Ambient Ionization and Miniature Mass Spectrometers: Fundamentals and Point-of-Care Diagnostics

R. Graham Cooks
Purdue University

Two Poplars on a Road through the Hills
Vincent van Gogh 1889
Cleveland Museum of Art
Mass Spectrometry

Not primarily about *mass*
Certainly not a type of *spectroscopy*

“*The science and technology of ions*”

1. Ambient Ionization, DESI and Paper Spray: Therapeutic analysis & tissue imaging
2. Miniature mass spectrometers
3. Analysis by MS + solution phase chemical reactions
4. Reaction monitoring and preparative ambient MS
Missing Capability in MS

- Mass spectrometry
- Tandem mass spectrometry
- Exact mass
- Powerful separation/MS combinations
- Electrospray ionization, MALDI, etc.
  - give sensitivity, specificity, wide applicability, quantitation

BUT SPEED,

CONVENIENCE,

IN SITU CAPABILITIES
ARE MISSING
Ambient Mass Spectrometry: direct analysis of objects in their native environment

Ambient Ionization: Desorption & ionization in concert, in air

Takats, et al Science, 2004
Analyte ‘pick-up’ by micro-scale solvent extraction and pneumatic transport
Three Ambient Ionization Methods

Ambient Ionization: Desorption & ionization in concert, in air

DESI \([V, \text{ pneumatic}, \text{ solvent}]\)
- HV
- Solvent
- Secondary charged droplets with analyte
- To Mass Analyzer
- Sample

PAPER SPRAY \([V, \text{ solvent}]\)
- HV
- Charged droplets with analyte
- To Mass Analyzer
- Sample

LTP \([V, \text{ gas}]\)
- Grounded electrode
- Discharge Gas
- Sample

Simple, fast, chemical methods

Sample Workup
Chromatography
Touch Spray for Direct Microorganism Detection

- **E. coli**
- **C. albicans**
- **Rickettsia**
- **Staphylococcus a.**
- **Salmonella**
- **C. botulinum**
- **M. tuberculosis**

1. Touch
2. Spray

**MS**

- Inlet

**Negative ion**

- **Citrobacter fameri**

- **Citrobacter freundii**

Ahmed Hamid
Paper Spray: Therapeutic Drug Monitoring

1. Prick Finger
2. Load Sample
3. Apply HV
4. Apply Solution
5. Acquire Data
6. Report Results

60s Analysis of Biological Samples

Point-of-Care Analysis

Paper Spray


![Graph showing the relationship between amitriptyline blood concentration (ng/mL) and the ratio of [2H6]amitriptyline to amitriptyline, with a correlation coefficient of r² = 0.9936.](Actual Image)
Quantitation from DBS

2 μL IS Solution

10 μL Blood sample

Allow to dry

![Chemical structure of amitriptyline]

<table>
<thead>
<tr>
<th>Actual concentration (ng/mL)</th>
<th>4.4</th>
<th>8.9</th>
<th>44</th>
<th>88</th>
<th>443</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean measured concentration (ng/mL)</td>
<td>4.9</td>
<td>8.3</td>
<td>42</td>
<td>84</td>
<td>450</td>
</tr>
<tr>
<td>Std. Dev. (ng/mL)</td>
<td>0.4</td>
<td>0.8</td>
<td>5</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>Imprecision (%CV)</td>
<td>9%</td>
<td>10%</td>
<td>11%</td>
<td>7%</td>
<td>8%</td>
</tr>
<tr>
<td>Accuracy (% bias)</td>
<td>110%</td>
<td>94%</td>
<td>96%</td>
<td>95%</td>
<td>102%</td>
</tr>
</tbody>
</table>

*Signal at 0.89 ng/mL = >5 X blank signal
Paper Spray Technology

- Analyte opening
- Solvent reservoir
- Steel ball electrode
- Cellulose paper
Imatinib Quantitation from Blood

- Slopes obtained for 3 days:
  - Day 1 = 0.0119
  - Day 2 = 0.0115
  - Day 3 = 0.0116
  - Acc. & Prec. < 5%

- HPLC-MS method:
  - 0.0125  0.0013

R² = 0.9996

Data courtesy of Nick Manicke
Point-of-Care Analysis

Quantitation of Imatinib in Whole Blood by Paper Spray-MS/MS vs. HPLC-MS/MS

**Sample Prep.**
1. Precipitate protein – add acetonitrile, seal 96 well plate, mix
2. Centrifuge for 10 minutes
3. Transfer supernatent to fresh 96 well plate. Seal. Ready for injection

**Analysis Time (excl. sample prep)**
- Paper Spray: 45 seconds
- HPLC-MS/MS: 2.5 minutes

**Linear dynamic range**
- Paper Spray: 4 – 10000 ng/mL
- HPLC-MS/MS: 4 – 10000 ng/mL

**Precision**
- Paper Spray: 4.9%
- HPLC-MS/MS: 5.0%

\*calculated as an average across all quality controls

Nick Manicke, QuantIon Inc.

