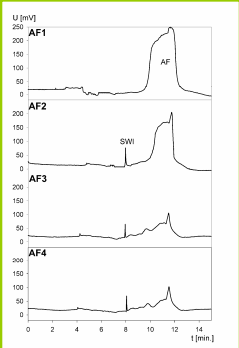
S. epidermidis




S. aureus

9/21/2009 IEF 39/60

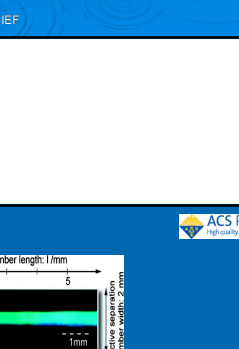
CIEF virů s UV detekcí



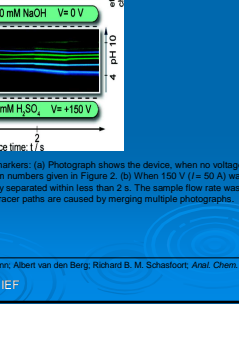
AF1



AF2



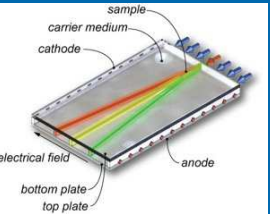
AF3



AF4

9/21/2009 IEF 40/60

Microfluidic Free-Flow Electrophoresis for Proteomics on a chip



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.

9/21/2009 IEF 41/60

Free-flow isoelectric focusing of 7 fluorescent IEF markers

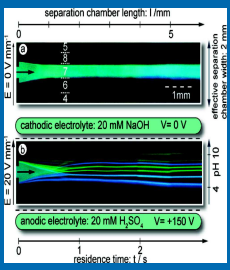


Figure 4 Free-flow isoelectric focusing of 7 fluorescent IEF markers: (a) Photograph shows the device, when no voltage was applied. The white numbers directly correspond to the stream numbers given in Figure 2. (b) When 150 V ($I = 50$ A) was applied, the markers (pI 4, 5.1, 6.2, 7.2, 8.1, 9, and 10.3) fully separated within less than 2 s. The sample flow rate was 0.4 L min⁻¹ ($v = 2$ mm s⁻¹) The apparent kinks in the fluorescent tracer paths are caused by merging multiple photographs.

Published in: Dietrich Kohlheyer, Jan C. T. Eijkel, Stefan Schaubmann, Albert van den Berg, Richard B. M. Schaafsma, *Anal. Chem.* 2007, 79, 8190-8198
DOI: 10.1021/ac071419b
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9/21/2009 IEF 42/60

Preparativní IEF proteinů

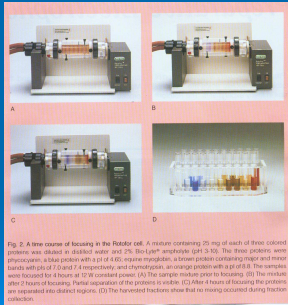


Fig. 2. A time course of focusing in the Rotofor cell. A mixture containing 25 mg of each of three colored proteins was added to distilled water and 2% (w/v) urea (pH = 4.55). The three proteins were phosphorylated in a basic buffer with a pH of 4.55, equine myoglobin, a bovine protein containing major and minor bands with pI of 3.9 and 5.3 respectively and phosphorylated salmon protein with pI of 5. The samples were focused for 3 hours at 20 kV constant power. (A) The sample mixture prior to focusing. (B) The mixture after 1 hour of focusing. (C) The separation of the proteins in order. (D) After 3 hours of focusing the proteins are separated into distinct regions. (E) The separated fractions show that no mixing occurred during fraction collection.

9/21/2009

IEF

43/60

Preparativní autofokuse peptidů



pI = 3.9

pI = 5.3

9/21/2009

IEF

44/60

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.

