

# Electrospray Ionization Efficiency Scale of Organic Compounds

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## Outline

- ESI Ionization
  - Mechanism
  - Defining the problem
- Quantifying ESI ionization efficiency
  - Definition
  - Assumptions
  - Method
- ESI ionization efficiency Scale
  - Compounds and their IE-s
  - Consistency
- ESI ionization efficiency and molecular structure
- Still to be done

2

## ESI Ionization

- The most widely applied ionization method in MS
- Ionization via
  - Protonation
  - Deprotonation
  - Adduct formation
- Complex mechanism
  - Ion Evaporation Model (IEM)
  - Charged Residue Model (CRM)

3

## ESI in action (Positive ions)

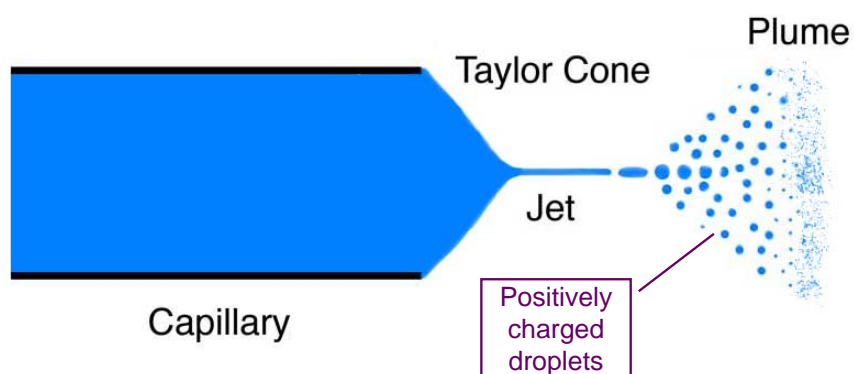
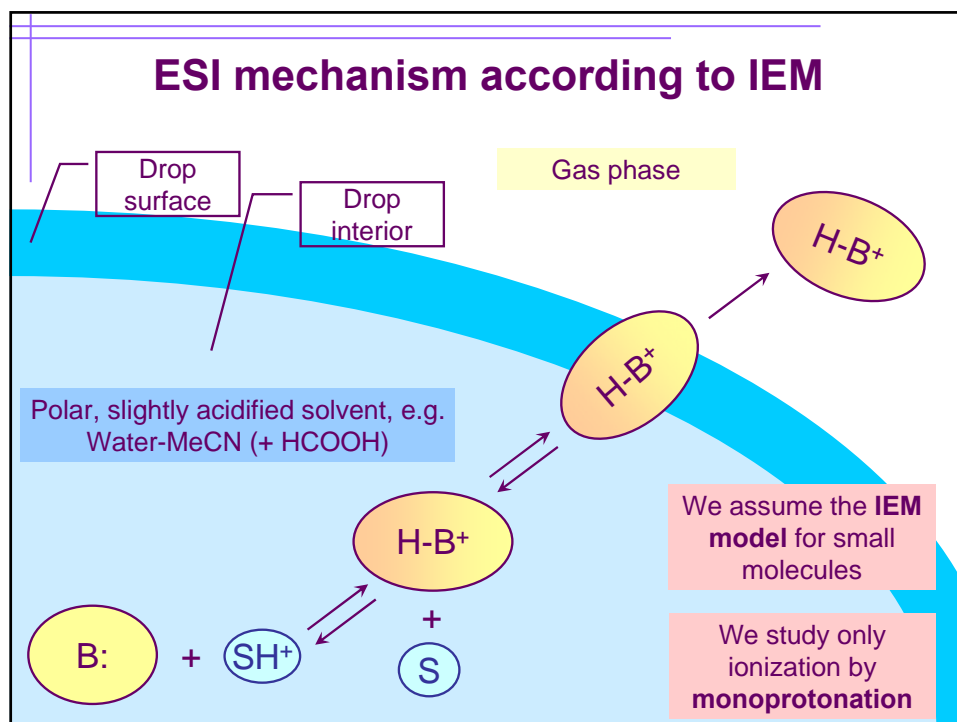


Image by K. K. Murray (via Wikipedia)

4



## Efficiency of ion formation in ESI

- **Not all** molecules in a droplet are converted to gas-phase ions
- Ionization efficiency: what proportion of the molecules (or ions) present in the sprayed solution are converted to gas-phase ions

## ESI Ionization Efficiency (IE)

- IE depends on
  - Molecular structure
  - Solvent
  - ESI and MS Conditions

**Different molecules have vastly differing ionization efficiencies in the ESI source**

**Being able to predict ionization efficiencies of molecules would be very useful**

7

## What determines ionization efficiency of a molecule?

- The main influence factors are known
  - Ionizability
  - Surface activity
  - ...

