Trends in analytical chemistry

Application of membrane-based pre-separation techniques in analysis of environmental, biological and clinical samples

Lecture for students of MU in Brno, 19.10.2017

Pavel Kubáň (kuban@iach.cz)

Department of Electromigration Methods

Institute of Analytical Chemistry of the Czech Academy of Sciences

- Complex samples and their pretreatment
- Membrane techniques
- Electrically induced transfer of ions across membranes
- Applications
- Coupling to standard analytical instrumentation
- Conclusions and future perspective

Pretreatment of complex samples

Clean-up

- **Matrix effects**
- **High concentrations of proteins and salts**

↓

- **Deteriorated performance**
- **Analytical system poisoning**

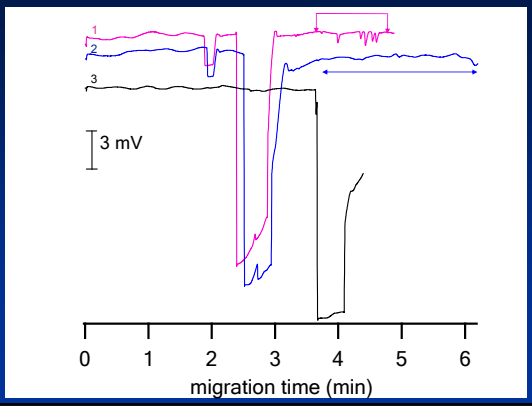
Preconcentration

- **Low concentrations of analytes**

↓

- **Analytes not detected**
- **Poor quantitative results**

Human plasma 1:1, essential amino acids



Standard methods for pretreatment of complex samples

Liquid-liquid extraction (LLE)

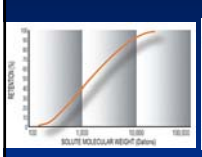
Solid phase extraction (SPE)

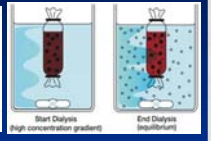
☺ Automation (SPE)

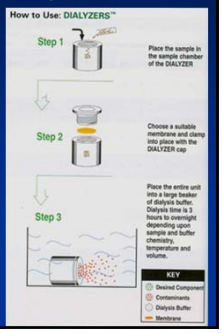
☹ High consumption of organic solvents and complex samples
Time consuming
Costly
Additional instrumental equipment

Membrane techniques for pretreatment of complex samples

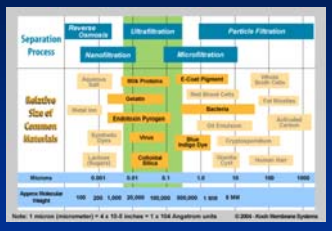
Dialysis (MWCO membranes, hollow fibers)

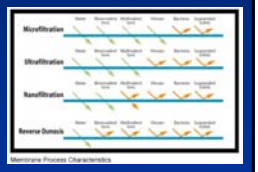






Ultrafiltration (flat sheet membranes, hollow fibers)

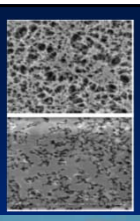


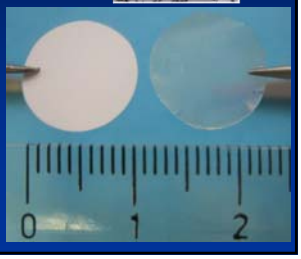


7

Supported liquid membrane (SLM)

Support – porous PP, PTFE (thickness 25 – 300 μm)

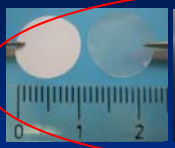


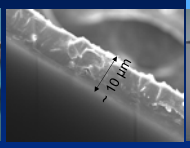


8

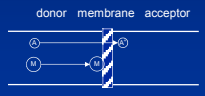
Liquid phase microextraction

Phase interfaces – membranes

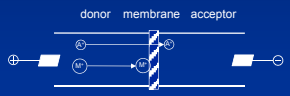




Diffusion



Electric potential



9

Electric potential in sample treatment

- Short extraction times
- High extraction efficiencies
- High selectivity
- Simple instrumentation

- Membrane selection ?
- Electrode reactions ?
- High electric current → collapse of system!

10

Electromembrane extraction – EME

- LLE → large volumes of organic solvents !
- 1996 – LPME (μL volumes of organics)

Liu and Dasgupta, Anal. Chem. 68 (1996) 1817-1821

Jeannot and Cantwell, Anal. Chem. 68 (1996) 2236-2240

- Stability of organic phase !
- 1999 – HF-LPME (inert porous polypropylene hollow fibre)

Pedersen-Bjergaard and Rasmussen, Anal. Chem. 71 (1999) 2650-2656

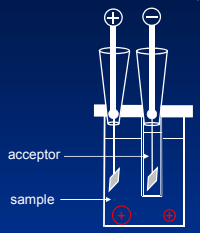
- Long extraction times !
- 2006 – EME (short extraction times due to use of DC voltage)

