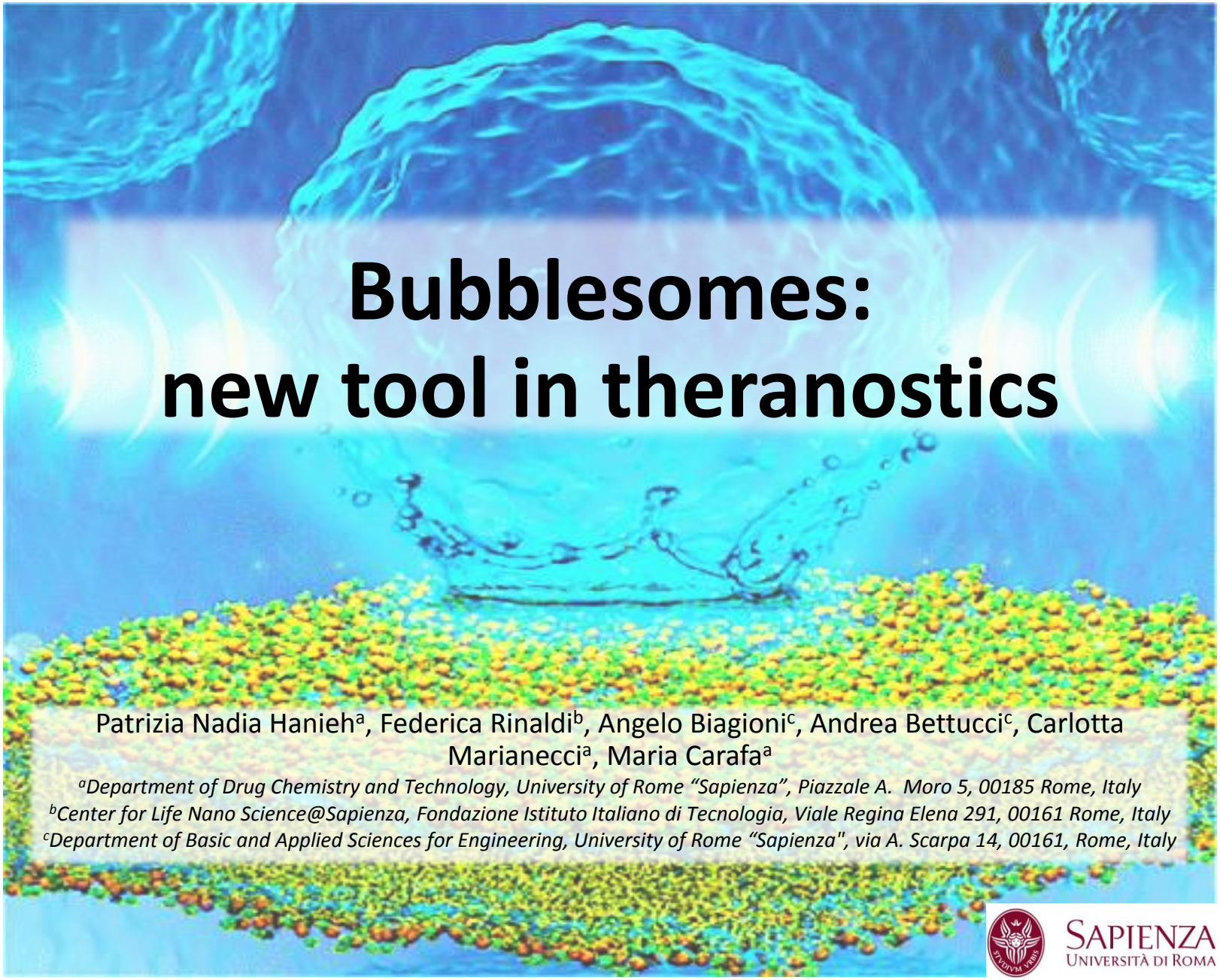
Bubblesomes: new tool in theranostics



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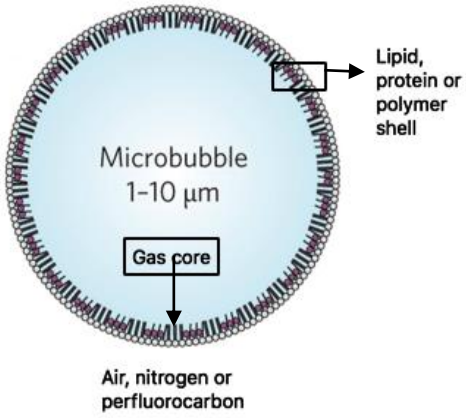
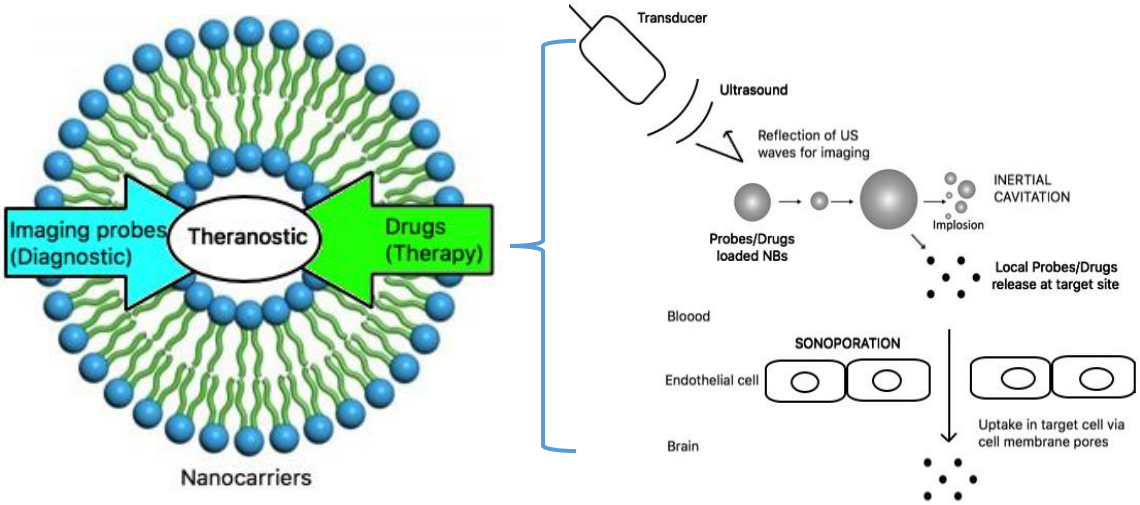
Aim





Ultrasound Contrast Agents (UCAs)

- **Application only as contrast agents**
- **Micrometric dimensions**
