DYNHOM project state-of-the-art

Dr Michel Van Wassenhoven UNIO Vienna 17th of November 2016 **DYNHOM** project

- **Main question**: What is the **Signature** of a homeopathic dynamized medicine?
- Secondary questions: Homeopathic Medicines Stability (expiring date – preservation); Best Manufacturing Methodology (GPP & GMP).



Signature ?

Ink added to paper but significance is not coming from the material?



Already published :

- ✓ Modulation of genes' activity (Epigenetic reaction)
- ✓ Discriminant signal between remedies (NMR)
- Persistence of stock material in high dynamizations
- Quality requirements (European Pharmacopoeias)
- $\checkmark\,$ Clinical surveys, clinical cases
- ✓ Provings, Materia medica
- ✓ AVOGADRO ?



A comprehensive approach

✓ Nano particles search

✓ Solvent (water) behaviour

✓ Electrons behaviour

The Golden Team

- Teams logistic
 - Manufacture GPP: PhD Martine Goyens
 - Fund raising, Secretarial Office, Management, Coordination, publications: MD Philippe Devos.
- Teams measurements
 - UHPLC-UV (plants) and lyophilisations : Prof. PhD Joëlle Quetin-Leclercq. Dean UCL-Faculty of Pharmacy.
 - SP-ICP-MS/TEM (metals): PhD Nadia Waegeneers. Veterinary and Agrochemical Research Centre + Sysmex.
 - DLS/ZP/NTA/SEM-EDX: Sysmex company Holland.
 - NMR: Prof. Luce Vander Elst. Mons University. Biomedical chemistry. Nanotechnology.
 - Electro-photonic analyse: Prof. Marc Henry. Strasbourg University. Molecular chemistry.















Preparations GPP







A comprehensive approach

✓ Nano particles search

✓ Solvent (water) behaviour

✓ Electrons behaviour

SP-ICP-MS

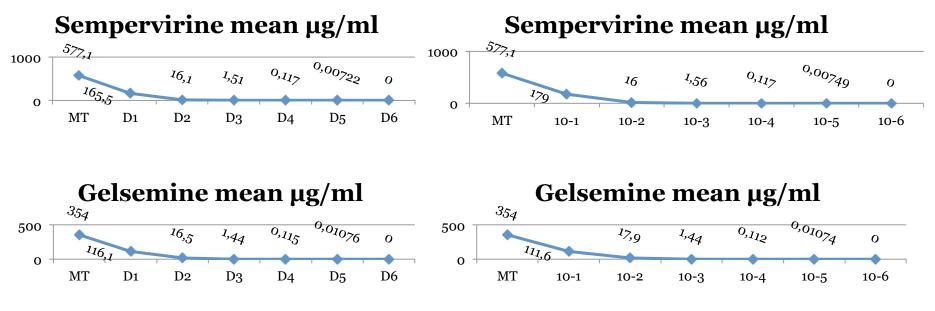


- Cuprum metallicum.
 - SP-ICP-MS/TEM (metals): Single Particle Inductively Coupled Plasma Mass Spectrometry-Transmission Electronic Microscopy. in 20cc of 4CH dynamized water solution maximum 0,02µg of cuprum would be expected and 0,2g of Lactose.
 - Results in Cupr 4CH: In the solution, there is a huge background signal but these particles are far too small to be detected by single particle ICP-MS, the detection limit for copper particles is 45 nm (52 nm for Cu2O). Later on we did the same using a concentrate after lyophilisation of 200cc of solution with a similar outcome.

HPLC-UV

• Gelsemium sempervirens

• HPLC-UV: High Performance Liquid Chromatography using UV detector. Dynamized versus simply diluted.



• No difference with or without dynamization / Quantification limit = D6

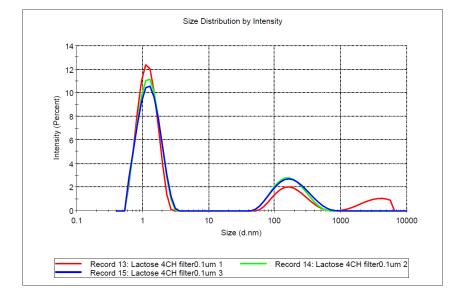


DLS: Dynamic Light Scattering

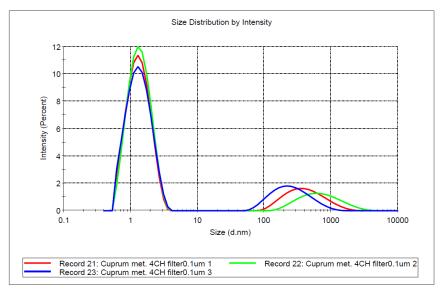
• Cuprum metallicum 4CH.

Results

Lactose



Cuprum

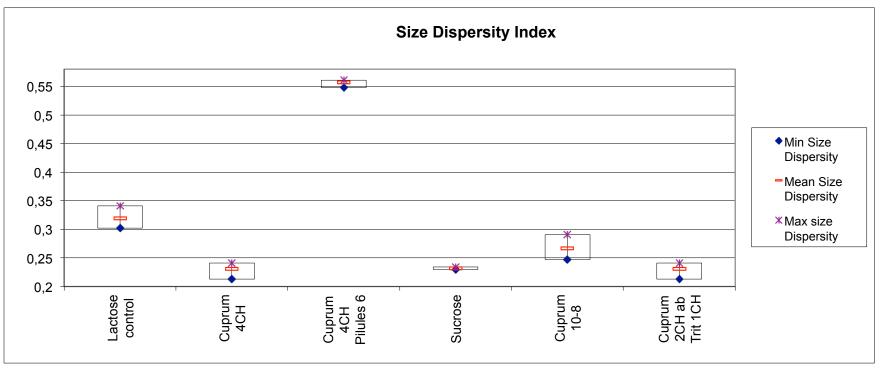


DLS: Dynamic Light Scattering



• Cuprum metallicum 4CH.

The PDI (Poly Dispersity Index) of DLS measurements shows that measurements are valid (>0,7 would indicate a too broad size distribution). The particles size distribution in Cuprum 4CH is more homogeneous than in the lactose control.



DLS: Dynamic Light Scattering

• Cuprum metallicum.