**However,  
there is still a long way to go to gain complete understanding**

8

## How to measure/quantify/express ionization efficiency of a molecule?

- For applying the scientific method to a phenomenon, its extent needs to be measured

**There is currently no generally accepted way to measure/quantify ESI IE**

9

## Goals

- Devise a parameter and measurement method that can be widely used for quantifying ESI IE
  - Measurable with routine MS equipment
- Determine this parameter for a wide selection of molecules of diverse structure under the same conditions
- Relate the ESI IE data to molecular structure
  - Ideally: predict ESI IE from structure

10

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11

## Defining ionization efficiency

- Fundamental:

$$IE_{\text{fundamental}} = \frac{n(\text{gas - phase ions})}{n(\text{solution - phase molecules})}$$

- $n$  in the gas phase is very difficult to determine

12

## Defining ionization efficiency

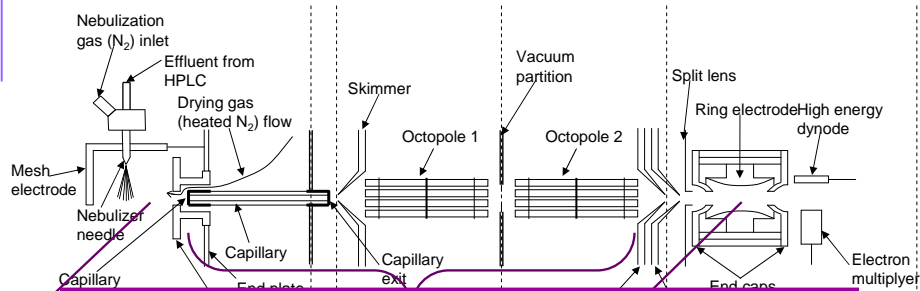
- Practical:

$$IE = \frac{R_{BH^+}}{C_B}$$

- $R_{BH^+}$  – MS Response of the  $BH^+$  ion in the mass spectrum
- $C_B$  – molar concentration of the base B in the mass spectrum

13

## Agilent XCT ion trap MS with ESI Source



**Conclusion:  $R$  does not measure just ESI IE but the efficiency of the whole system**

Not all ions reach the entrance

Ion losses during ion transport

Response depends on trapping efficiency

14

## Dependence of $R_{\text{BH}^+}$ on conditions

$$R_{\text{BH}^+} = Pf \frac{K_{\text{BH}^+} [\text{BH}^+]}{K_{\text{BH}^+} [\text{BH}^+] + K_{\text{E}^+} [\text{E}^+]} [Q]$$

- $K_{\text{BH}^+}$  partition coefficient of  $\text{BH}^+$  between drop surface and interior
- $[\text{BH}^+]$  equilibrium concentration of  $\text{BH}^+$  in the drop
- $K_{\text{E}^+}$  generalized partition coefficient of all other ions between drop surface and interior
- $[\text{E}^+]$  equilibrium concentration of all other ions in the drop
- $f$  the fraction of droplet charge that is converted into gas-phase ions
- $P$  "sampling efficiency" of the mass spectrometer
- $[Q]$  excess charge in the drop

C. Enke *Anal. Chem.* 1997, 69, 4885

15

## Assumptions (I)

- Drop surface and interior are two distinct phases
- Excess charge is on the drop surface
  - Drop interior is neutral
- Ion partition between these phases is rapid
- The amount of  $\text{BH}^+$  on the surface is small compared to the interior
- $R_{\text{BH}^+}$  is proportional to the amount of  $\text{BH}^+$  on the drop surface

C. Enke *Anal. Chem.* 1997, 69, 4885

16



## Two Compounds simultaneously

- If
  - the assumptions hold
  - And if two ions  $B_1H^+$  and  $B_2H^+$  are infused **in the same solution** at significantly lower concentration than buffer electrolytes
- then the following holds:

$$\frac{K_{B_1H^+}}{K_{B_2H^+}} = \frac{R_{B_1H^+} [B_2H^+]}{R_{B_2H^+} [B_1H^+]}$$

17

## Two Compounds simultaneously

- Considering that

$$\alpha_{B_1H^+} = \frac{[B_1H^+]}{C_{B_1H^+}} \quad \alpha_{B_2H^+} = \frac{[B_2H^+]}{C_{B_2H^+}}$$

- We get:

$$RIE(B_1, B_2) = \frac{IE(B_1)}{IE(B_2)} = \frac{K_{B_1H^+} \alpha_{B_1H^+}}{K_{B_2H^+} \alpha_{B_2H^+}} = \frac{R_{B_1H^+} C_{B_2}}{R_{B_2H^+} C_{B_1}}$$

18

## Relative Ionization Efficiency of B<sub>1</sub> and B<sub>2</sub>

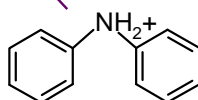
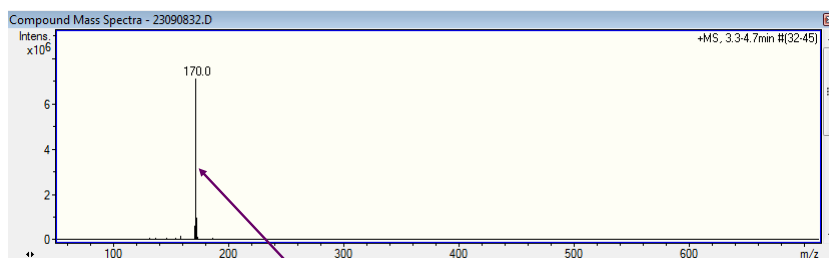
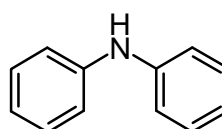
$$RIE(B_1, B_2) = \frac{IE(B_1)}{IE(B_2)} = \frac{R_{B_1H^+} C_{B_2}}{R_{B_2H^+} C_{B_1}}$$

- It is more convenient to use logRIE values
- Measured by infusion of a solution containing two analytes B<sub>1</sub> and B<sub>2</sub> into ESI MS
- Concentrations of B<sub>1</sub> and B<sub>2</sub> are known
- R are measured from mass spectra as peak heights (areas)