Pedersen-Bjergaard and Rasmussen, J. Chromatogr. A 1109 (2006) 183-190

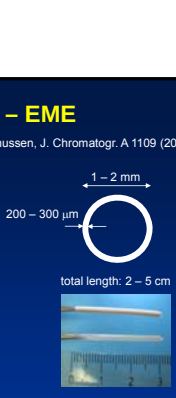
11

Electromembrane extraction – EME

Pedersen-Bjergaard and Rasmussen, J. Chromatogr. A 1109 (2006) 183



Hollow fiber:



- Hollow fiber impregnated with organic solvent (~ 10 μL) – SLM
- Cheap disposable extraction units (< 10 h/cm), no carry-over
- DC voltage source (0 – 400 V)
- Donor (~ mL) and acceptor (~ 20 μL) are aqueous

12

Parameters affecting EME

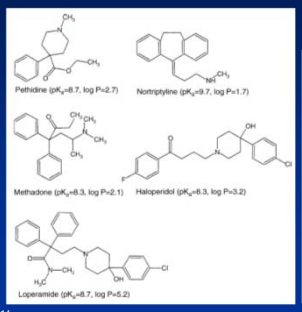
- Composition of liquid membrane
- pH a composition of acceptor and donor
- Electric potential / current

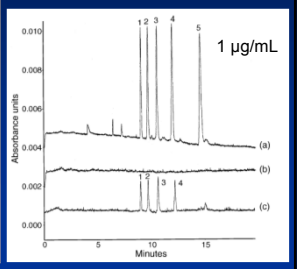
- Extraction time
- Stirring/agitation

13

EME of basic drugs – model example

- SLM – NPOE
- Acceptor – 10 mM HCl
- Donor – drugs in 10 mM HCl
- Extraction time – 5 min at 300 V





14

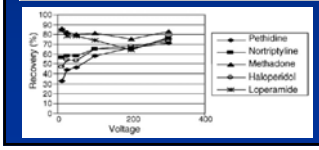
Pedersen-Bjergaard and Rasmussen J. Chromatogr. A 1109 (2006) 183

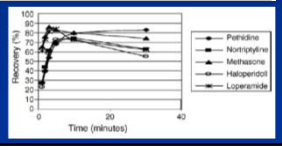
Recovery with different artificial liquid membranes

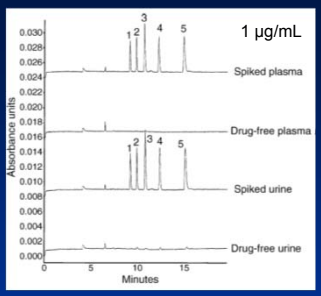
	Recovery (%)[a]				
	Pethidine	Nortriptyline	Methadone	Haloperidol	Loperamide
2-Nitrophenyl octyl ether	70	70	79	72	76
Dihexyl ether	nd	nd	nd	nd	nd
1-Octanol	3	4	7	3	7
2-Octanone	b	b	b	b	b
Dodecylacetate	nd	nd	nd	nd	nd
2-Nitrophenyl octyl ether + 5% di(2-ethylhexyl) phosphate	57	13	26	3	4
Kerosene	nd	nd	nd	nd	nd
Silicone oil AS 4	nd	nd	nd	nd	nd
Soy-bean oil	nd	nd	nd	nd	nd
Peppermint oil	13	73	73	78	79

Recovery with different acceptor solutions

	Recovery (%)[a]				
	Pethidine	Nortriptyline	Methadone	Haloperidol	Loperamide
100 mM HCl	60	40	83	72	80
10 mM HCl	70	70	79	72	76
1 mM HCl	44	27	31	23	22
10 mM HCOOH	56	70	63	52	61
10 mM phosphate pH 6.0	24	24	26	9	15
10 mM phosphate pH 7.0	19	8	9	nd	4
10 mM phosphate pH 8.0	nd	nd	nd	nd	nd

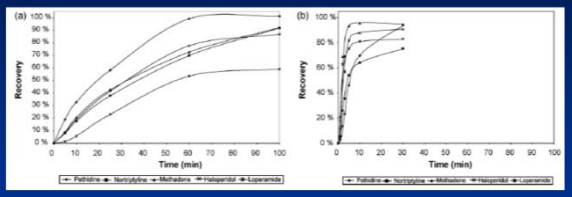






16

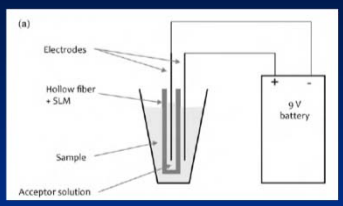
Comparison of LPME and EME of basic drugs

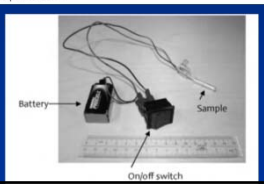


17

Gjelstad et al. J.Chromatogr. A 1157 (2007) 38

EME in portable format – use of 9 V battery



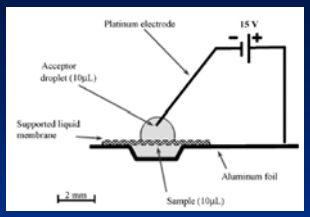


- SLM – ENB
- Acceptor – 10 mM HCl
- Donor – plasma, blood, urine in 10 mM HCl
- Extraction time – 5 min at 9 V