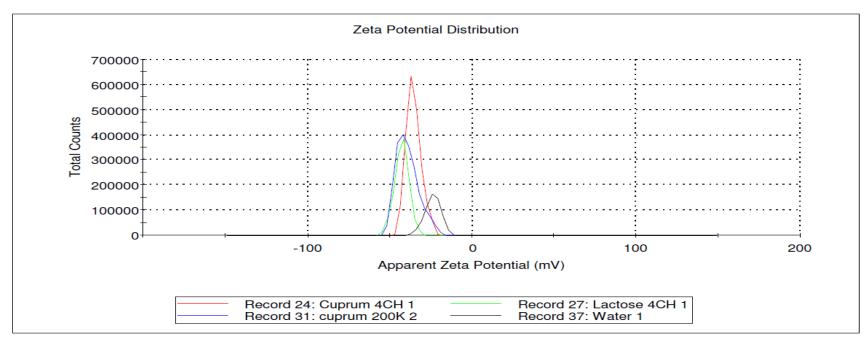
RESULTS conclusions: Same size of small nano-particles in cuprum 4CH and lactose 4CH, between (0,8nm/1,95nm). The presence of the expected 0,02µg of coper in 20cc cuprum metallicum 4CH dynamization is not yet confirmed. These nano particles are not detectable with DLS above 4CH. Greater heterogeneity of particles in lactose 4CH.

ZP : Zeta-Potential

• Cuprum metallicum.



- Zeta potential is a method for the measurement of the electrostatic potential at the electrical double layer surrounding a nanoparticle in solution.
- Zeta potential Cuprum 4CH median value -35,6mV, lactose -42,9mV, Water -24mV, Cuprum 200K
 -39,3mV. Note also that the total counts is significant higher and valid for Cuprum 4CH

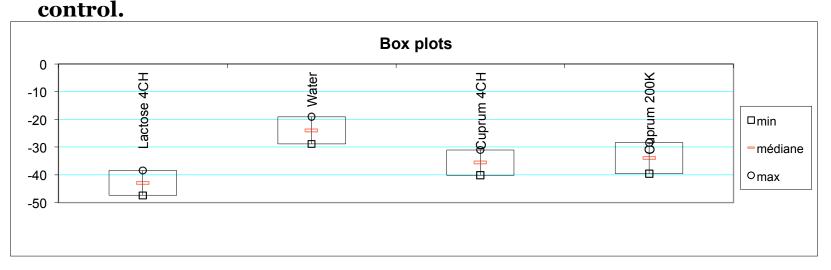


ZP : Zeta-Potential

• Cuprum metallicum.



- In opposition to DLS, if the preparation is filtered (filter 0,1μ) this signal became unstable and irrelevant. This means that other detected larger particles (see further) play a role in stabilisation of this information.
- With zeta potential the mean difference between water control and other samples is significant and possible between Cuprum 4CH and lactose



NTA : Nanoparticle Tracking Analysis

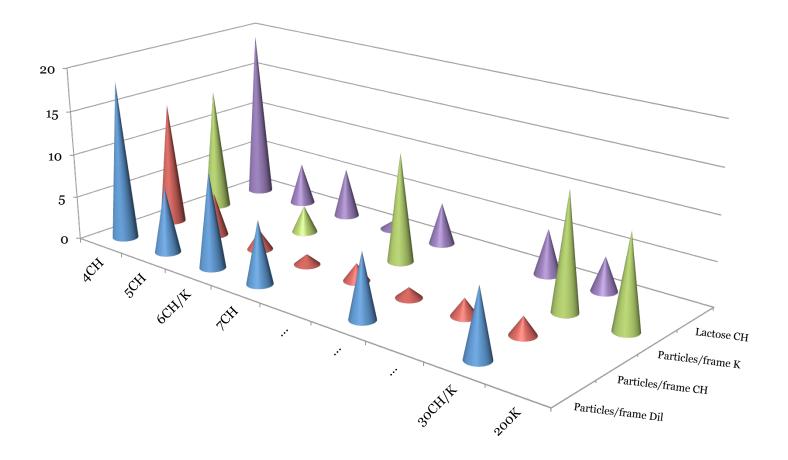
• Cuprum /Gelsemium.

Nanosight:

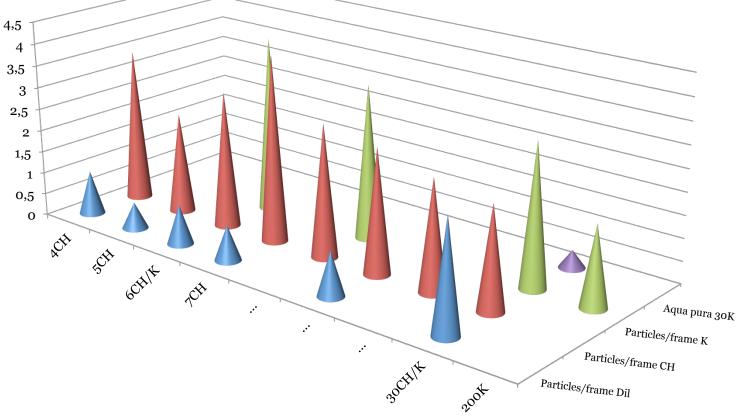


- This technology is limited to particles above 20nm, we could see a significant amount of particles only in unfiltered samples.
- \circ No particles in pure undynamised water control.