I. Leito et al *Rapid. Comm. MS* 2008, 22, 379

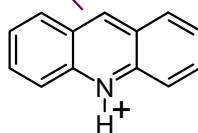
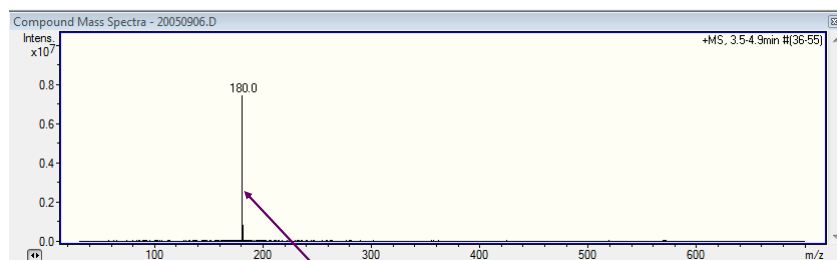
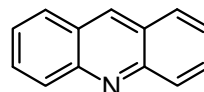
## Compound B<sub>1</sub>: Diphenylamine

- M = 169



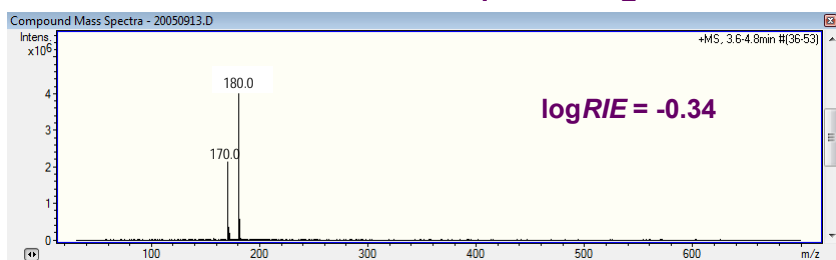
## Compound B<sub>2</sub>: Acridine

- M = 179



21

## Mixture of B<sub>1</sub> and B<sub>2</sub>



20050913			T=500	T=170	T=180	Isotopic correction
Compound	DPhA	akridiin	I	I	I	
C(ppm)	0.6	0.8				
M(g/mol)=	169.22	179.2173				
C(M)	3.018E-06	3.925E-06				
Infusion rate	0.3	0.2	170	1611978	2128693	2162743
C(M) in spray	1.811E-06	1.57E-06	180	3754692	3990029	4142303
		akridiin				
			180 corr	4332915	4604493	4780218
			sum corr	4332915	4604493	4780218
		DPhA				
			170 corr	1842491	2433096.1	2472015.2
			sum corr	1842491	2433096	2472015
		K_rie (frag)		0.369	0.458	0.448
		log(K_rie)		-0.433	-0.339	-0.348

22

## Assumptions (II)

- Linearity:  $R_{\text{BH}^+} \sim [\text{BH}^+]$ 
  - Holds if the concentration of  $\text{BH}^+$  in the drop interior is in the range of  $1 \cdot 10^{-6}$  M
- Compounds  $\text{B}_1$  and  $\text{B}_2$  do not suppress/enhance each other's ionization or do that in a proportional way
- Equal transmission efficiency and detector sensitivity for  $\text{B}_1\text{H}^+$  and  $\text{B}_2\text{H}^+$ 
  - Transmission efficiency is more important
  - In the used MS system transmission efficiency of different  $m/z$  ions can be tuned with a parameter *target mass*

I. Leito et al *Rapid. Comm. MS* 2008, 22, 379

## Advantages of relative measurement

- All experimental parameters (Numerous voltages in the MS, gas flow rates, temperatures, solution composition) are automatically the same for both compounds
  - Many of them difficult to control
- Ion losses **cancel** to a large extent
  - Similar transmission efficiencies

## Experimental details

- Solvent: MeCN : 0,1% HCOOH 80:20
- Concentrations:  $n \cdot 10^{-7}$  ...  $n \cdot 10^{-5}$  M
- For every concentration ratio ca 250 spectra were averaged
- Infusion rate: 0.5 ml/h
- Transmission efficiencies made as similar as possible
  - Using the *Target mass* parameter of the Agilent XCT IT MS
  - Other MS parameters at default values



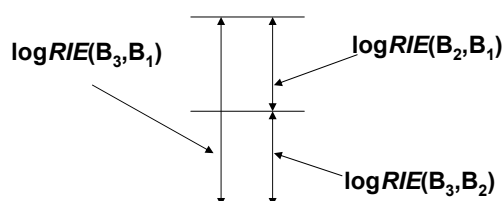
## QA of the results

- In all pairs parallel measurements were done with **different concentration ratios** usually differing by more than 10x
- Every measurement is **“circularly validated”** by at least one additional “path”
- A **“validation” pair** of compounds (DMS vs DMG) is measured every now and then to monitor the MS system