- **Instability over time**

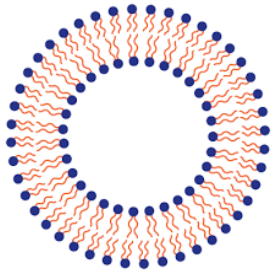
Nanobubbles (NBs)

✓Theranostic carrier

✓Nanometric dimensions

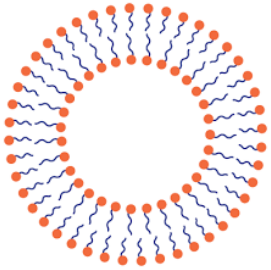
✓Increase the physico-chemical stability

Bubblesomes®

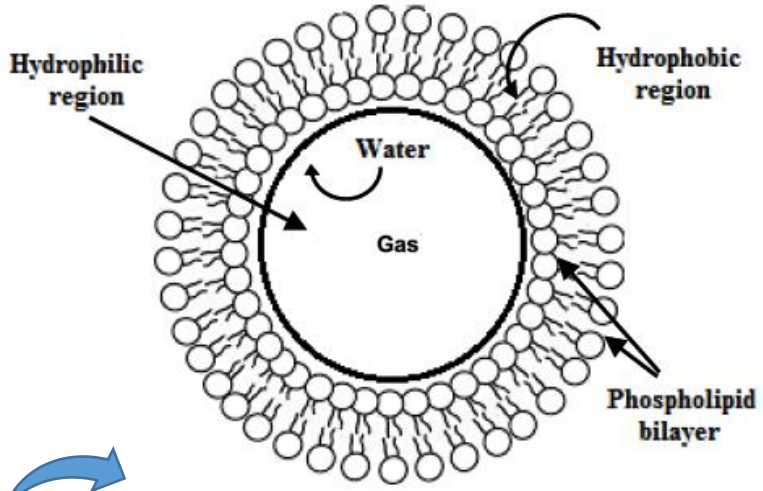


Phospholipid (P1 or P2 or P3)