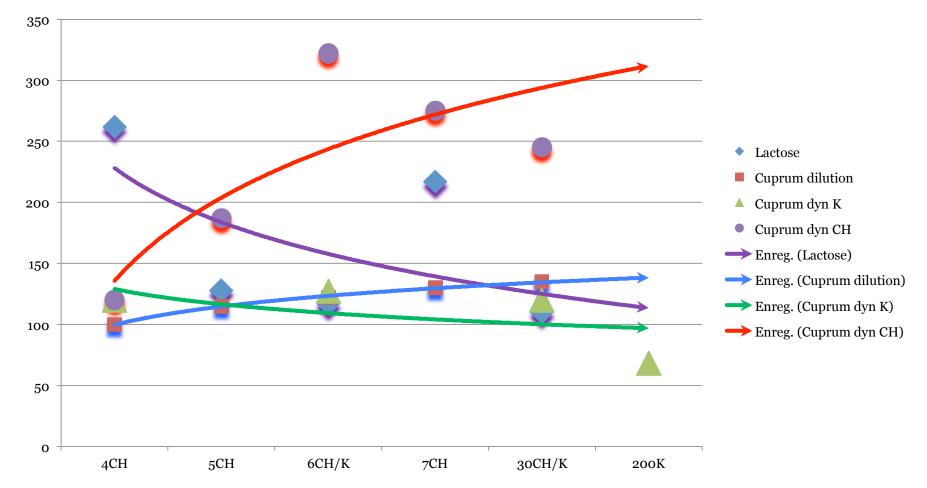
CUPRUM: Presence of particules above 20nm in successives dynamisation (Particules/frame). Blue are particles in simply diluted copper, red are CH potenties, green are K potencies and purple are dynamized lactose. "..." are extrapolated values.



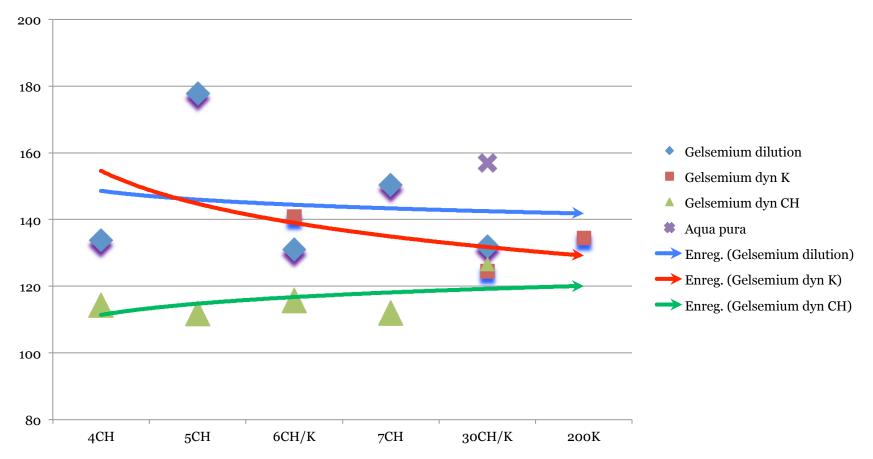
GELSEMIUM: Presence of particules above 20nm in successives dynamisation (Particules/frame). Blue are particles in simple diluted samples, red are CH potenties, green are particles in K potencies, purple are particles in potentized aqua pura 30K (PET container). "..." are extrapolated values.



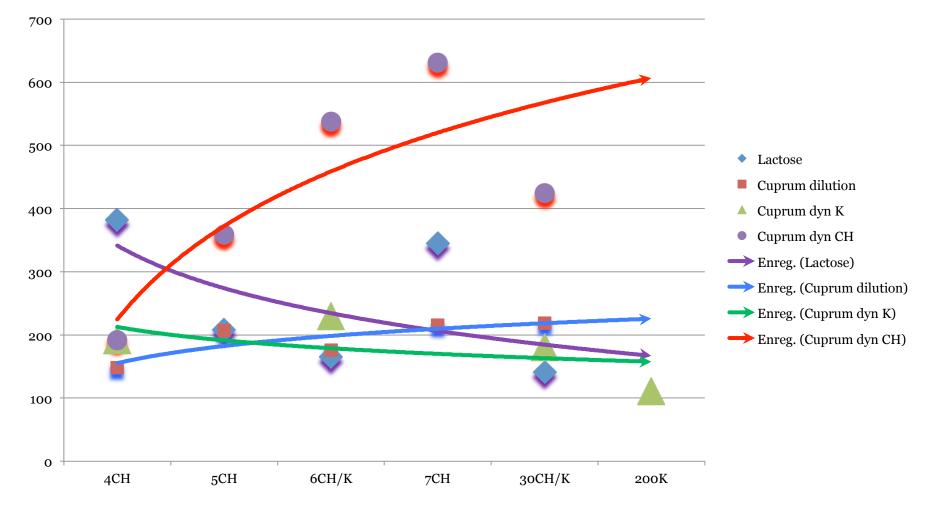
Mean particules sizes in nanometers (Cuprum metallicum and controls).



Mean particules sizes in nanometers. (Gelsemium and controls).



Particules sizes distribution (D90) in nanometers. (Cuprum metallicum)



Particules sizes distribution (D90) in nanometers. (Gelsemium)



NTA: Nanoparticle Tracking Analysis

• Conclusions.



- The presence of particles even in highest dynamisation stay in a relatively stable concentration.
- The particles sizes evolution for potentised Cuprum metallicum can clearly be differentiated from the two control groups. The sizes and the dispersion of the particles sizes is growing only in CH potentized Cuprum.
- For simply diluted samples of Gelsemium the quantity of particles is significantly lower than when a dynamisations proces has been invloved. The particles sizes and dispersion cannot clearly be differentiated between groups even if it is smallest for CH dynamisations (size just above 100nm).



A comprehensive approach

✓ Nano particles search:

are these particles specific for the remedy?

✓ Solvent (water) behaviour

✓ Electrons behaviour

SEM/EDX



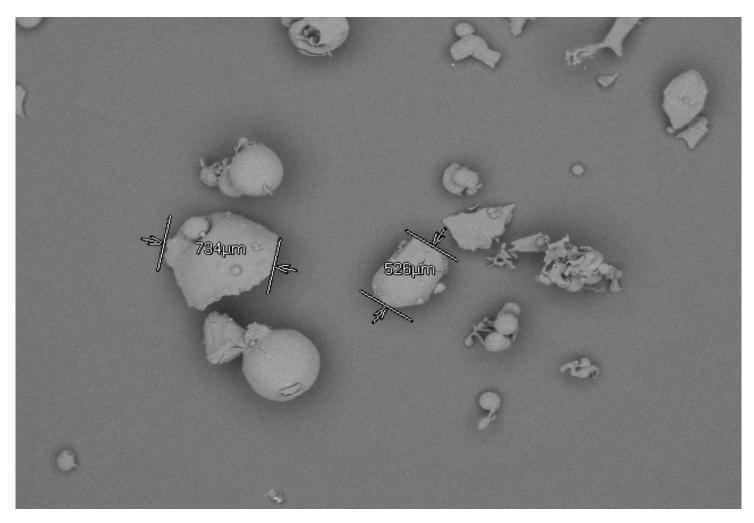
 Cuprum metallicum.
 SEM/EDX = Scanning Electron Microscopy with X-ray microanalysis.