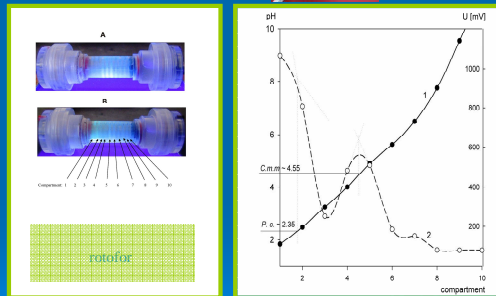
Abbreviation in Fig.	Strain	pI
C. michiganensis	Citrobacter michiganensis subsp. michiganensis CCM 1635	4.6
	Citrobacter michiganensis subsp. michiganensis VURV C254	4.6
	Citrobacter michiganensis subsp. michiganensis VURV 2459	4.7
	Citrobacter michiganensis subsp. michiganensis VURV 5390	4.6
	Citrobacter michiganensis subsp. michiganensis VURV 5395	4.7
	Citrobacter michiganensis subsp. michiganensis VURV 7036	4.6
C. michiganensis	Citrobacter michiganensis subsp. michiganensis VURV 7038	4.7
	Citrobacter michiganensis subsp. michiganensis VURV 7039	4.6
	Citrobacter michiganensis subsp. michiganensis VURV 7030	4.7
		pI = 4.7, RSD = 1.5%
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 2064-1	4.1
	Xanthomonas vesicatoria LMG 667	4.1
		pI = 4.1, RSD = 0.7%
P. syringae	Pseudomonas syringae pv. tomato CFBP 5432	4.0
	Pseudomonas syringae pv. tomato CFBP 2212	4.0
	Pseudomonas syringae pv. tomato IVA 1733.3	4.0
	P. syringae	4.0
		pI = 4.0, RSD = 1.9%
P. corrugata	Pseudomonas corrugata CFBP 4501	2.4
	Pseudomonas corrugata CFBP 5485	2.4
	Pseudomonas corrugata CFBP 6663	2.4
	Pseudomonas corrugata IVA 614-5.3	2.4
	P. corrugata	2.4
		pI = 2.4, RSD = 0.5%

9/21/2009

IEF

45/60

FF IEF

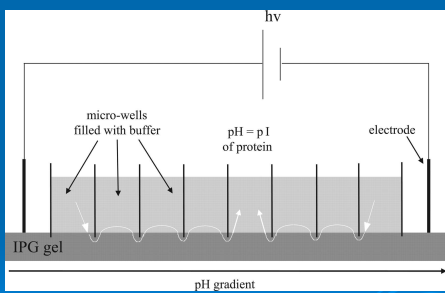


9/21/2009

IEF

46/60

Schematic presentation of the setup used for OFFGEL electrophoresis

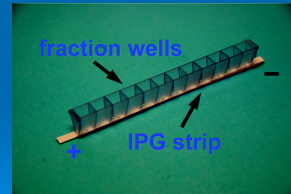


Horth, P. (2006) Mol. Cell. Proteomics 5: 1968-1974

9/21/2009

IEF

47/60



9/21/2009

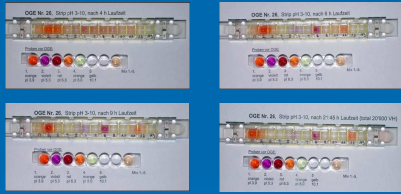
IEF

48/60

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

Heller, M.

DKF, University of Bern, Switzerland



- 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

9/21/2009

IEF

51/60

Preparativní free flow IEF

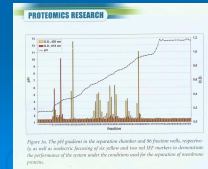
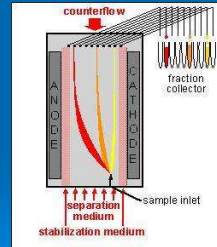


Figure 3. The pH profile in the separation medium and in fraction wells, respectively, as well as a schematic drawing of an off-line and free flow IEF method for preparative fractionation of the cytosolic fraction of the cytosolic fraction of a mammalian protein.

9/21/2009

IEF

50/60

IEF v rozbíhavém toku (divergent flow IEF, DF IEF)

Základní idea

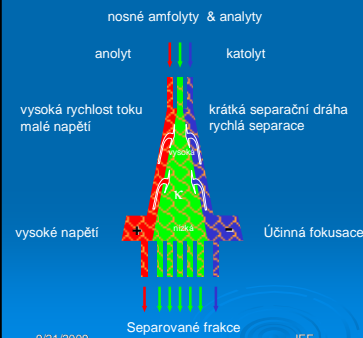
- Fluidika – kontinuální rozšiřování plochého kanálu při toku kapaliny od vstupu k výstupu při čemž je generován rozbíhavý tok
- a současně,
- IEF - malé příčné napětí na vstupu kanálu a vysoké příčné napětí na výstupu kanálu

9/21/2009

IEF

51/60

IEF v rozbíhavém toku



Fluidika – rozbíhavý tok

IEF – řízení elektrického proudu vodivostí kapaliny κ

Jednoduché zařízení:

Membrány eliminovány použitím porézního lože

Separační plocha a vstupy a výstupy kapaliny tvořeny netkanou textilií

Kontakty k elektrodám tvořeny netkanou textilií

Tok generovaný hydrostaticky

9/21/2009

IEF

52/60

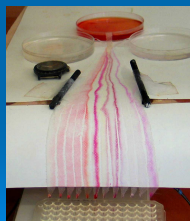
Divergent flow IEF

K. Slais

Electrophoresis 2008

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

9/21/2009

IEF

53/60

Dynamics of divergent flow IEF

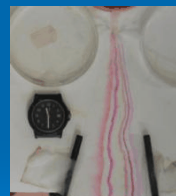
K. Slais

Electrophoresis 2008

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 H₂PO₄, 5.2 mS/cm, 1 mL/h
Catholyte: 0.05 M NaOH, 11 mS/cm, 1 mL/h
Carriers and pI markers: 0.75 mS/cm, 4 mL/h,

Holdup volume: 1 ml
Separation area: 71 cm²

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

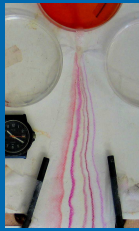
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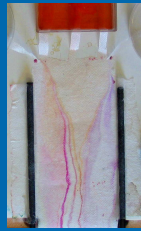
54/60

IEF v rozbíhavém toku

IEF v rovnoběžném toku



730 V



380 V

Materiál separačního prostoru, délka 15 cm, výstupní šířka 8 cm, výkon 1 W
Pracovní roztoky
Výstupní tok 6 mL/h = 0.9 cm/min
jsou **stejně** u obou geometrií

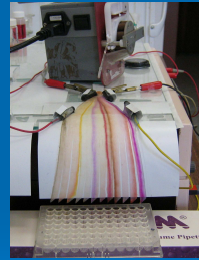
Šlais K. Electrophoresis 29 2008 2451-2457

9/21/2009

IEF

55/60

IEF v rozbíhavém toku s jedním vstupem



Linie červených pl markerů a proteinů od leva
oranžová - methyl oranž,
levandulová - marker pI 5.2
červená - marker pI 6.2
hnědá - hemoglobin, 0.5 mg/ml
čihlová - cytochrom C, 0.5 mg/ml
fialová - marker pI 11



Vstupní roztok s pl markery a proteiny má vodivost 1.05 mS.cm⁻¹.
průtok 0.18 mL.min⁻¹
Vstupní elektrody - 75 V, 1.5 mA
Výstupní elektrody - 380 V, 1. mA

Štátná M., Šlais K., Electrophoresis, přijato -00293

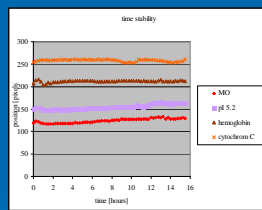
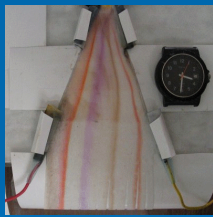
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56/60

IEF v rozbíhavém toku s jedním vstupem

Časová stabilita polohy linií



Linie od leva
oranžová - methyl oranž, levandulová - marker pI 5.2
hnědá - hemoglobin, 0.5 mg/ml, čihlová - cytochrom
C, 0.5 mg/ml, průtok 0.18 mL.min⁻¹

Kolísání linií 3.96 %, 3.94 %, 1.26 % a 1.88 %

Štátná M., Šlais K., Electrophoresis, přijato -00293

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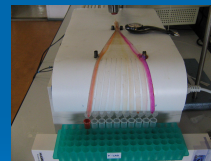
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57/60

Použití barevných pl markerů při preparativní SI DF IEF aplikované na extrakt ječmene

Kontinuálně dávkované pl markery:
oranžová - MO,
fialová - marker pI 11

1 kapka roztoku směsi pl markerů LM



Vstup - Extrakt ječmene (neodsolený) + pufrý + MO + marker pI 11
průtok - 0.23 ml/min,
vodivost - 1.0 mS/cm
Vstupní elektrody: 4 mA, 20 V
Výstupní elektrody: 6 mA, 800 V

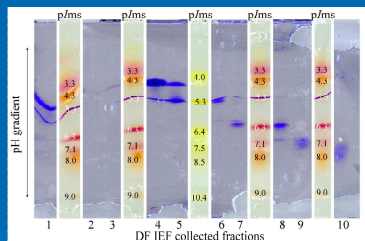
Mazanec K., Bobálová J., Šlais K., Anal. Bioanal. Chem, odesláno

9/21/2009

IEF

58/60

Kombinovaný sken IEF gelu frakcí z DF IEF piva



Barevné pl markery skenované ihned po gel IEF
Proteiny skenované po vybarvení Comassie

Mazanec K., Bobálová J., Šlais K., Anal. Bioanal. Chem, odesláno

9/21/2009

IEF

59/60



9/21/2009

IEF

60/60