Breath Analysis Summit 2011 - International Conference on Breath Research

Towards an Online Breath Cancer Diagnostic Tool.

G. Vidal-de-Miguel[1], A. Herrero[1], G. Bailador[2], E. Criado[1], F. Zambrana[3], C. Sánchez[2], E. Casado[3].

[1]Sociedad Europea de Análisis Diferencial de Movilidad (SEADM)
[2]Universidad Politécnica de Madrid (UPM)
[3]Unidad de Oncología, Hospital Universitario Infanta Sofía.

Parma 11-14 September 2011

Objectives

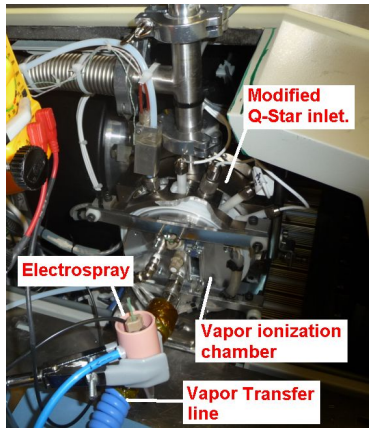
- Find reliable cancer biomarkers in breath.
  - Trained dogs show that there are biomarkers in breath.
- Develop a SESI-MS On-line breath analyzer.
  - Based on previous work by P.Martinez-Lozano and J. F. de la Mora [1,2].
  - Sensitivity below 1 ppt (measured with QStar and TNT vapors).
  - Capable of handling big populations.
  - Reducing contamination (real sensibility limiting factor).
  - Reducing required flow of breath.
- Study Healthy vs Cancer breath differences.
  - Once found, biomarkers could be tested with more specific analyzers.

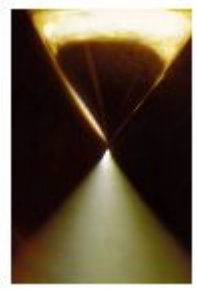
[1] P. Martinez-Lozano and J. F. de la Mora, "Electrospray Ionization of volatyles in breath" Aug 2007.

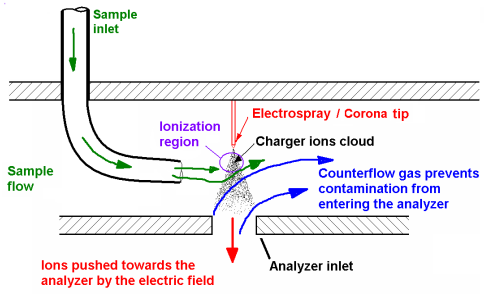
[2] P. Martinez-Lozano and J. F. de la Mora, "Direct analysis of fatty acid vapors in brath by electrospray ionization and atmospheric pressure ionizzation-mass spectrometry" Nov. 2008

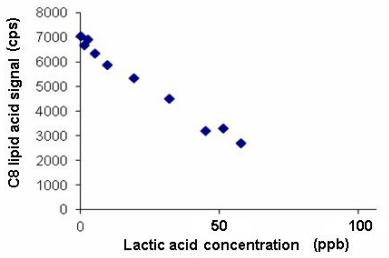
SESI Ionizer

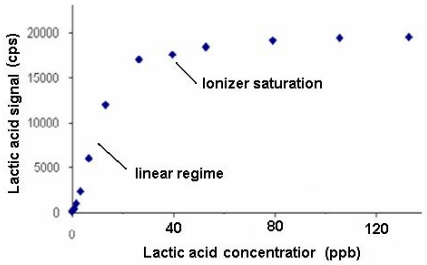
- Noted by J. Fenn et al and used for breath ionization by P. Martinez-Lozano and J. F. de la Mora
- Electrospray cloud produces OH- ions.
  - Soft ionization technique at atmospheric pressure. Ions transfer Charge to acidic molecules.
  - Ionizes polar molecules.
  - Ionization efficiency: 10^{-3} ion/molecule.
  - Sensibility measured with TNT vapors and Q-Star XL: 0.8ppt
- Improved fluid configuration reduces required flow rate (fron 5 lpm to 1.5 lpm) and increases ionization efficiency.
- Humidity increases ionization efficiency
  - Contaminant signals rise with breath though not included in breath.
  - System contamination measurement requires introducing humid air.
- High ionization efficiency permits eliminating the preconcentration step and allows On-line analysis.