Starting from 400cc (20 x 20cc 4CH samples), lyophilized (concentrated) we are able to identify these particles. 200cc of 200K and 30CH, contains also particles !

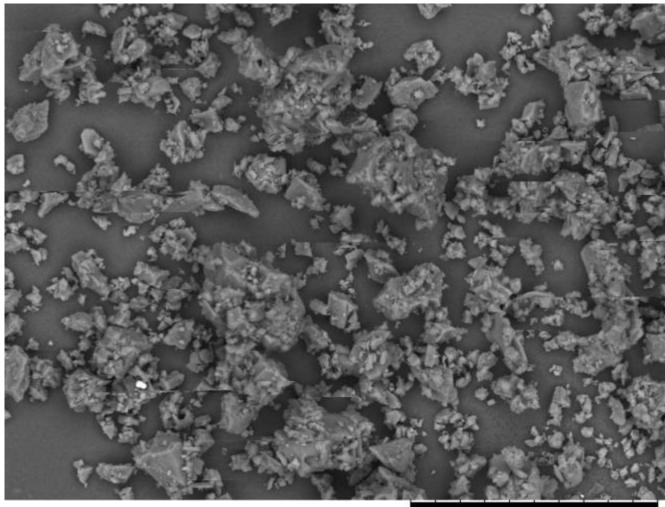
Obtained dry lyophilized material

	Cu /g	lactose/g	Incertitude/g	Dry material/g
1 CH	0.01	0.99	0	
2 CH	0.0001	0.9999	3x10 ⁻⁹	
3 CH	0.000001	0.999999	3x10 ⁻⁹	
4CH aqua	+/-10 ⁻⁸	+/-0,01	3x10 ⁻⁹	10mg (=expected)
5CH aqua	10 ⁻¹⁰	10 ⁻⁴	3x10 ⁻⁹	
6CH aqua	10 ⁻¹²	10 ⁻⁶	3x10 ⁻⁹	
7CH aqua	10 ⁻¹⁴	10 ⁻⁸	3x10 ⁻⁹	
8CH aqua	10 ⁻¹⁶	10 ⁻¹⁰	3x10 ⁻⁹	
9CH aqua	10 ⁻¹⁸	10 ⁻¹²	3x10 ⁻⁹	
30CH aqua	10 ⁻⁶⁰	10 ⁻⁵⁴	3x10 ⁻⁹	0,001mg = 1 μg
200K aqua	10 ⁻⁴⁰⁰	10 ⁻³⁹⁶	3x10 ⁻⁹	0,0025mg = 2,5 μg
Diluted 10 ⁻⁶⁰ aqua	10 ⁻⁶⁰	10 ⁻⁵⁴	3x10 ⁻⁹	0,003mg = 3 μg
Pure aqua 30CH	0	0	0	0,002mg = 2 μg

lyophilised Cuprum 4CH magnified x40 pure sample (without crushing)

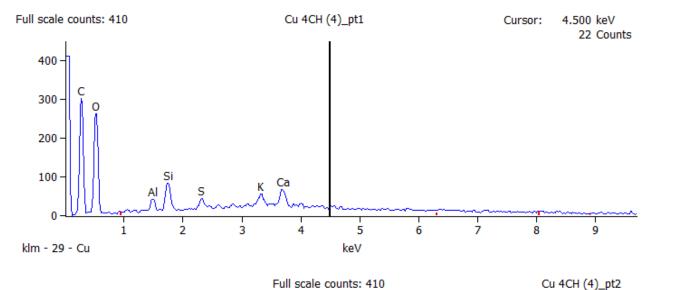


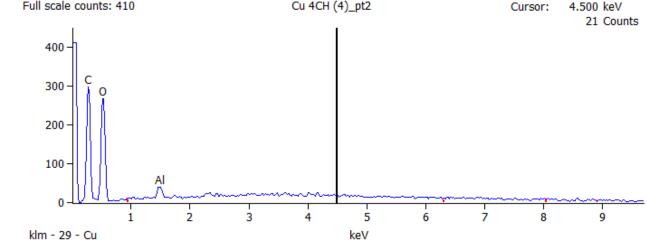
lyophilised Cuprum 4CH magnified x800 (after new crushing)



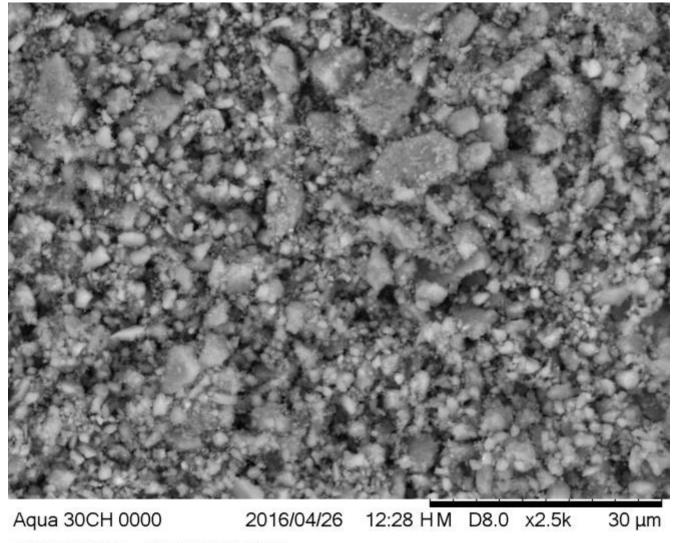
Cu 4CH 0005 2016/04/26 11:31 H M D7.9 x800 100 μm SYSMEX - Hitachi TM3030PLUS