Gas (Contrast agent)

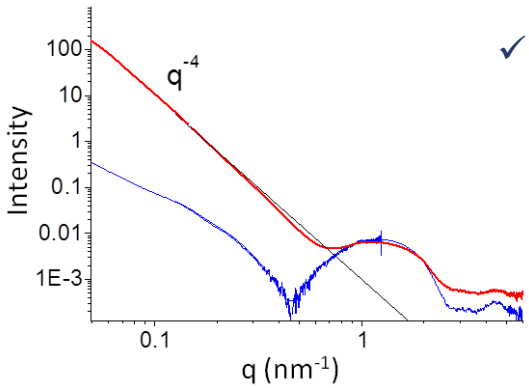


Surfactant (S1)



Small Angle X-Ray Scattering (SAXS) measurements:

- **Red line**: the intensity profile shows the characteristic features of a lipophilic bilayer and the system is unilamellar
- **Blue line**: in the high q region, the intensity profile of nanobubbles is superimposed to the previous line. Instead, in the low q region, the q^{-4} intensity decay shows the presence of a nanobubbles.



- ✓ **Spherical vesicles**
- ✓ **Presence and localization of the gas in the vesicles**
- ✓ **The NBs mean dimensions were greater than 120 nm**

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Dynamic Light Scattering (DLS) characterization

Sample	Size (nm)±SD	ζ-Potential (mV) ±SD	Polydispersion Index
NBs (P1)	151,90 ± 3,55	-4, 35 ± 0,15	0,25
NBs (P2)	153,10 ± 2,20	-70,90 ± 1,60	0,20
NBs (P3)	166,00 ± 1,88	-15,70 ± 0,57	0,10
NBs (S1)	243,30 ± 3,70	-41,90 ± 1,82	0,12

- ✓ **Nanometric size**
- ✓ **Monodisperse**

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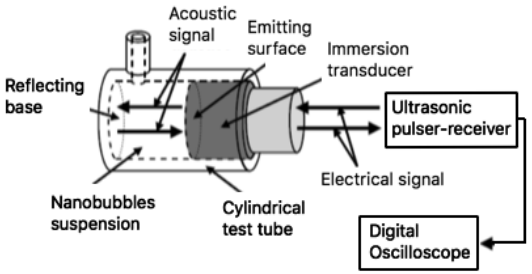
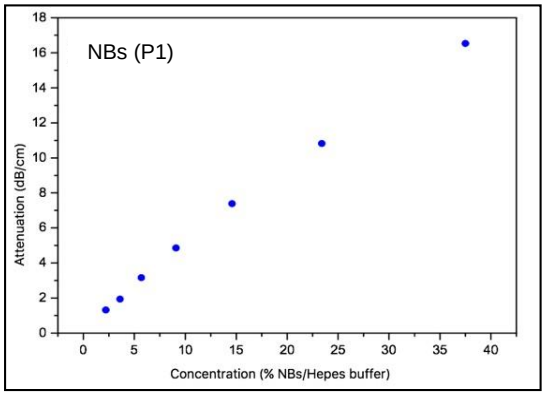
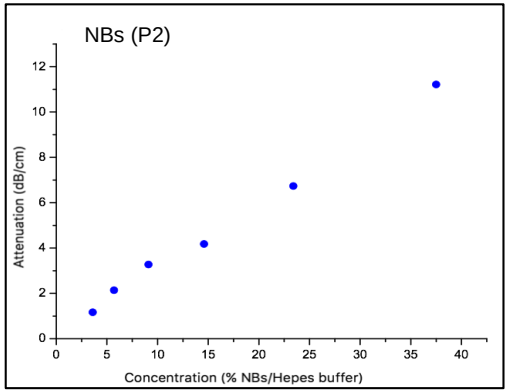
Acoustical studies