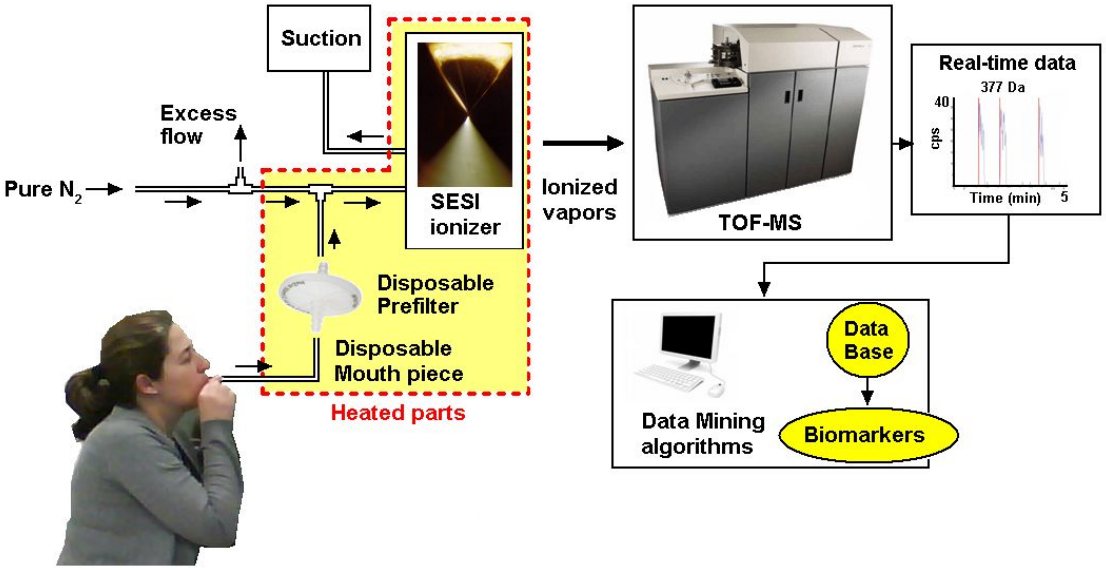


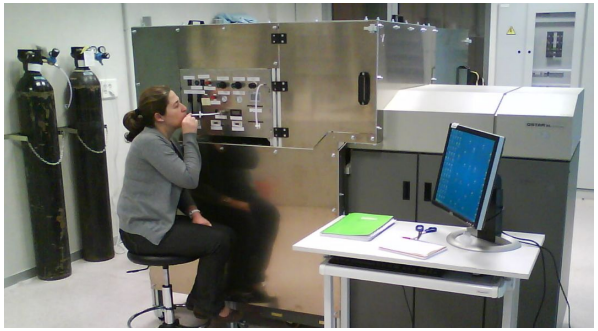




System architecture.

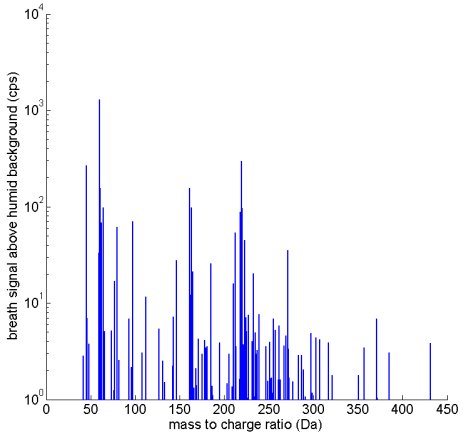
- Problems to be addressed:
  - Heavier species are typically more specific, but less volatile. VOCs are typically in the ppt level and below.
  - Breath is a complex process affected by many confounding variables (inhaled air, spirometric profile, state of humor, bacterial metabolism, food, ... and health)
- Requirements:
  - High sensitivity required to detect more specific VOCs.
  - On-line system helps scientists to understand dynamic processes.
  - High selectivity required to distinguish biomarkers from confounding species.
  - Easy and safe for patients.