After crushing: expected lactose + points with Calcium/Silicium in Cuprum 4CH



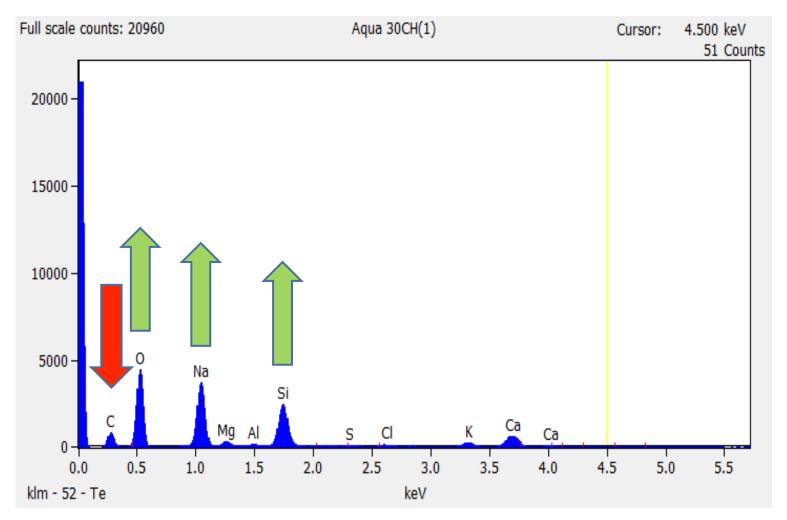


lyophilised Aqua pura 30CH magnified x2500 (triturated)

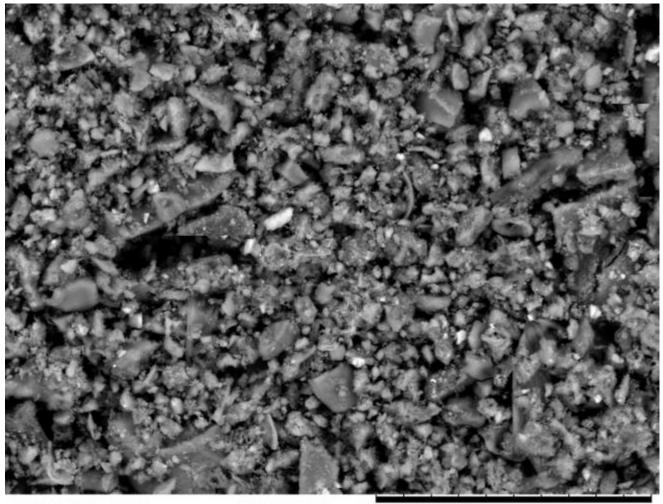


SYSMEX-Hitachi TM3030PLUS

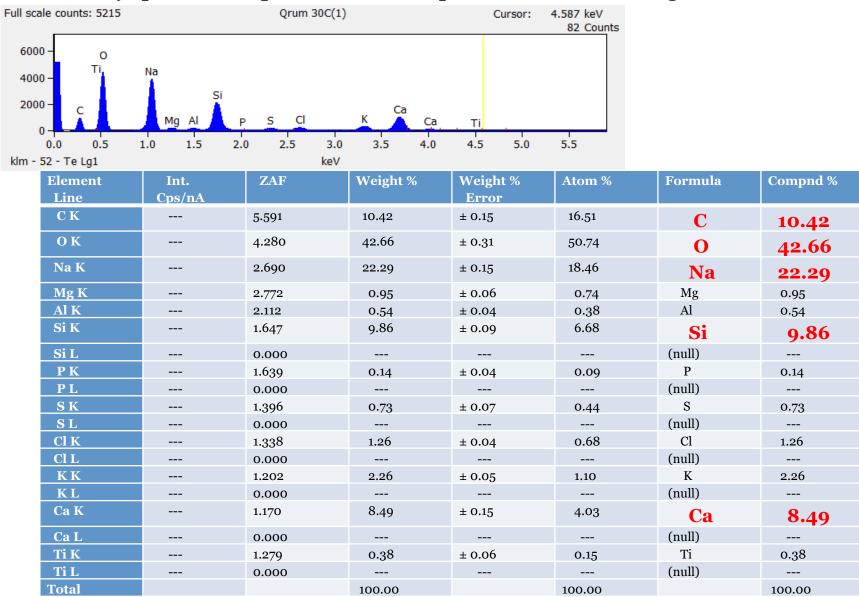
lyophilised Aqua pura 30CH composition after trituration



lyophilised Cuprum 30CH magnified x1800 (crushed)

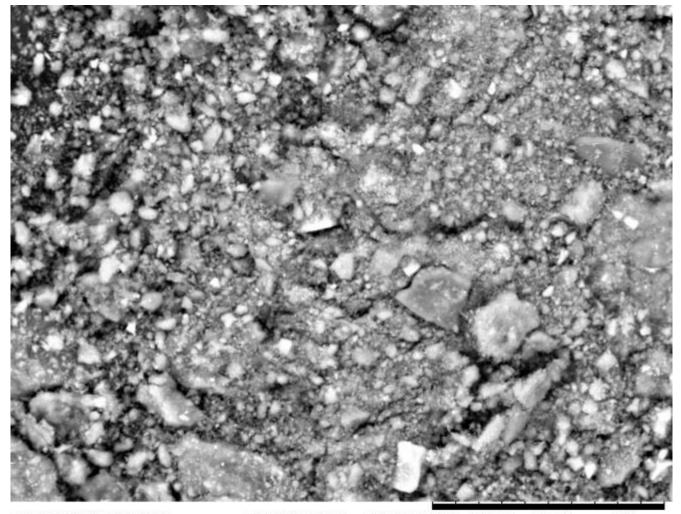


CUPR 30C 0000 2016/04/26 12:13 H M D8.0 x1.8k 50 μm Hitachi TM3030PLUS Qrum30C

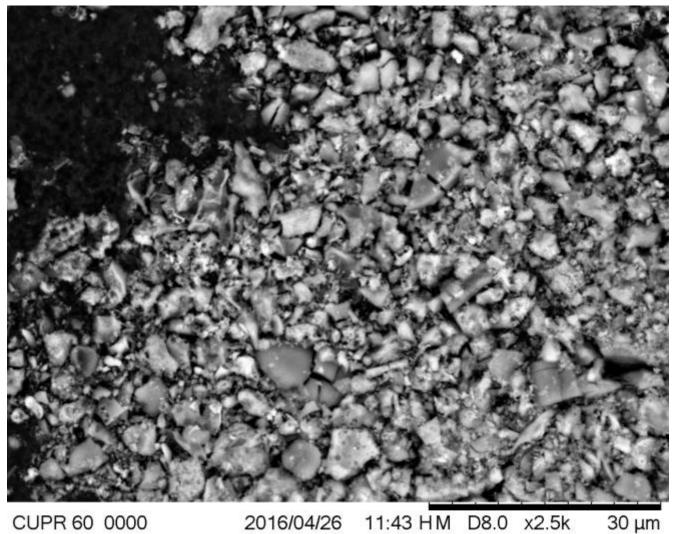


lyophilised Cuprum 30CH composition after crushing

lyophilised Cuprum 200K magnified x2500 (crushed)