## Circular validation

$$\log RIE(B_1, B_2) = \log \frac{IE(B_1)}{IE(B_2)}$$

$$\log RIE(B_2, B_1) = \log RIE(B_3, B_1) - \log RIE(B_3, B_2)$$



27

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28

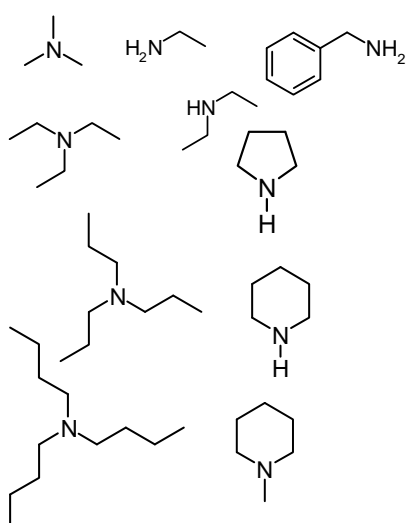
## Compounds

- **62 compounds** from the families of
  - Aliphatic and aromatic amines
  - Amidines, guanidines
  - Phosphazenes
  - tetraalkylammonium salts
  - Heterocycles
  - Amides, Esters, Acids
  - some others

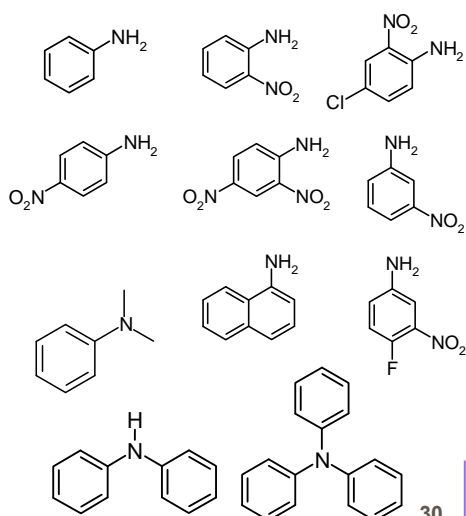
29

## Compounds (I)

### Aliphatic amines



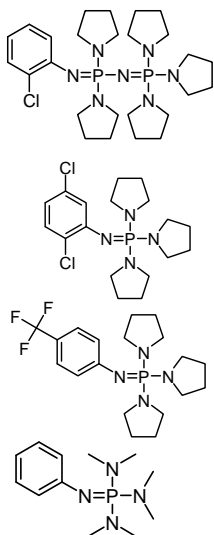
### Aromatic amines



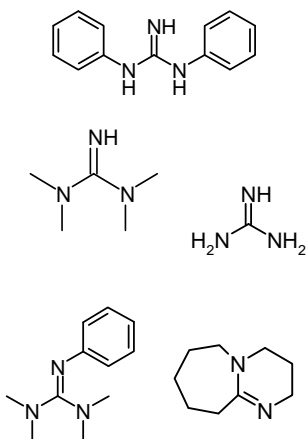
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## Compounds (II)