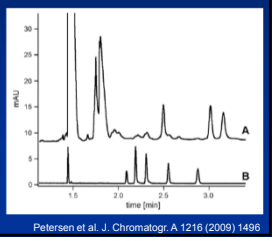
18

Eibak et al. J. Chromatogr. A 1217 (2010) 5050

Drop-to-drop EME



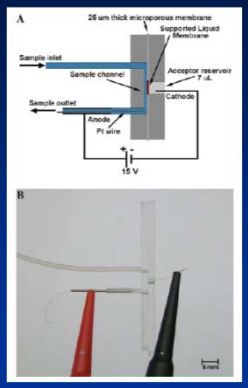
- SLM – NPOE
- Acceptor – 10 mM HCl
- Donor – plasma, urine in 10 mM HCl
- Extraction time – 5 min at 15 V

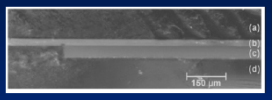


A – spiked urine no EME
B – spiked urine after EME

Petersen et al. J. Chromatogr. A 1216 (2009) 1496

Microchip EME

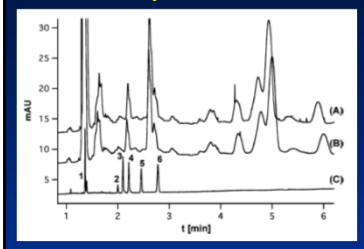




a) PMMA – top cover
b) PP membrane
c) 50 μm channel
d) PMMA – base plate

Petersen et al. Microfluid Nanofluid 9 (2010) 881

Microchip EME



- SLM – NPOE
- Acceptor – 10 mM HCl
- Donor – urine in 10 mM HCl
- Extraction time – 10 min at 15 V
- F_R donor = 3 μL/min

A – raw urine
B – urine spiked with drugs
C – urine spiked with drugs + EME

EME at constant electric current

1. Faraday's law $m = A \cdot I \cdot t$

A – electrochemical equivalent

2. Faraday's law $A = \frac{M_m}{F \cdot z}$

F = 96485 C mol^{-1}
z – charge number

$$n = \frac{I \cdot t}{F \cdot z}$$

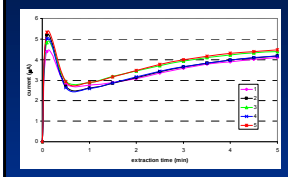
Ohm's law $I = \frac{U}{R}$

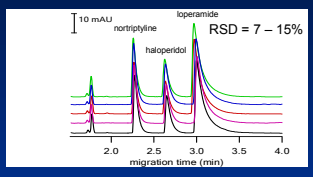
⇒ Poor repeatability of EME (RSD up to 30%)

EME of basic drugs

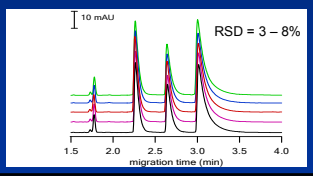
- SLM – ENB
- Acceptor – 10 mM HCl
- Donor – STD, urine in 10 mM HCl
- Extraction time – 5 min

Constant U (5 V)



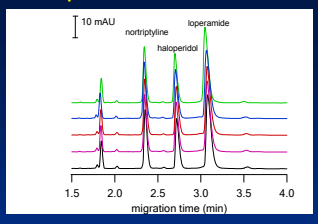


Constant i (4 μA)



CE-UV in 15 mM phosphate buffer (pH 2.9)

EME of spiked urine at constant electric current



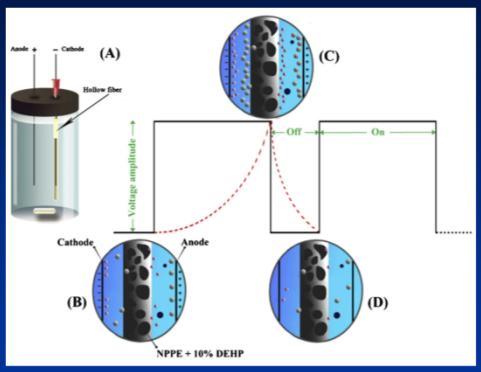
Constant U (5 V)
RSD = 6 – 12%

Constant i (4 μA)
RSD = 3 – 7%

- Improved repeatability
- Similar performance for other analytical parameters
  - Linearity
  - LOD
- Supplies operating at constant electric current are not cheap !