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**Schematic:**
Basic LC System

- Solvent Inlet Filter
- Pumps
- Injection Valve
- Column
- Detector
- Recorder/Monitor
- Backpressure Regulator
- Waste Reservoir
The road Menders, Vincent van Gogh, 1889, near St. Remy
Cleveland Museum of Art
Outline

1. Ambient Ionization, DESI and Paper Spray: Therapeutic analysis & tissue imaging
2. Miniature mass spectrometers
3. Analysis by MS + solution phase chemical reactions
4. Reaction monitoring and preparative ambient MS
Ambient Ionization using Miniature Mass Spectrometers

DESI, Mini 11, neg. ion

Leaf spray, Mini 11

Mol.wt. 1034

ESI, Mini 11

Paper spray, MS/MS, Mini 10.5
Paper Spray: Immediate Point-of-Care Analysis

1. Prick Finger
2. Load Sample
3. Apply Solution
4. Apply HV
5. Acquire Data
6. Report Results

- One drop of blood
- 60 second analysis of biological samples
Paper Spray on Mini 12 Quantification

R² = 0.99566

Therapeutic Range

Ratio of amitriptyline/amitriptyline d-6 vs. Concentration (ng/ml)

Concentration (ng/ml): 0, 100, 200, 300, 400, 500, 600
Ratio: 0, 0.5, 1, 1.5, 2, 2.5, 3
Paper Spray of Drugs in Biofluids

Multiplex Analysis

N.E. Manicke et al., Int. J. Mass Spectrom. 2011, 311, 123-129

Excellent precision (< 5%)

Cocaine

Blank
Zoom X50
10 ng/mL Zoom X50
500 ng/mL

10 ng/mL

50 ng/mL

100 ng/mL
# Miniature Mass Spectrometers

<table>
<thead>
<tr>
<th>System</th>
<th>Self-sustainable Portable Systems</th>
<th>Portable Systems without rough pumping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mini 10/11/12</td>
<td>Palm-portable MS</td>
</tr>
<tr>
<td>Developer</td>
<td>Purdue University</td>
<td>HAPSITE®</td>
</tr>
<tr>
<td>Weight</td>
<td>10kg/4kg/15kg</td>
<td>Samyang Chemical Co.</td>
</tr>
<tr>
<td></td>
<td>9kg/14.9kg</td>
<td>Inficon</td>
</tr>
<tr>
<td>Power</td>
<td>70W/30W/65W</td>
<td></td>
</tr>
<tr>
<td>Mass Analyzer</td>
<td>Rectilinear ion trap</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadrupole mass filter</td>
<td></td>
</tr>
<tr>
<td>MS/MS</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Sampling /Ionization</td>
<td>MIMS, direct leak, GDEI, APCI, ESI, DESI, LTP, PS, LS</td>
<td>Pulsed gas leak EI</td>
</tr>
<tr>
<td></td>
<td>SPME, EI</td>
<td></td>
</tr>
<tr>
<td>Mass range /Resolution</td>
<td>m/z 700, R = 700; m/z 1500, R = 750</td>
<td>m/z 300, R = 300</td>
</tr>
<tr>
<td></td>
<td>m/z 600, R = 400; m/z 400, R = 200</td>
<td>m/z 300, R = 150</td>
</tr>
<tr>
<td></td>
<td>m/z 70,000, R = 70</td>
<td>m/z 300, R = 300</td>
</tr>
<tr>
<td></td>
<td>m/z 425, R = 400</td>
<td>m/z 500, R = 300</td>
</tr>
<tr>
<td></td>
<td>m/z 500, R = 500</td>
<td>m/z 300, R = 300</td>
</tr>
<tr>
<td></td>
<td>m/z 300, R = 150</td>
<td>m/z 300, R = 300</td>
</tr>
<tr>
<td></td>
<td>m/z 300, R = 300</td>
<td>m/z 300, R = 300</td>
</tr>
</tbody>
</table>