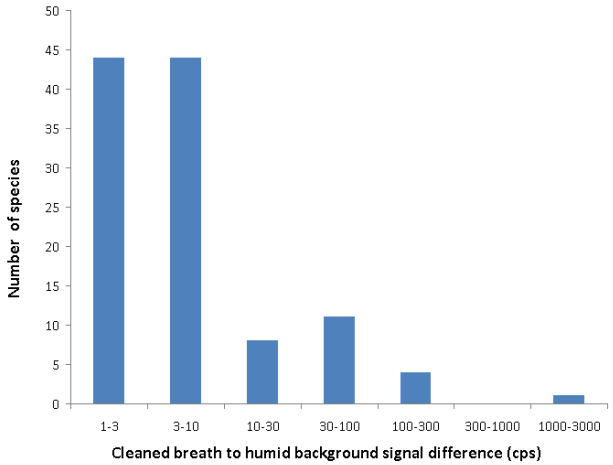




First tests. Detected species.

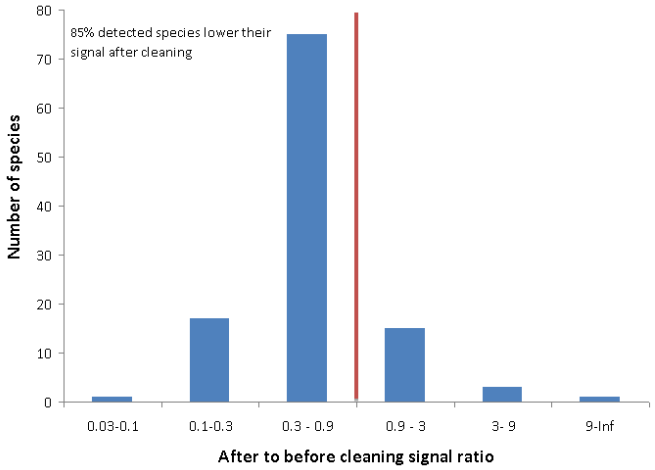
- How to differentiate real breath signals from contamination signals?
  - To eliminate room air contamination:
    - Subject breaths purified medical grade air.
  - To eliminate equipment and medical grade air contaminants:
    - Background measured by introducing humidified medical grade air at the same flow rate as breath.
  - Signals are accounted for after 'cleaning process' if:
    - Breath signal rises more than 3 times humid background variance above humid background and
    - Breath signal rises more than 1 cps above humid background.
- Heaviest detected specie: 431 Da
- Around 110 detected species coming from breath.

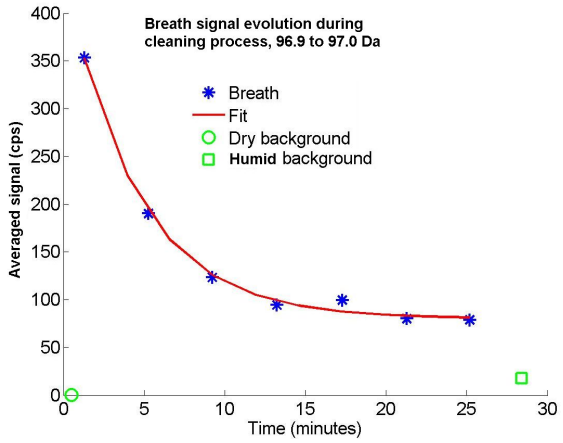




First tests. Room air effect and cleaning.

- Room air is a known confounding variable in breath analysis.
- Subject starts breathing medical grade pure air and breath is analyzed every 5 minutes.
- Most species reach an stationary state after 10 minutes.
- Around 85% of detected species are also present in room air.