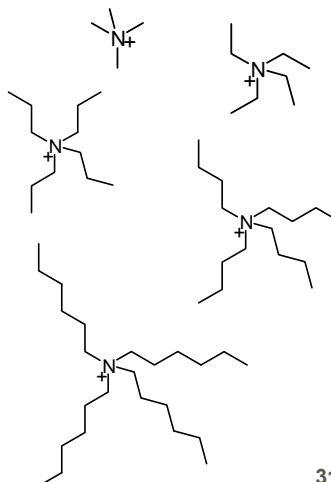
### Phosphazenes



### Guanidines, amidines



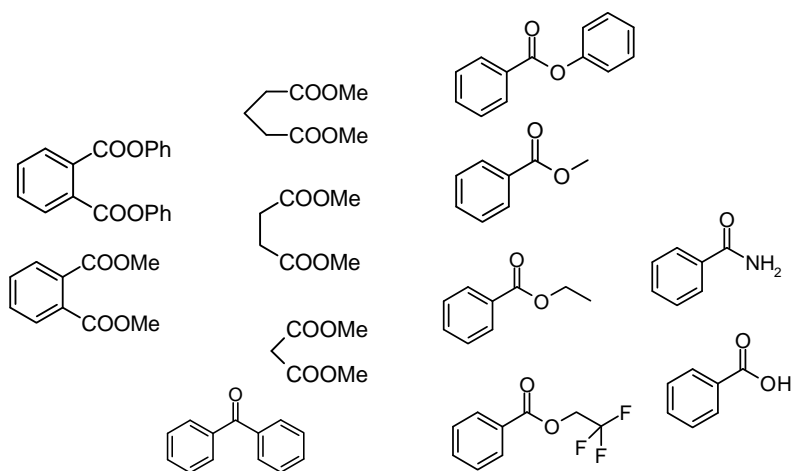
### Tetraalkylammonium



31

## Compounds (III)

### Carbonyl compounds

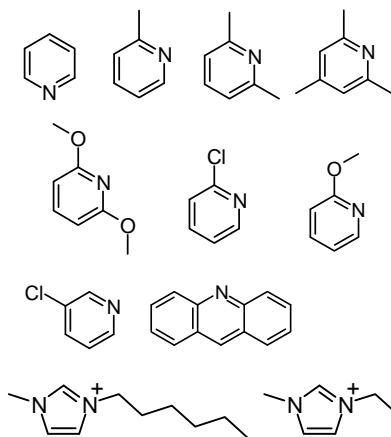


32

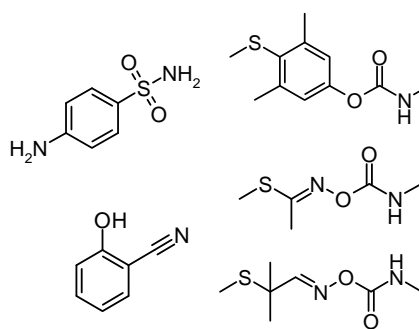


## Compounds (IV)

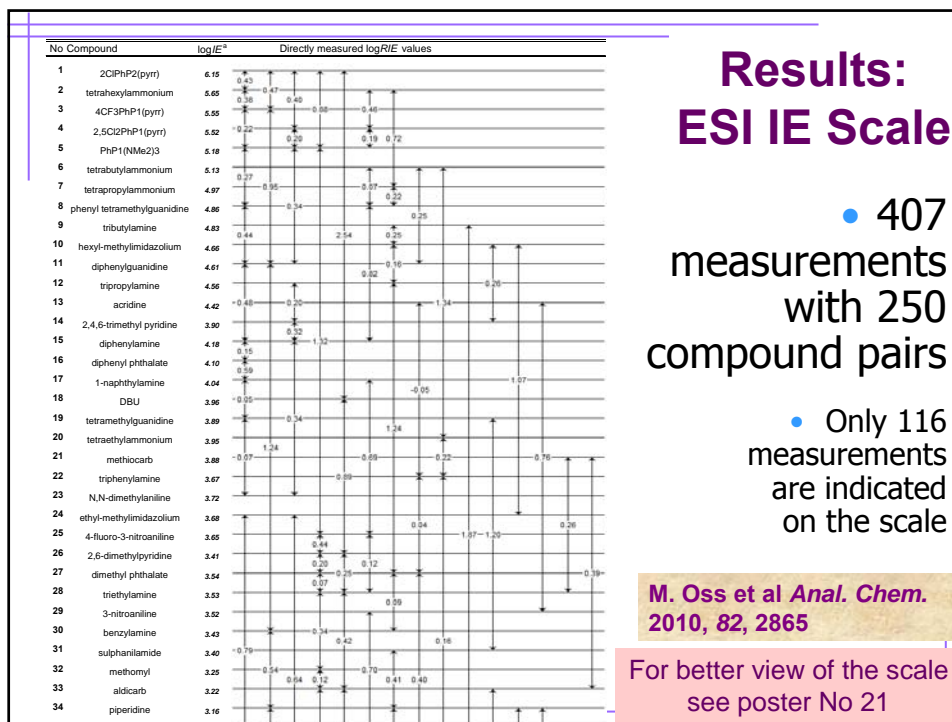
### Heterocycles

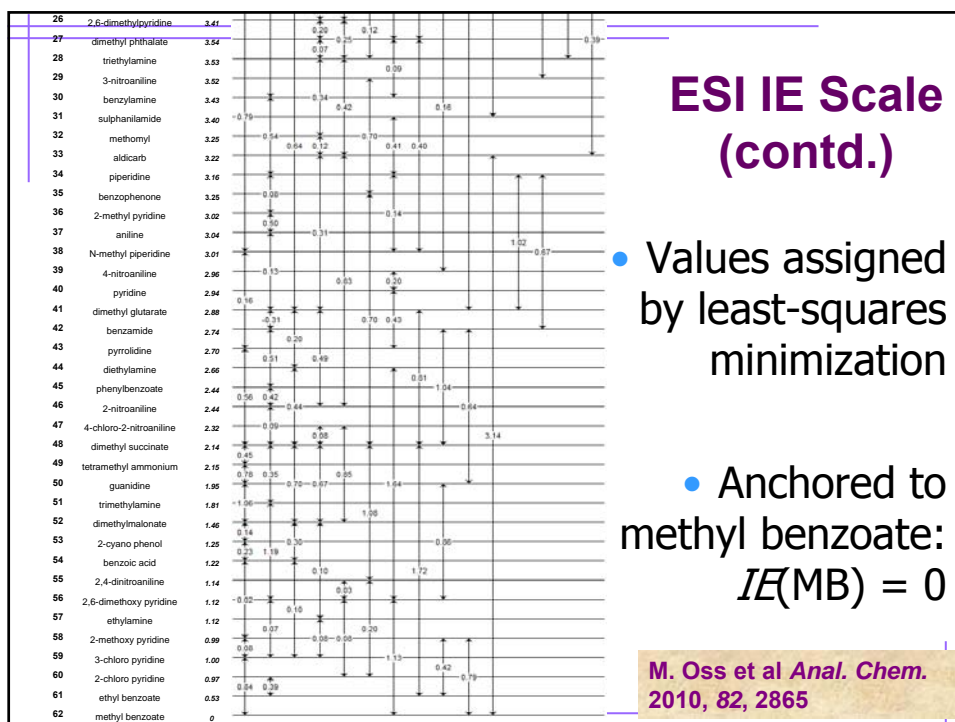


### Others



33





Compd.	logIE	Compd.	logIE	Compd.	logIE	Compd.	logIE
2CIPh <sub>2</sub> (pyrr)	6.15	1NaNH <sub>2</sub>	4.04	Aldicarb	3.22	Me <sub>4</sub> N <sup>+</sup>	2.15
Hex <sub>4</sub> N <sup>+</sup>	5.65	DBU	3.96	Piperidine	3.16	Guanidine	1.95
4CF <sub>3</sub> PhP <sub>1</sub> (pyrr)	5.55	TMG	3.89	Benzophenone	3.25	Me <sub>3</sub> N	1.81
2,5Cl <sub>2</sub> PhP <sub>1</sub> (pyrr)	5.52	Et <sub>4</sub> N <sup>+</sup>	3.95	2MePy	3.02	DMM	1.46
PhP <sub>1</sub> (NMe <sub>2</sub> ) <sub>3</sub>	5.18	Methiocarb	3.88	Aniline	3.04	2CNPh	1.25
Bu <sub>4</sub> N <sup>+</sup>	5.13	Ph <sub>3</sub> N	3.67	NMP	3.01	PhCOOH	1.22
Pr <sub>4</sub> N <sup>+</sup>	4.97	N,N-DMA	3.72	4NA	2.96	2,4DNA	1.14
PhTMG	4.86	EMIM <sup>+</sup>	3.68	Pyridine	2.94	2,6DMeOPy	1.12
Bu <sub>3</sub> N	4.83	4F3NA	3.65	DMG	2.88	EtNH <sub>2</sub>	1.12
HexMIM <sup>+</sup>	4.66	2,6MePy	3.41	Benzamide	2.74	2MeOPy	0.99
DPhG	4.61	DMP	3.54	Pyrrolidine	2.70	3CIPy	1.00
Pr <sub>3</sub> N	4.56	Et <sub>3</sub> N	3.53	Et <sub>2</sub> NH	2.66	2CIPy	0.97