![System Photos](image-url)
Mini 12 nanoESI Mass Spectra

1 ppm clebuterol
Resonance ejection
350 kHz, 1.5-3.0 V_{p-p}

5 ppm tacrolimus,
1 ppm imatinib,
1 ppm amitriptyline,
Resonance ejection
300 kHz, 3.5 – 3.75 V_{p-p}

277
278 Amitriptyline [M+H]^+

279
494 Imatinib [M+H]^+

281

Tacrolimus [M+Na]^+

826

Linfan Li
Electrosonic spray ionization (ESSI) MS/MS L-arginine clusters; 30ppm L-arginine, 8ppm caffeine and 60ppb cocaine in MeOH/H₂O

Ewa Sokol, unpublished
Backpack MS with Handheld LTP Sampling Probe Mini 12

5x4x30 mm
6.5 kV @ 1MHz
1 – 2 amu FWHM
> m/z 900

Paul Hendricks, et al. 2012s
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DESI Imaging

Remarkable match between analytical performance using DESI, 30 - 200 µ resolution, and needs in

1. Forensics .....fingerprints
2. Cultural studies ......art objects
3. Surgery ..........tumor margins
Latent Fingerprint Chemical Imaging by Mass Spectrometry (Ifa et. al., Science, 2008)
Ambient Imaging

Painting (Original)

Mass Spectrum

- m/z 207: blue
- m/z 421: red

False color image

DESI creating chemical image

Christina Ferreira
Applications of DESI-MS Imaging

DESI-MS Imaging

Latent Fingerprint

Tumor Diagnostics

Drug Mapping

Hormones

Analysis of Inks

- Ifá et al, Science, 2008, 321 (5890)
- C. Wu et al, Analyst, 2010, 135, 20
- Eberlin et al, Angew. Chemie, 2010, 49,
- Eberlin et al, Angew. Chemie, 2010, 49,
Tissue Analysis: Metabolite Profiling

a) Line Scans

Signal ratios from MS

b) Tissue Imaging

Image construction

Abundance of Phosphatidylserine in Human Liver Adenocarcinoma

Tumor margin

nontum
tumor

log (abundance ratio of m/z 788 : 448)

Lateral distance (mm)

Analysis of Images
(spatial distribution of chemicals)

* Creation of Images
(spatial distribution of chemicals)
Human Glioma – Towards Intrasurgical MS

DESI imaging; 200 micron, 1 sec/pixel; compound maps
=> Cancer margins; cancer staging

a) PS (36:1) m/z 788.8
b) PS (40:6) m/z 834.2
c) PI (38:4) m/z 885.4
d) ST (24:1) m/z 888.4
e) ST (26:1) m/z 916.5
f) Optical Image
H&E stained

Sample G2
Astrocytoma
WHO grade II

Sample G4
Astrocytoma
WHO grade III

Sample G5
Astrocytoma
WHO grade IV

Livia S. Eberlin, Allison L. Dill, Alexandra J. Golby, Keith L. Ligon, Justin M. Wiseman, R. Graham Cooks* and Nathalie Y.R. Agar Angew. Chem. 2010
Human Brain Cancers

1. What the pathologist sees:

2. MS -- distribution of hundreds of chemicals:

3. Automatic interpretation of MS data:

Visualization of Tumor Characteristics and Location Combining Pre-operative MRI with DESI-MS

Surgical CASE 1 Visualization in segmented pre-operative 3D-MRI volume of glioma tumor cell concentration (A) and grade classification (B) for surgical case 1 of tumor and surrounding region.

Stereotactically registered samples correlated to pre-operative MRI by neuronavigation and visualized over segmented 3D MRI reconstruction.

Tumor volume is light purple.