CUPR 200K 0000 2016/04/26 12:01 H M D8.1 x2.5k 30 μm Hitachi TM3030PLUS Qrum 200K lyophilised Cuprum 10⁻⁶⁰ magnified x2500 (crushed)

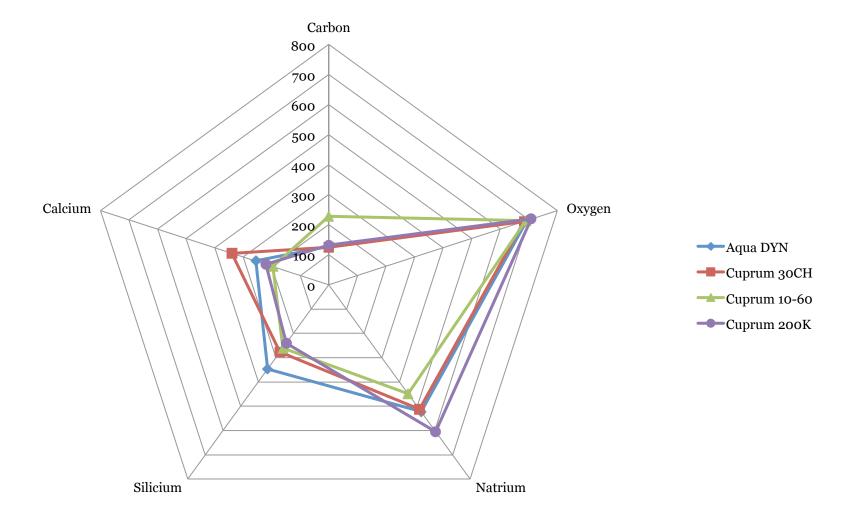


Hitachi TM3030PLUS Qrum 10 -60

Composition of dry lyophilized material

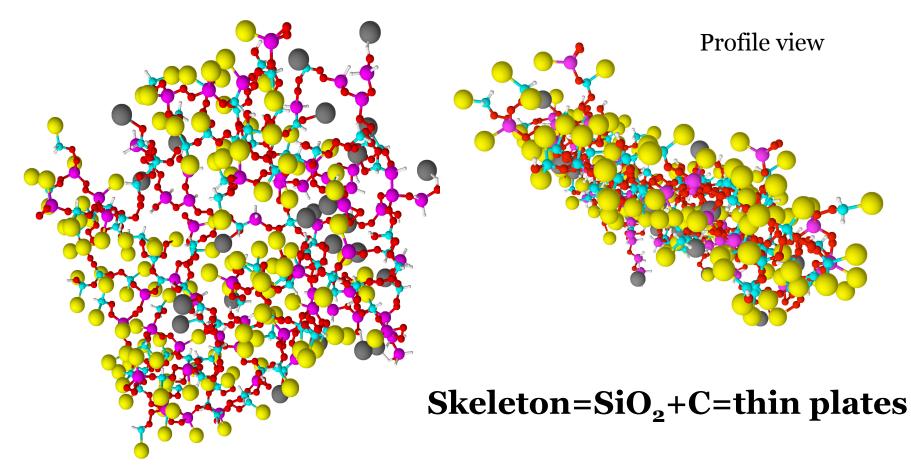
Cu	Dry material/g	С	0	Si	Na	Са	/g
4CH	10mg	49,9%	47,4%	1%			9,7 mg (lactose)
30CH	0,001mg = 1 μg	10,4%	42,7%	9,9%	22,3%	8,5%	= 1ppm
200K	0,0025mg = 2,5 μg	11%	44,3%	8,6%	26,3%	5,5%	= 2,5ppm
Diluted 10 ⁻⁶⁰	0,003mg = 3 μg	19%	43,1%	9,3%	19,5%	4,9%	= 3ppm
AQUA 30CH	0,002mg = 2 μg	10,7%	43,3%	12,4%	22,7%	6,4%	= 2ppm

Identified particles in dilutions/dynamizations (% * molecular mass).



Possible modelisation of these particles (100 smaller than in reality)

C112 H164 Ca24 Na 128 O352 Si64: Lmin 1,4nm; Lmax 1,4nm; Thickness 0,6nm. **Yellow = Na**; Red = O; Magenta = Si; Blue = C; **Grey = Ca**; White = H.



Temporary conclusions SEM/EDX (1)

- The detected particles' chemical components in **Cuprum 4CH** are essentially "C" and "O", as such it are almost pure **lactose** agglomerates (C12H22O11).
- After crushing of the powder obtained by lyophilisation of Aqua 30CH, Cuprum 200K, 30CH and the simply diluted Cu (10⁻⁶⁰) small particles are identified containing dominantly **oxygen**, **carbon**,
 silica, **natrum and calcium**. These are clearly due to the step by step preparations in glass containers (also used for simple dilutions).
- The chemical composition of the different particles is similar but not identical. More controls are needed to conclude about the specificity of these differences.

Conclusions SEM/EDX (2)

- Hypothesis: The specificity of the homeopathic remedy Cuprum metallicum may be related to the relative composition of the compounds but also to their organization, electric potential, size, size distribution and specific structure (see NTA results). More controls are in progress including other metals, chemicals and plants.
- Submission of 2 publications foreseen begin 2017.