acridine	4.42	3NA	3.52	PhB	2.44	EtB	0.53
2,4,6TMePy	3.90	BA	3.43	2NA	2.44	MB	0
DPhA	4.18	sulfa	3.40	4Cl2NA	2.32		
DPhP	4.10	Methomyl	3.25	DMS	2.14		

## Consistency of the scale

$$s = \sqrt{\frac{SS}{n_m - n_c}}$$

- $s = 0.30 \log RIE$  units
  - 143 measurements deviate by more than 0.2 log units
  - Their exclusion almost did not change  $\log IE$  values
  - Therefore no measurement was excluded

37

## Reasons of deviations?

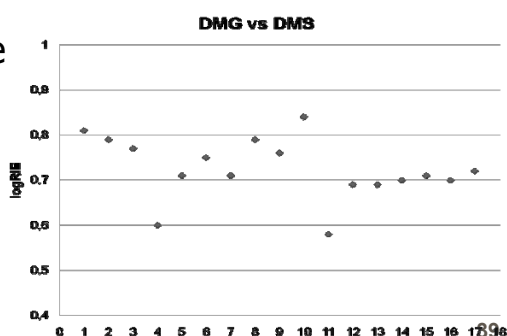
- Too different molecular masses of  $B_1$  and  $B_2$ ?
  - 2,5-Cl<sub>2</sub>PhP<sub>1</sub> (pyrr) (M = 400) vs DPhG (M = 211) : deviation -0.001602 units
  - Sulfanyl amide (M = 172) vs Et<sub>2</sub>NH (M = 73) : deviation 0.04054 units
  - aniline (M = 93) vs pyridine (M = 79): deviation 0.401235 units
- Differences due to different concentrations of  $B_1$  and  $B_2$ ?

DMP vs DMG	$\log IE$
• 6.81 ppm ja 13.71 ppm	0.50
• 0.61 ppm ja 6.81 ppm	0.50
• 23.19 ppm ja 7.89 ppm	0.52
• 23.19 ppm ja 10. 28 ppm	0.56
• 11.19 ppm ja 6.53 ppm	0.44
• 9.88 ppm ja 9.38 ppm	0.53

38

## Reasons for deviations?

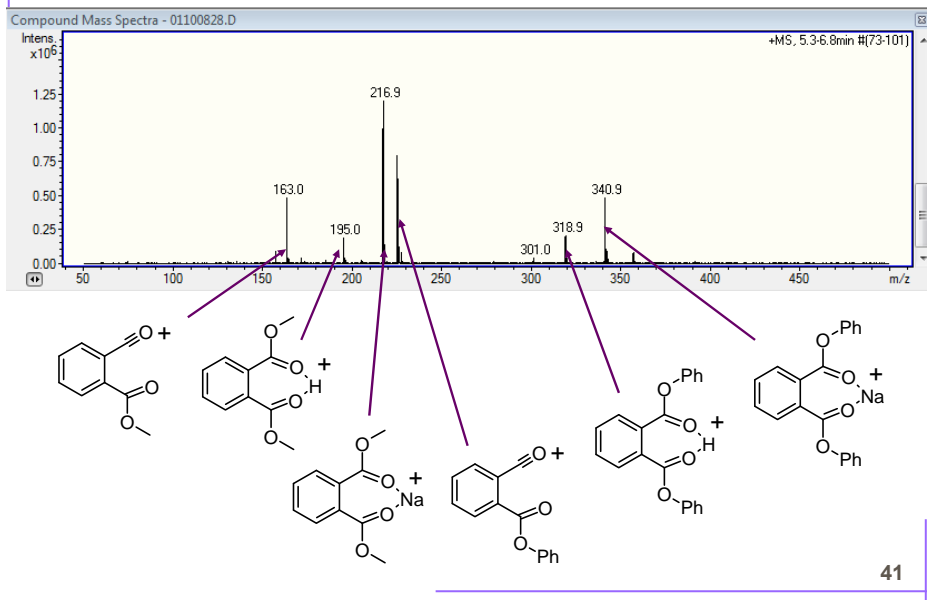
- Loss of linearity  $R_{\text{BH}^+} \sim [\text{BH}^+]$
- Mutual ionization suppression of  $\text{B}_1\text{H}^+$  and  $\text{B}_2\text{H}^+$ ?
- Additional processes?
  - Should be independent
- Long-term drift of the instrument?