Future in surgical applications

1. Lipids metabolites characterize the biosystem ....prognotics?

2. Miniature mass spectrometers preferred if this is widespread

3. More rapid non-imaging methods of diagnosis
1. Ambient Ionization, DESI and Paper Spray: Therapeutic analysis & tissue imaging

2. Miniature mass spectrometers

3. Analysis by MS + solution phase chemical reactions

4. Reaction monitoring and preparative ambient MS

Gabriel Dante Rossetti, 1863 Beata Beatrix
Phases in chemical analysis -----

19th C / early 20th C chemical reaction based esp. color – essentially sensors

Early 20th C instrumental chemical methods e.g. electrochemical elemental

Mid 20th C instrumental molecular methods, spectroscopy

Late 20th C beginning of specific biochemical methods – enzyme assays

Early 21st C integration of advanced instrumental and chemical methods
Fast Steroid Screening in Urine: Hydroxylamine

\[ R_1\text{O} \xrightarrow{\text{H}_2\text{NOH}} R_1\text{O}H \xrightarrow{-\text{H}_2\text{O}} R_1\text{NH} \]

Product ion Schiff base

**DESI:** MOST CHEMICAL OF IONIZATION METHODS --- SOLN PHASE CHEMISTRY

**Steroids Mixture in Urine**

**Conventional DESI**
- Epitestosterone: 289
- 5α-androstan-β, 17β-diol-16-one: 307
- Androsterone hemisuccinate: 391

**Reactive DESI**
- Androstane: 322
- Androstane 3B-17β-diol: 340
- Epitestosterone: 424

Reactive DESI & Microdroplet Girard T Reaction

10 μm, 150 m/s

Secondary droplets as reaction vessels

10 μm droplet at 90°;
1. React at 90°
2. Analyze at 60°

Acid catal. dehydration

Girod, Moyano, Campbell & Cooks, Chemical Science, 2(2011), 501-510

RATE ENHANCEMENT
3 ORDERS
Nanospray Girard T/Benzaldehyde

Reaction: Benzaldehyde (2μL) + Girard T in ACN (1000 ppm)

a) 1 h bulk solution-phase

(b) Nanospray Girard T/Benzaldehyde

Nanospray Girard T/Benzaldehyde

(b) Nanospray Girard T/Benzaldehyde

Nanospray Girard T/Benzaldehyde

(c) Nanospray Girard T/Benzaldehyde

Nanospray Girard T/Benzaldehyde

(d) Reactive DESI (5 μL/min 1000 ppm benzaldehyde)

Dahlia Campbell & Xin Yan
Droplet 2 μ diam., 4 μ^3 vol., 10^8 surface sites
i.e. 40 mM or less allows all permanent ions on surface
20 mM of each allows both reagents to take surface sites
*Rates are increased*

**SHRINKING DROPLETS**

**FALLING pH**

**SURFACE EFFECTS**

(3D case → 2D)
C-C Formation: Synthesis by Mass Spectrometry

MS synthesis / off-line

\[
\begin{align*}
\text{1.47 mg} & \quad (92 \text{ % yield}) \\
\text{Electrospray} & \quad (-5kV) \\
\text{KOH} & \quad 2.5 \text{ min}
\end{align*}
\]


\[
\begin{align*}
\text{0.83 mg} & \quad \text{0.88 mg} \\
\text{0.83 mg} & \quad \text{0.88 mg}
\end{align*}
\]
Synthesis by Mass Spectrometry

1-indanone + 4-chlorobenzaldehyde → KOH → 92.2% yield

Carbon-carbon bond formation: Claisen-Schmidt condensation

UV:

MS:

HPLC:

Thomas Müller
Increased reaction rate is observed for solution-phase chemical reactions during desorption electrospray ionization (DESI). Reactions occur in the departing secondary droplets.

**Piperidine (MW 85)**

**Acrylamide (MW 71)**

**Reaction Product (MW 156)**

*Instantaneous (milliseconds)*

**Product m/z 157**

**Acrylamide**

**Piperidine**

**Reaction Product (MW 156)**

**Bulk Solution-Phase**

**nESI-MS**

**60 min**

Accelerated Reactions in Thin Films: Mannich Reaction on Ambient Surfaces

Rapid small scale chemical synthesis by dropcasting on ambient surfaces

Amine + aldehyde + ketone

Benzaldehyde (10 mM), ethanolamine (16 mM) and cyclohexanone (0.1 M) in acetonitrile (c) at ambient surface and (d) bulk solution-phase.