A comprehensive approach

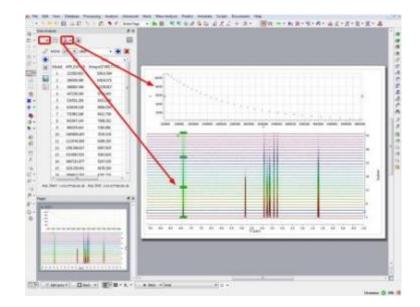
 \checkmark Nano particles search

✓ Solvent (water) behaviour

✓ Electrons behaviour



- $_{\circ}\,$ NMR: Prof. Luce Vander Elst.
- Nuclear Magnetic Resonance Spectroscopy. Calculation
 of Relaxation Times 1, 2, for the full range of
 dynamization up to 30CH.
- Aim: Collection of all values
 for Cuprum and Gelsemium.



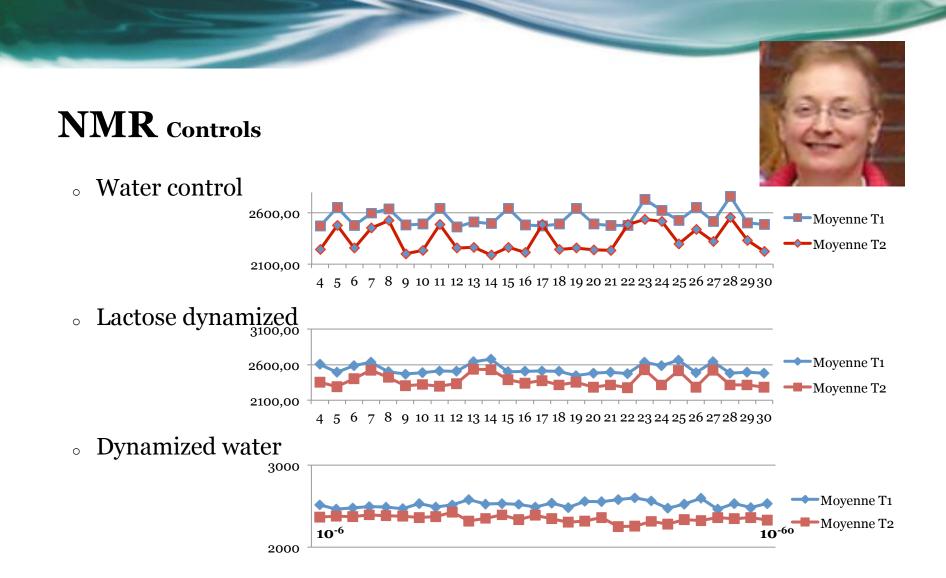


- NMR Relaxation times are correlated to the water behaviour.
- Aim: statistical discriminant analyse of NMR signals from different homeopathic remedies and different dynamizations versus 3 controls : pure water, dynamized lactose (for triturated stocks), dynamized water and simply diluted stocks.



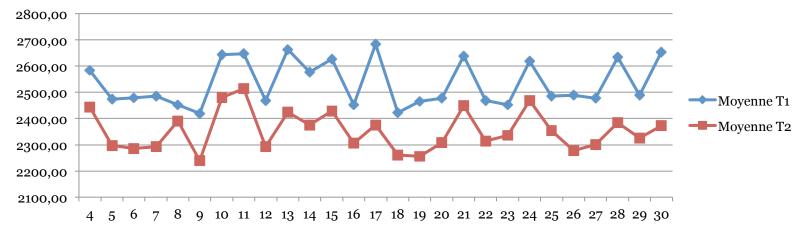




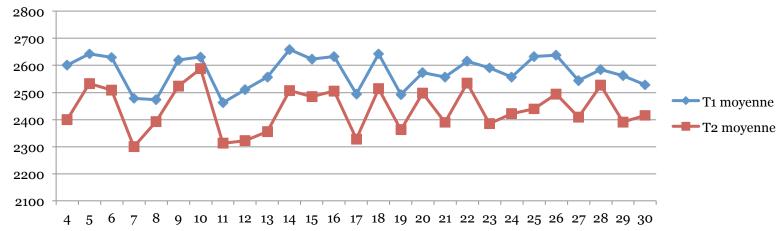




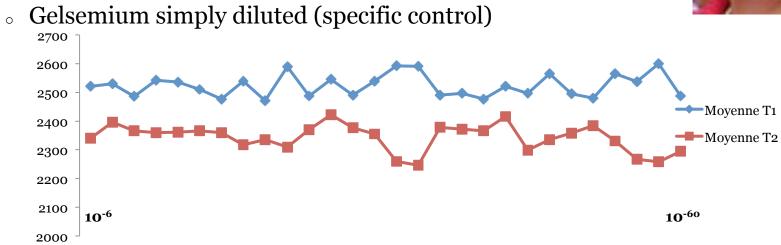
Cuprum simply diluted (specific control)



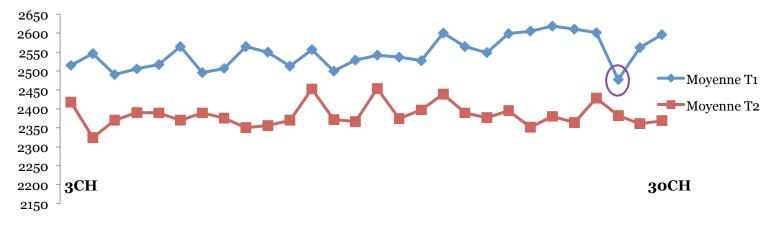
• Cuprum dynamized







。 Gelsemium dynamized



NMR Cuprum metallicum



- The measured values are mathematically and statistically significant, they are **not random noise!**
- There is a clear significant difference in water behaviour
 between the verum's and their 3 controls but also between
 Cuprum metallicum and Gelsemium sempervirens
 (specific "signatures" of the remedies). These findings
 confirm previous publications of 'Prof. Demangeat'.

NMR Cuprum metallicum



- The NMR answer of <u>simply diluted stocks</u> cannot significantly be discriminate between each other.
 The specificity of the electromagnetic signature is due to the potentization process.
- Paper will be submitted for publication very soon.