## Additional processes

- With O-protonating compounds:  $\text{Na}^+$  adducts
  - Not taken into account on the assumption that  $\text{Na}^+$  adduct formation does not significantly decrease  $[\text{B}]$
- With many compounds: fragmentation
  - Taken into account on the assumption that fragmentation occurs in the gas phase after the ESI process
    - Intensities of fragment ions were added to that of  $\text{BH}^+$

## Dimethyl phthalate vs diphenyl phthalate



## Usefulness of the scale

- Classifying compounds, which would aid in predicting ESI ionizability of structurally similar substances
- Semiquantitative determinations to be made without the need to calibrate with each analyte
- $\log IE$  as a descriptor in QSAR/QSPR
- Better understanding of the ESI mechanism at the molecular level

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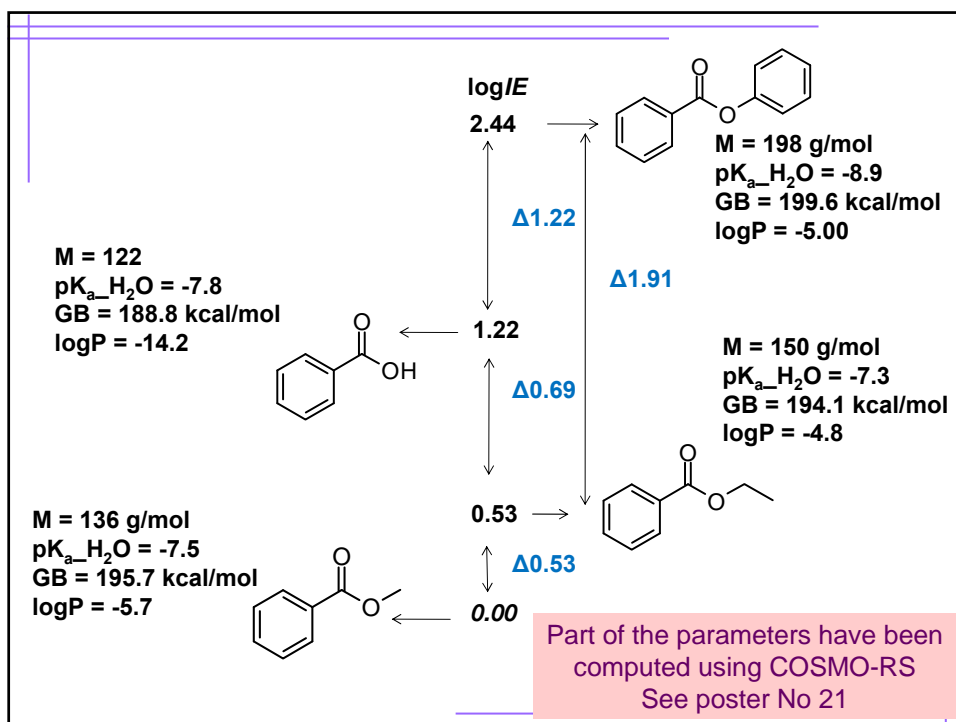
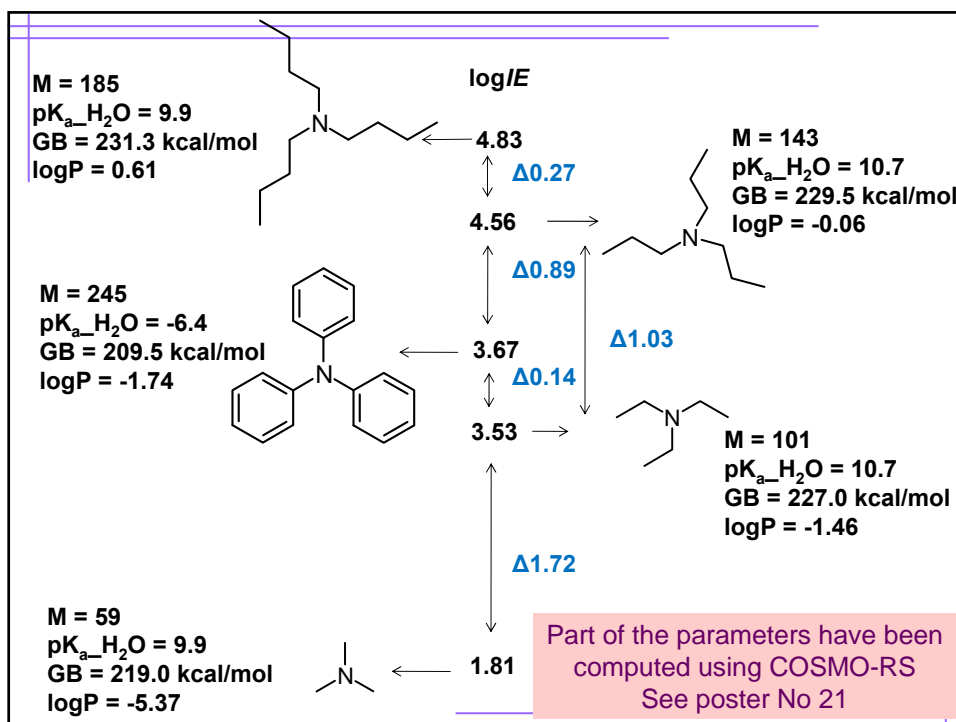
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43