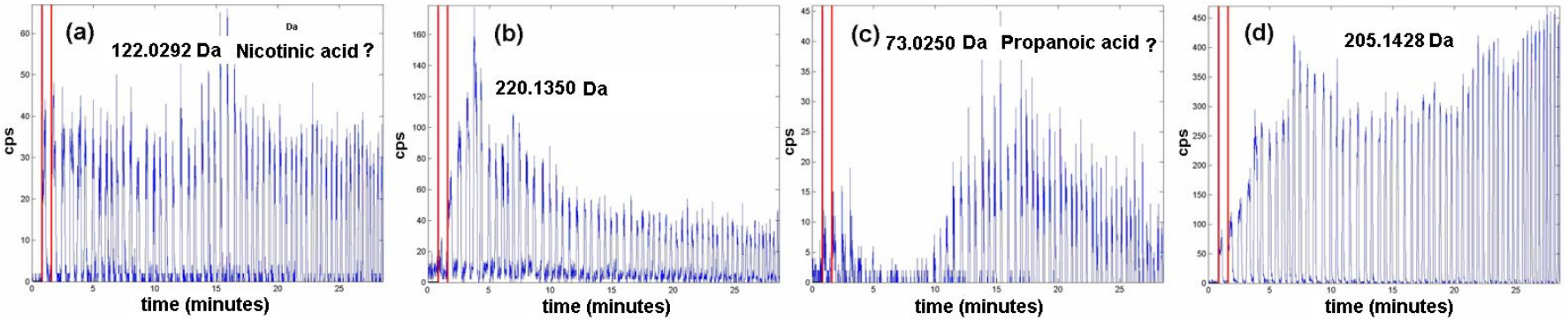




Ilía SISTEMAS SL · Hospital Infanta Sofía SaludMadrid · Centro tecnológico CARTIF · POLITÉCNICA · SEADM

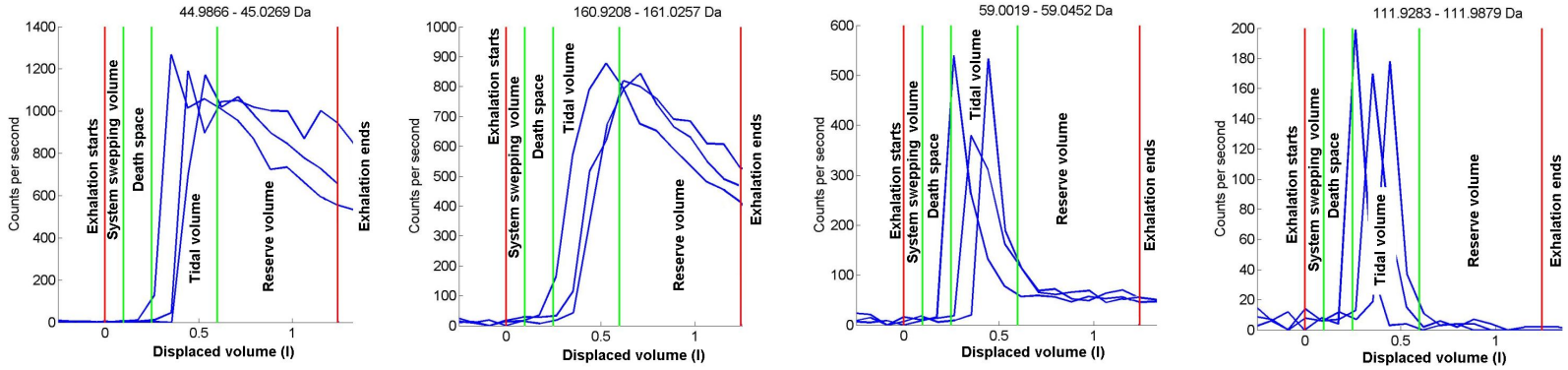
First tests. Mouth bacterial metabolism

- Subject rinses mouth with water and glucose and breathes normally exhaling through the analyzer.
- Results show the behavior of different substances:
  - Constantly produced substances (a), immediate short response (b), delayed response (c) and Immediate and long lasting response (d).
- This experiment illustrates the complexity of the dynamics of breath.