A comprehensive approach

 \checkmark Nano particles search

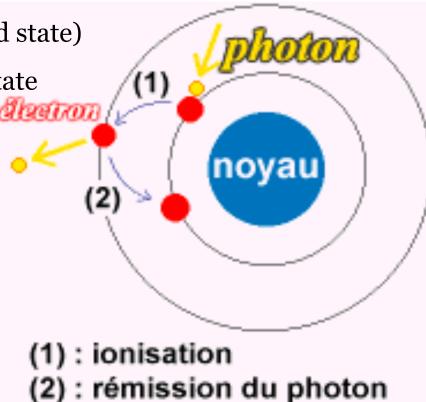
✓ Solvent (water) behaviour

✓ Electrons behaviour

Atomic ionisation process

- Electric shock of very high intensity
- Electron orbital change (excited state)
- Spontaneous return to initial state
- Photon emission

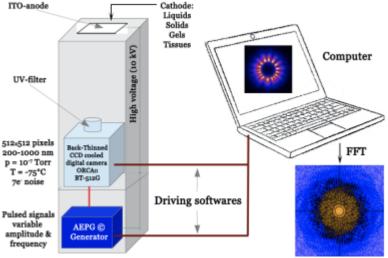
Emitted light wavelength
 linked to initial electron spin and
 orbit depending of the specificity
 of the atom.



EPA

- Electro Photonic Analyse of any "material"
- Aim: precise discrimination of each remedy but also of the dynamizations of a same stock. Allowing to test the different methods and number of dynamizations; top, midden, bottom pipetting.
- Very sensitive and specific analyse.
- One drop or one pilule is enough!





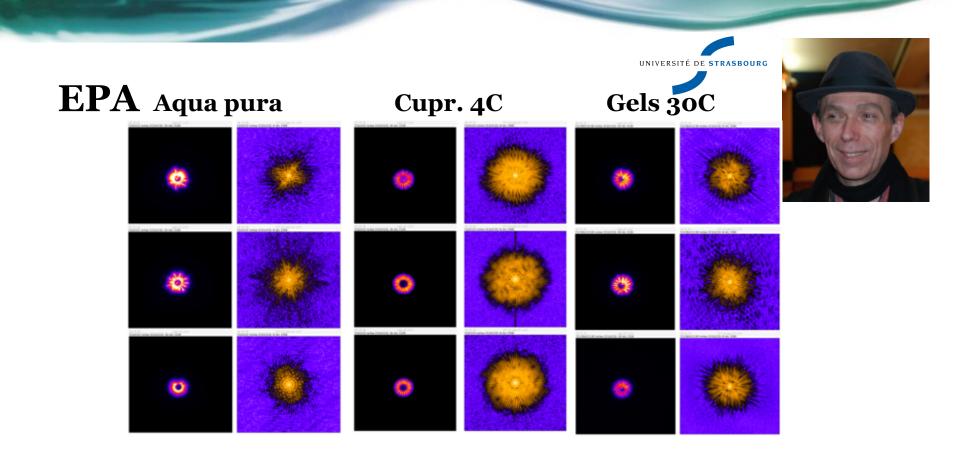
EPA

- April 2016: 607 images : 3 for 175 liquid samples and 1 for 82 impregnated pilules (size 6) samples including several controls.
- Liquids one drop of 15µl suspended at the top of the pipette tip and in contact with the electrode (10000 Volts, 400 Htz). When electric stimulation stop the emitted light is photographed.
- For globules the electrode is in contact at the top of the pilule (11000 Volts, 120 Htz).





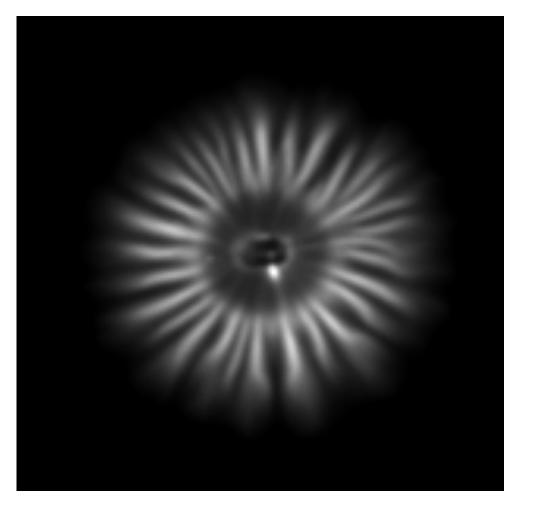




Light Energy: Unstable +/- 6.000 +/- 12.000

Ongoing Systematic Statistical Analyse. Publication submission foreseen in 2017.

EPA granules **Cuprum 4CH = low intensity 30 rays**

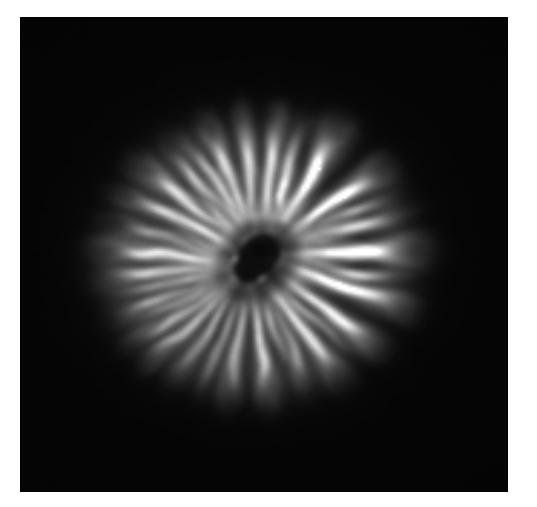




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EPA granules **Gelsemium 4CH = high intensity 34 rays**





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Signature ?

Ink added to paper. Significance is not coming from the chemical composition but from its structure and interference with solvent! A comprehensive global presentation of all results, explanations and implications for practice: May 2017.

2nd DYNHOM COLLOQUIUM **Medicine & Homeopathy** FLOICA **Basic Research** 2 **Homeopathic Practice** Saturday 13 May 2017 from 9:00 to 18:00 Crowne Plaza Brussels Airport Hotel

Da Vincilaan 4 1831 Diegem (Brussels) Belgium MediCongress Noorwegenstraat 94 9940 Evergem (Gent) Belgium

or

charlotte@medicongress.com

DYNHOM

Thanks for your attention!