Šlampová et al. J. Chromatogr. A 1234 (2012) 32

Pulsed EME

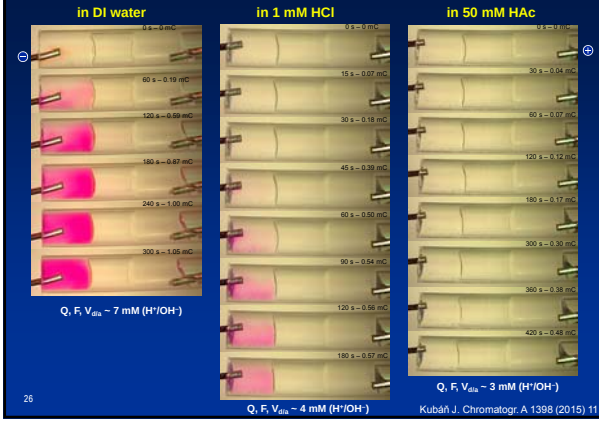


25

Rezazadeh et al. J. Chromatogr. A 1262 (2012) 214

EME and electrolysis

phenolphthalein, colorless → pink at pH 8 – 10



Q, F, V_{dia} ~ 7 mM (H^+/OH^-)

Q, F, V_{dia} ~ 4 mM (H^+/OH^-)

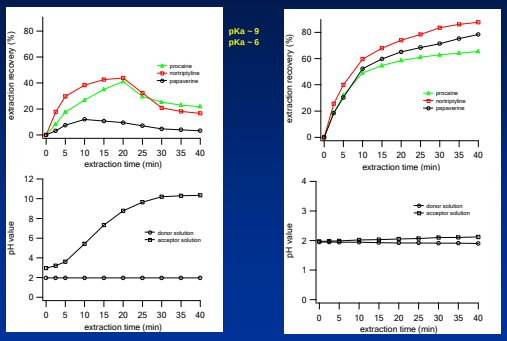
Q, F, V_{dia} ~ 3 mM (H^+/OH^-)

26

Kubáň J. Chromatogr. A 1398 (2015) 11

Non-optimized acceptor 1 mM HCl

Optimized acceptor 500 mM formic acid



27

Šlampová et al. Anal. Chim. Acta 887 (2015) 92

Selected applications of EME in analysis of biological, environmental and other complex samples

28

EME of drugs used for treatment of alcohol and opiates abuse in biological fluids

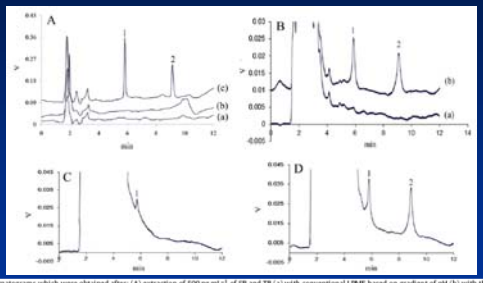


Fig. 3. Chromatograms which were obtained after: (A) extraction of 500 ng mL^{-1} of SB and TB (a) with conventional LPME based on gradient of pH (b) with the extraction conditions same to EME method but in the absence of electrical field (c) with a 100 V electrical potential difference, (B) EME of (a) nonspiked plasma sample, (b) plasma sample spiked at a concentration level of 60 ng mL^{-1} of the drugs. (C) EME of nonspiked urine sample, (D) EME of urine sample spiked at a concentration level of 60 ng mL^{-1} of the drugs. 1: naltrexone, 2: nalmefene.

29

Rezazadeh et al. J.Chromatogr. B 879 (2011) 1143

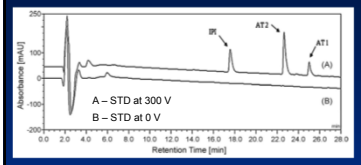
- SLM –NPOE/DEHP
- Acceptor – 100 mM HCl
- Donor – plasma and urine in 10 mM HCl
- Extraction time – 20 min at 100 V

Sample	Analyte	C_{spik} (ng mL^{-1})	C_{added} (ng mL^{-1})	C_{found} (ng mL^{-1})	RSD% (n = 3)	RR%	Error%
Plasma 1	Nalt	nd[a]	80.0	81.6	5.1	102	+2
	Nalm	nd	80.0	76.3	2.6	95	−5
Plasma 2	Nalt	nd	80.0	84.0	6.2	105	+5
	Nalm	nd	80.0	77.3	3.4	97	−3
Urine 1	Nalt	22.8	60.0	80.4	7.0	96	−4
	Nalm	nd	60.0	57.5	5.3	96	−4
Urine 2	Nalt	nd	60.0	62.4	4.7	104	+4
	Nalm	nd	60.0	61.7	3.1	103	+3
Urine 3	Nalt	20.0	60.0	77.2	6.5	95	−5
	Nalm	nd	60.0	59.0	4.5	98	−2

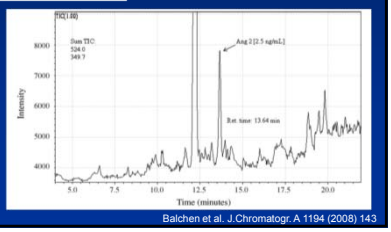
[a] Not detected.