## ESI IE and molecular structure

- Likely to be important:
  - Basicity of B (in water, acetonitrile, GP)
  - Polarity of BH<sup>+</sup> (dipole moment, polar surface area)
  - Hydrophobicity of BH<sup>+</sup> ( $\log P_{\text{solvent:hexane}}$ )
  - Size of BH<sup>+</sup> (M, MV, surface area)

44



## Attempt to predict ESI IE: Training set

- Training set of 42 random compounds
  - Scaled and centered parameters
- Only  $pK_a$  and  $\log MV$  statistically significant:

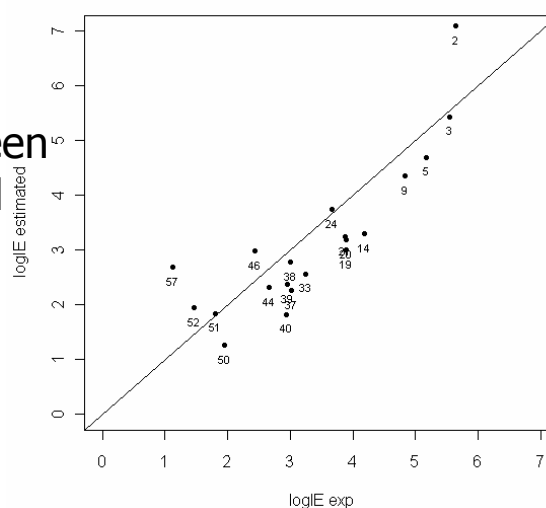
$$\log IE^s = (0.4109 \pm 0.0934)pK_a^s + (0.6359 \pm 0.0934)\log MV^s$$

- $R^2 = 0.67$ ,  $s = 0.86$  log units

M. Oss et al *Anal. Chem.*  
2010, 82, 2865

## Attempt to predict ESI IE: Test set

- Test set of 20 compounds
- Differences between measurement and prediction range from -1.6 to 1.1



M. Oss et al *Anal. Chem.*  
2010, 82, 2865



## ESI IE and molecular structure

- Certain trends are seen
  - Size matters
  - Hydrophobicity matters
  - Basicity matters
    - But compounds protonated to a negligible extent in solution may well ionize
- Prediction ability still low
  - Several parameters are computational
  - Strong correlation between some molecular parameters can distort the picture
  - It would certainly work better within compound families
    - More data are needed

49

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50

### Still to be done

- Measurements with different MS systems to test the universality of the approach
  - In principle similar IE order should be obtained
  - Different MS systems have different tuning parameters
  - Preliminary attempts were unsuccessful

51

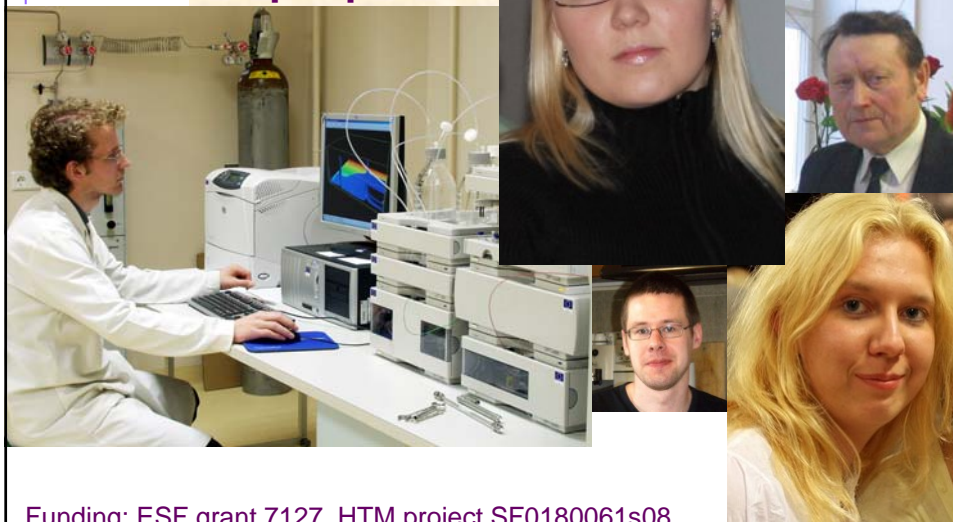
### Still to be done

- More thorough investigation of influence of ESI and MS parameters
  - Possible mass discrimination
  - Linearity  $R_{\text{BH}^+} \sim [\text{BH}^+]$
- Different solvents
  - Preliminary results show that with common LC-MS solvent mixtures the picture is similar

**Your ideas/comments/criticism is most welcome!**

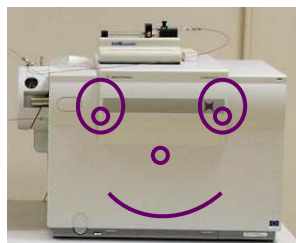
52

Thanks  
to all these  
people!



Funding: ESF grant 7127, HTM project SF0180061s08

- This talk is available from:
  - [http://tera.chem.ut.ee/~ivo/Chrom\\_MS/](http://tera.chem.ut.ee/~ivo/Chrom_MS/)
- Papers:
  - M. Oss et al *Anal. Chem.* **2010**, *82*, 2865
  - I. Leito et al *Rapid. Comm. MS* **2008**, *22*, 379



Thank you for your attention!

54