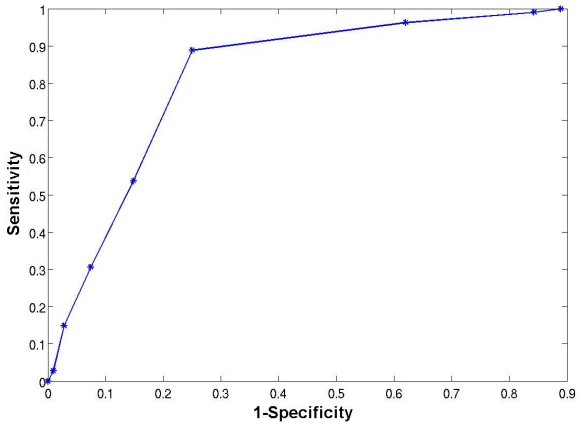
First tests. Spirometric profiles.

- A healthy subject inspires normally and exhales through the analyzer at constant flow rate until depletion.
- Spirometric profile:
  - shows different patterns for different species belonging to different parts of the lungs.
  - could be used as separation technique in breath analysis to improve species separability.
  - Permits differentiating mouth originated VOCs (associated to bacterial metabolism) from deeper lungs VOCs.



First Colon Cancer Diagnosis Campaign.

- Population:
  - Colon Cancer Diagnosed Patients (Colonoscopy diagnosed) CCDP - 36 people.
  - Healthy Diagnosed Patients (from Early Detection Preventive Program, Colonoscopy diagnosed) HDP 36
  - No breath cleaning procedure available for this campaign.
- Variable pre-selection:
  - Averaged signal during exhalation.
  - Only signals rising above dry background when blowing.
  - Only signals found in at least 25% of HDP or CCDP
  - Signals having coherent time evolution during campaign eliminated.
    - Circadian cycles? Long term system contamination or cleaning process? environmental changes?
- Algorithm
  - Training Set: CCDP and HDP
  - Empirical optimization or ROC curves and signal thresholds and Forward characteristics.
  - Sensitivity: 88.9%, Specificity: 75%
- Conclusions:
  - Results are promising.
  - But couldn't find obvious differences.
  - Uncontrolled confounding variables are blurring the results.
    - Inhaled air is very variable during campaign.
    - Long term transient effects produce time varying signals that blind the algorithms if CCDP and HDP are appointed at separated periods.



Ilía SISTEMAS SL · Hospital Infanta Sofía SaludMadrid · Centro tecnológico CARTIF · POLITÉCNICA · SEADM

Conclusions

- High sensitivity SESI based analysis allows On-line analysis without a pre-concentration step.
- Sampling system precludes room air from contaminating the analyzer.
- The system permits spirometric analaysis of exhaled breath.
- Spirometric analysis allows for separation of species.
- Inhaled air effect characterized in laboratory environment.
  - It can be one of the main components of exhaled breath.
  - Most detected species are cleaned after 10 minutes breathing pure medical grade air.
- Bacterial mouth metabolism could be a potential confounding variable if subjects didn't fasten before test.
- A test on Cancer and Healthy subjects:
  - Shows promising results.
  - But also the need for cleaning confounding variables (Specially inhaled air.)
- Inhaled air is one of the most important confounding variables found in our research.
  - Cleaning the breath seems to be a necessary step to find biomarkers.

Future work.

- Prototype 2:
  - Ionizer and sampler temperature increased to prevent deposition and long term contamination.
  - Positive and negative mode analysis using two Electro-Sprays in parallel.
- Campaign 2:
  - Breath cleaning procedure implemented during next campaign.
- Spirometric analysis to be implemented in postprocessing algorithms.

Thanks for your attention

Contact:
Guillermo Vidal
Head of the R&D team at SEADM
guillermo.vidal@seadm.com
+34 983 130 154