30

EME of peptides in biological fluids



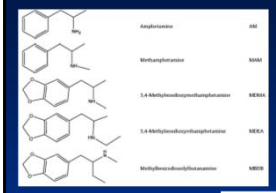
- SLM – 1-octanol/DEHP
- Acceptor – 0.1 M HCl
- Donor – plasma in 0.1 M HCl
- Extraction time – 5 min at 50 V



31

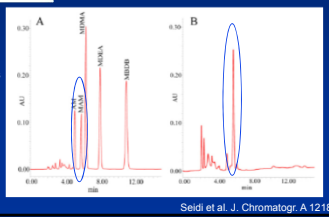
Balchen et al. J.Chromatogr. A 1194 (2008) 143

EME of amphetamines in biological fluids



- SLM – NPOE/TEHP
- Acceptor – 100 mM HCl
- Donor – urine in 1 mM HCl
- Extraction time – 7 min at 250 V

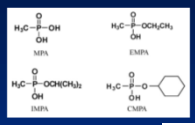
A – urine spiked with amphetamines
B – urine of amphetamine user



32

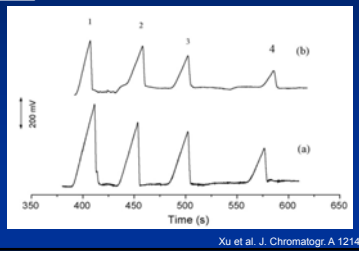
Seidi et al. J. Chromatogr. A 1218 (2011) 3958

EME of decomposition products of nerve agents in environmental samples



- SLM – 1-octanol
- Acceptor – DI water
- Donor – samples in DI water
- Extraction time – 30 min at 300 V
- Real samples – environmental waters

(a) – STD
(b) – river water spiked with phosphonic acids

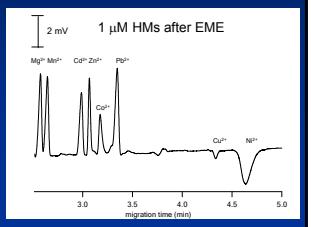


33

Xu et al. J. Chromatogr. A 1214 (2008) 17

EME of heavy metals in water samples and food supplements

- SLM – 1-octanol/DEHP
- Acceptor – 100 mM HAc
- Donor – samples in DI
- Extraction time – 5 min at 75 V
- Real samples – tap water, infant food supplements

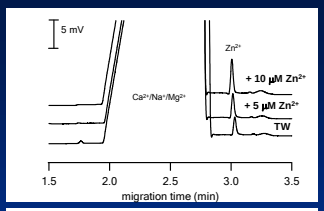


34

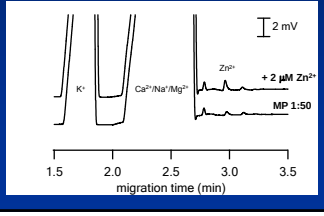
Kubáň et al. Electrophoresis 32 (2011) 1025

EME and CE-C^{4}D of zinc

Tap water



Milk powder



35

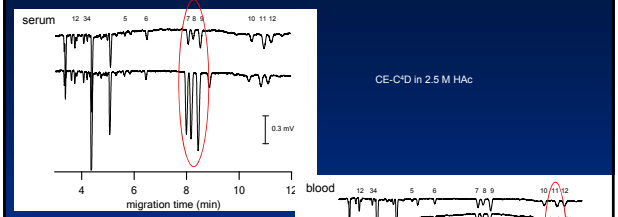
EME of amino acids in biological fluids

- Endogenous concentrations ~ 100 μM
- Metabolic disorders concentration (MSUD ~ 500 μM)
  (PKU ~ 350 – 1500 μM)

- SLM – ENB/DEHP
- Acceptor – 2.5 M HAc
- Donor – serum, plasma, blood, urine in 2.5 M HAc
- Extraction time – 10 min at 50 V

36

EME of amino acids in biological fluids



MSUD – branched amino acids (Val, Leu, Ile)

PKU – phenylalanine (Phe)

37

Strieglerová et al. J. Chromatogr. A 1218 (2011) 6248

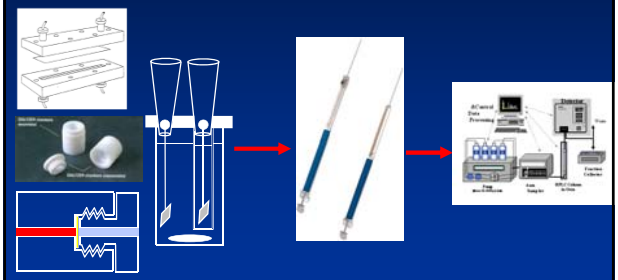
EME summary

- Clean-up and preconcentration in one step
- ~ 10 μL of organic solvent/analysis
- Disposable extraction units
- Short extraction times
- High selectivity of SLM
- Suitable for biological samples
- SLM selection !
- EME parameters !