Surface film: premix amine and aldehyde 2min then ketone then 10 min rxn

Bulk soln: premix amine and aldehyde 1 hr then ketone then 10 min rxn

Bulk Solution Phase
Peptide X-linking Reaction

Reactants: KKK (1000 ppm) + SBS (1000 ppm)
Solvent: MeOH/ H₂O/ acetic acid {49.5:49.5:1}
Reaction time: 1 hr & (inset) 24 hr

(b)

\[ [M+2H]^{2+} \]

\[ 100\% = 6.72E5 \]

\[ 202 \]

\[ [2+2H]^{2+} \]

\[ [M+H]^+ \]

\[ 403 \]

\[ 369 \]

\[ 466 \]

\[ m/z \]

1 hr

No m/z 541

No reaction

Abraham K. Badu-Tawiah, Anyin Li, Fred P.M. Jjunju, and R. Graham Cooks, Angew. Chem.. 2012
Peptide crosslinking is achieved nearly instantaneously using charged microdroplets to deliver reagent to a surface in ambient environment.
Peptide Cross-linking at Ambient Surfaces by Reactions of Nanosprayed Molecular Cations

1. Acidic SBS (M.W. 528)
   
   ![Acidic SBS Structure](image)
   
   m/z 529

   Peptide

   covalently modified peptide
   (M.W. 333+ M)

2. 0.60 μA or 3.6 × 10^{12} charges/s in area 0.8 cm²

3. cross-linked peptide
   (M.W. 138+ M)

   ![Cross-linked Peptide Structure](image)

   m/z 541

Linker on surface/ Peptide sprayed
3.6 × 10^{-10} mol/min of tripeptide KKK, (2+)
mol. wt. 402 i.e. 3.8 × 10^{-8} g /min;
peptide x-section 0.15 nm² so 0.02 monolayers
X-linker present in excess
reaction ~ 100% efficient

Abraham K. Badu-Tawiah, Anyin Li, Fred P.M. Jjunju, and R. Graham Cooks, Angew. Chem.. 2012
# Katritsky Reaction on Paper in Air

![Reaction Scheme](image)

### Substituent effects

<table>
<thead>
<tr>
<th>Substituent</th>
<th>position</th>
<th>product : reactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxy-</td>
<td>o-OMe</td>
<td>1:3</td>
</tr>
<tr>
<td></td>
<td>m-OMe</td>
<td>1:1</td>
</tr>
<tr>
<td></td>
<td>p-OMe</td>
<td>99:1</td>
</tr>
<tr>
<td>Nitro-</td>
<td>o-NO2</td>
<td>1:99</td>
</tr>
<tr>
<td></td>
<td>m-NO2</td>
<td>20:1</td>
</tr>
<tr>
<td></td>
<td>p-NO2</td>
<td>1:99</td>
</tr>
</tbody>
</table>

### Reaction Rate (pyrylium+p-OMe aniline)

<table>
<thead>
<tr>
<th></th>
<th>time</th>
<th>product : reactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>droplet</td>
<td>0.5 min</td>
<td>99:1</td>
</tr>
<tr>
<td></td>
<td>3 min</td>
<td>99:1</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>99:1</td>
</tr>
<tr>
<td>solution</td>
<td>1 min</td>
<td>1:99</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>36 min</td>
<td>1:10</td>
</tr>
</tbody>
</table>

### Intercharge distance effect

<table>
<thead>
<tr>
<th>n</th>
<th>Mono-cation product</th>
<th>Dication product</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

### Product collection

![Product Collection](image)

- m/z 414.36
- Xin Yan
Reaction Monitoring and Preparative MS
Simultaneous positive/negative ion spectra

Huang, Li, Ducan, Ouyang, Cooks Angew. Chem. 2011
Inductive nanoESI for artificial urine for 50 mins

Artificial Urine (undiluted)
Sunitinib MW 398 (100 ppb)
Spray flow rate (30 nL/min)
Spray tip id (~10 um)

TIC

SIC (326)

m/z 399

Signal lasts for 50 mins

Some interference over the last 10 min
Paper Reaction Scale-up

2,5-H (TPP)

aromatic H

D$_2$O

MeOD

CH$_3$O-(anisidine)

No product!

Paper reaction

6 min

10 mg

Yield 70%

2,5-H (TPP)

aromatic H

D$_2$O

MeOD

CH$_3$O-(Product)

CH$_3$O-(anisidine)
Alkanes at charged surfaces undergo C-C bond functionalization with N$_2$ to give imines

\[ \text{CH}_2\text{CH}_2\text{N}^+\text{CH}_2\text{C}_n\text{H}_{2n+1} \]
Protein Soft-Landing

Isolation from mixture and subsequent collection of intact proteins

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