38

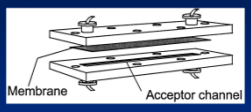
Coupling of membrane extraction techniques with state-of-the-art analytical instrumentation

Extraction and subsequent analysis off-line

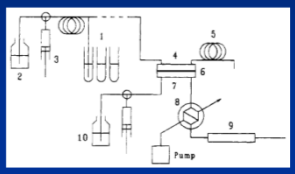


39

1. On-line coupling of SLM to HPLC



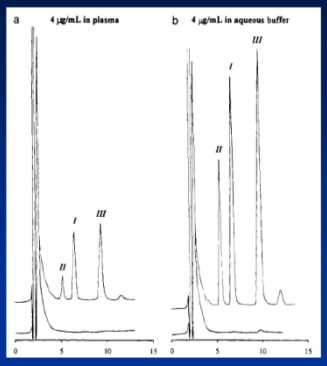
Acceptor channel volume ~ 10 μL



48

Lindegard et al. Anal. Chem. 66 (1994) 4490

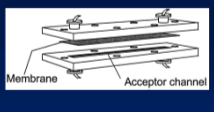
Determination of basic drugs in plasma

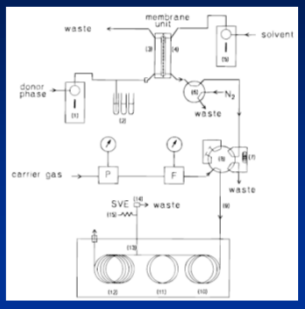


I – Amperozide
II – Amperozide metabolite
III – unknown compound

41

2. On-line coupling of SLM to GC





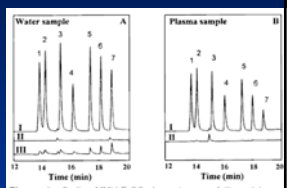
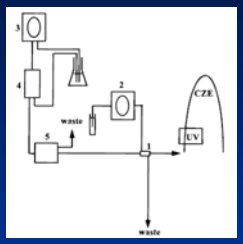


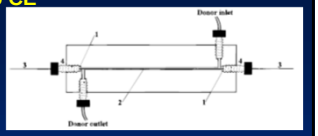
Figure 4. On-line MMLLE-GC chromatogram of (I) model com- pounds, (II) subsequent blank, and (III) memory effect due to adsorption in acceptor, from (A) water sample and (B) blood plasma sample. Peak identities: (1) prilocaine, (2) lidocaine, (3) mesocaine, (4) mepivacaine, (5) ropivacaine, (6) bupivacaine, and (7) pentycaine

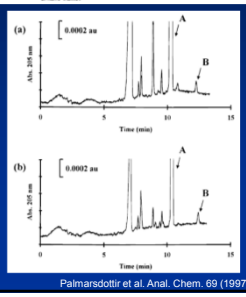
42

Shen et al. Anal. Chem. 70 (1998) 946

3. On-line coupling of SLM to CE





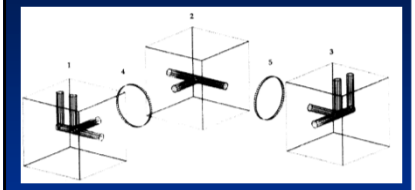


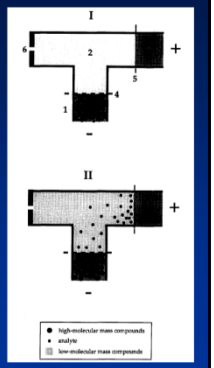
(a) – STD
(b) – plasma
A – IS
B – bambuterol

43

Palmarsdottir et al. Anal. Chem. 69 (1997) 1732

4. On-line coupling of ED to CE

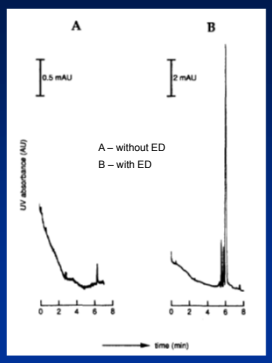


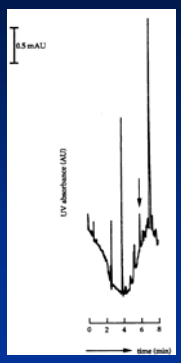


44

Buscher et al. J. Chromatogr. A 788 (1997) 165

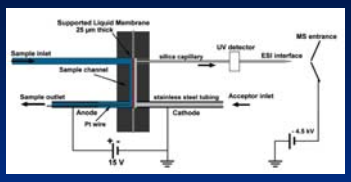
On-line coupling of ED to CE – inositol triphosphates analysis





45

5. On-line microchip EME

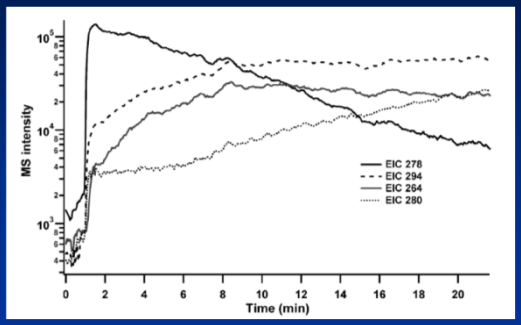


- SLM – 0.2 μL NPOE
- Acceptor – 100 mM HCOOH
- Donor – urine in 10 mM HCl
- Extraction time – 10 min at 15 V
- F_R donor = 9 μL/min
- F_R acceptor = 0 – 3 μL/min

46

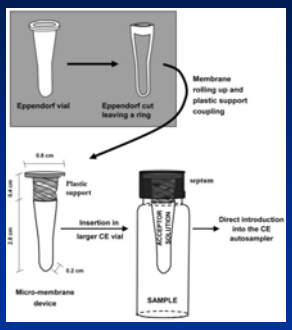
Petersen et al. Anal. Chem. (2011) 44

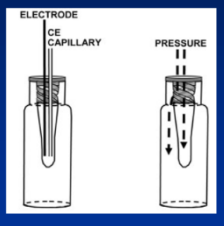
On-line monitoring of amitriptyline metabolism



47

6. On-line coupling of SLM to commercial CE – Beckman

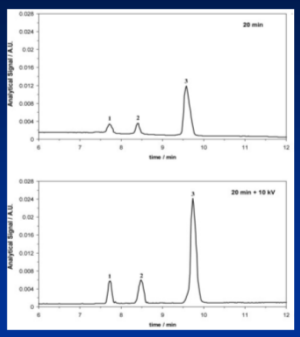


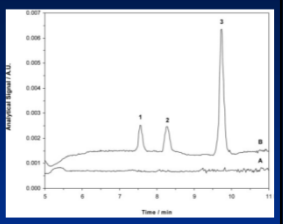


48

Nozal et al. Electrophoresis 27 (2006) 3075

Determination of nitroimidazoles in liver



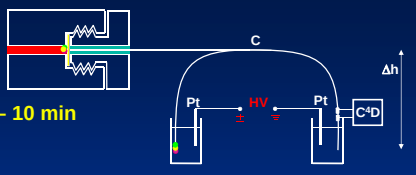


1 – metronidazole
2 – ronidazole
3 – dimetridazole

A – sample
B – spiked sample

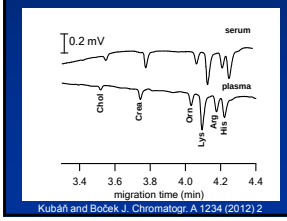
49

7. In-line coupling of disposable SLM to lab-made CE

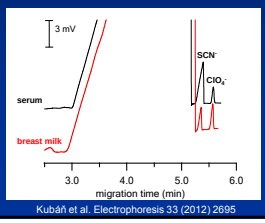


Extraction ~ 0 – 10 min

Applications

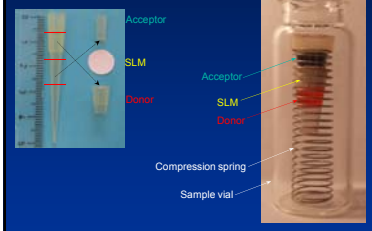


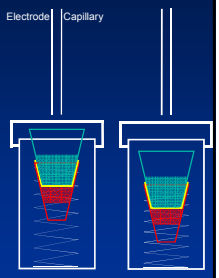
Kubáň and Boček J. Chromatogr. A 1234 (2012) 2



Kubáň et al. Electrophoresis 33 (2012) 2695

8. In-line coupling of disposable SLM devices to commercial CE



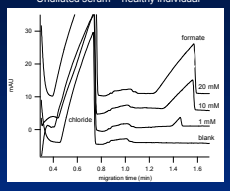


- SLM ~ 5 µL of organic solvent
- Sample volume – 10 – 40 µL
- Disposable extraction devices !!!

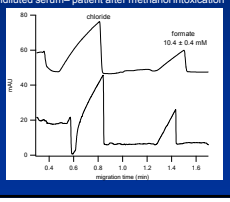
51

Formate in human serum and whole blood

Undiluted serum – healthy individual



Undiluted serum– patient after methanol intoxication



Endogenous concentrations: 0 – 0.4 mM
Methanol poisoning: 1 – 40 mM
> 10 mM serious health consequences

- Support: PP foil (100 µm)
- SLM: 10 µL MeOH
- Donor: undiluted serum, whole blood
- Acceptor: DI water
- Volume: 10 µL
- Extraction time: 60 s !!!

LOD: 30 µM undiluted serum,
35 µM undiluted whole blood

Total analysis time: ~ 4 min!!!

52

Pantůčková et al. J. Chromatogr. A 1299 (2013) 33

Polymer inclusion membranes (PIMs)

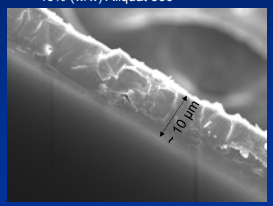
Base polymer – Cellulose triacetate (CTA)
Plasticizer – 2-nitrophenyloctyl ether
Ion carrier – Aliquat 336

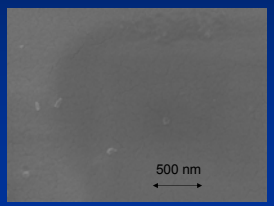
Schow et al. J. Membr. Sci. 111 (1996)

Dissolve in dichloromethane and evaporate

PIM – dry, homogenous, non-porous

Resulting PIM:
60% (w/w) CTA
40% (w/w) Aliquat 336

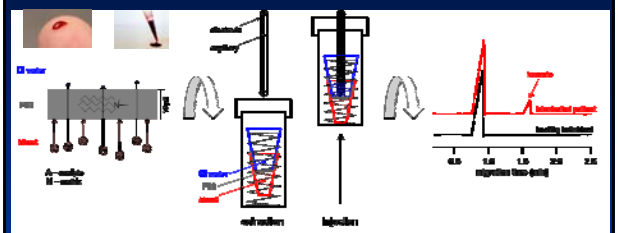




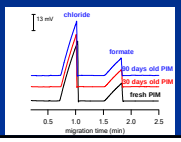
53

Formate in whole blood after methanol poisoning

PIM extraction | Coupling to CE | CE-C4D analysis of raw blood



Long-term performance of PIM



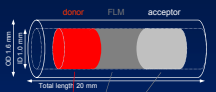
Total time ~ 7 min

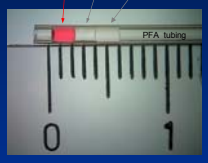
? **Pre-assembled disposable units for clinical applications**

54

Pantůčková et al. Anal. Chim. Acta 887 (2015) 111

10. Free liquid membranes (FLMs)

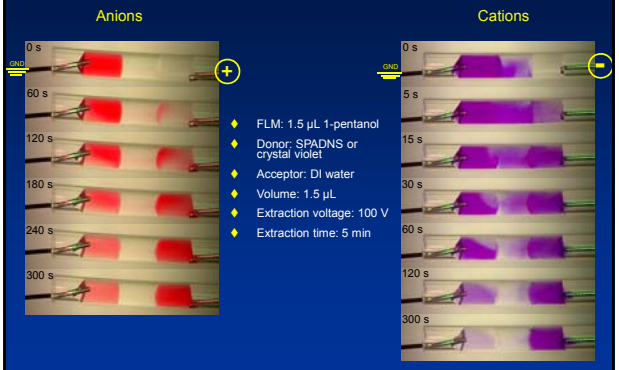




- Extraction units – PFA, PTFE, PP tubing (ID 0.5 – 1.0 mm)
- Minimum consumption of solvents/samples (350 nL – 1.5 µL/extraction)
- Cheap, disposable extraction units (~ 1 kč/cm), no sample carry-over
- Stable and precisely defined phase interfaces

55

µ-EME across FLM



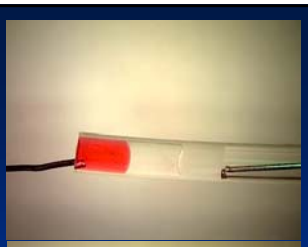
- FLM: 1.5 µL 1-pentanol
- Donor: SPADNS or crystal violet
- Acceptor: DI water
- Volume: 1.5 µL
- Extraction voltage: 100 V
- Extraction time: 5 min

56

µ-EME across FLM

µ-EME principle

Donor: $SPADNS^{3-}$ in DI water
Acceptor: DI water
FLM: 1-pentanol
Voltage: 100 V
Time: 3 min
Volumes: 1.5 µL

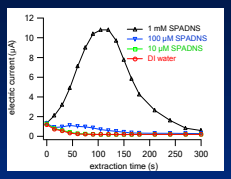


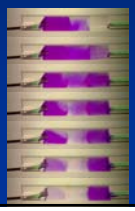
EME fundamentals

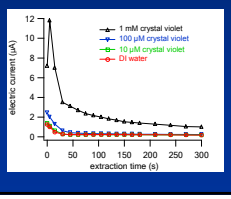
Donor: phenolphthalein in 1 mM HCl
Acceptor: DI water
FLM: 1-pentanol
Voltage: 100 V
Time: 3 min
Volumes: 1.5 µL

57

µ-EME process – electric currents monitoring

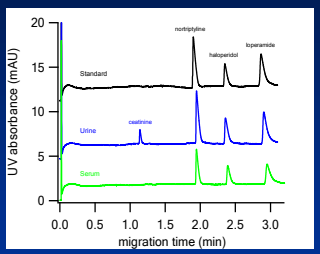






58

µ-EME of basic drugs across FLM



- FLM: 1.5 µL ENB
- Donor: urine and serum + 20 µg/mL drugs
- Acceptor: 10 mM HCl
- Volumes: 1.5 µL
- Voltage: 100 V
- Time: 5 min

59

CONCLUSIONS AND PERSPECTIVES

- Membrane extraction techniques are green, cheap, fast and efficient
- Electric potential is suitable for pretreatment of complex samples
- Resulting acceptors are compatible with standard analytical instruments
- On-line and in-line coupling to analytical instrumentation is very attractive

Ph.D. positions available

60