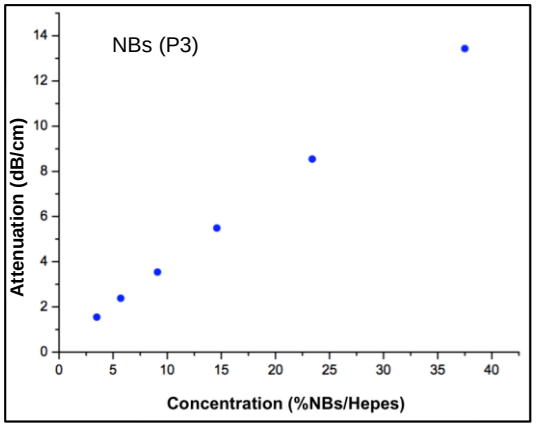
Pulse-echo

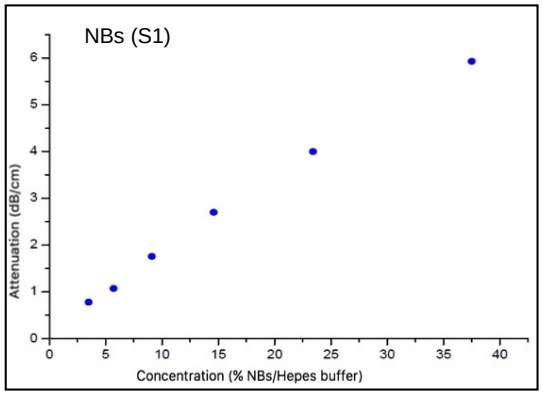


Physical technique used in clinic for the image creation
(the reflection in the human body of acoustic waves)









The dependence of the attenuation coefficient on the NBs concentration in hepes buffer.

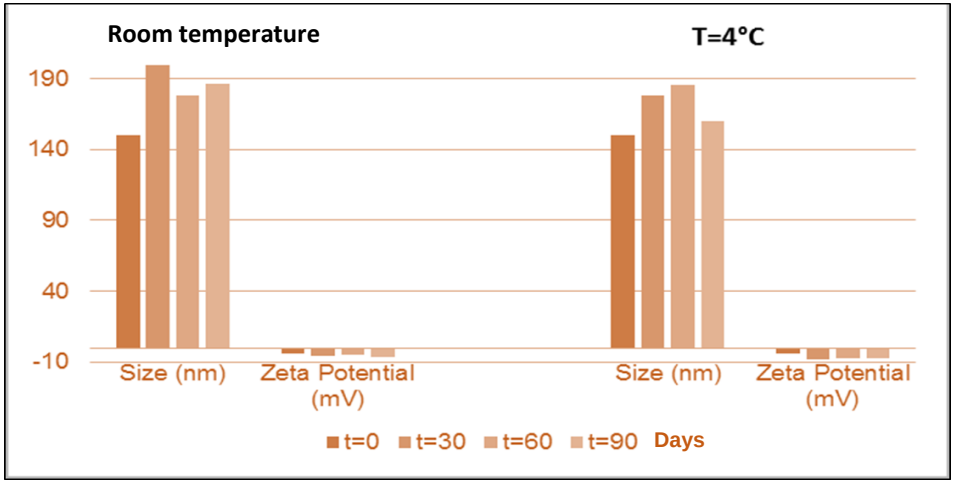
At the 14 MHz (central frequency), the several formulations of NBs show an attenuation value of 6 - 20 dB/cm.

The Bubblesomes® show an attenuation value equal or greater than the commercially UCAs

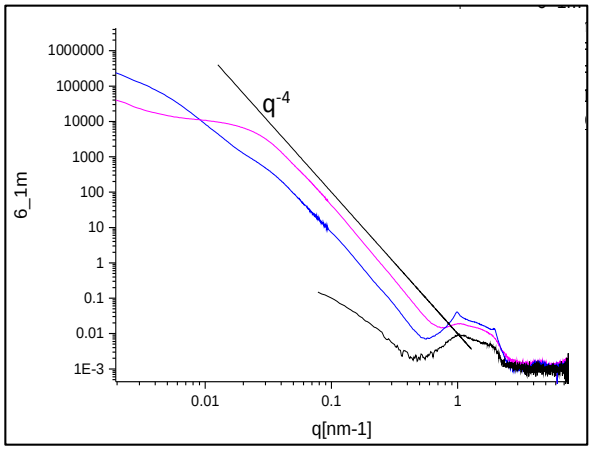
Stability studies

- DLS measurements: size and ζ-potential variations measured during a period of 3 months at two different storage conditions (4°C and room temperature);
- Pulse-echo measurements: attenuation variations measured immediately after the preparation and then after 3 months;
- SAXS measurements: structural variations measured immediately after the preparation and then after one month.

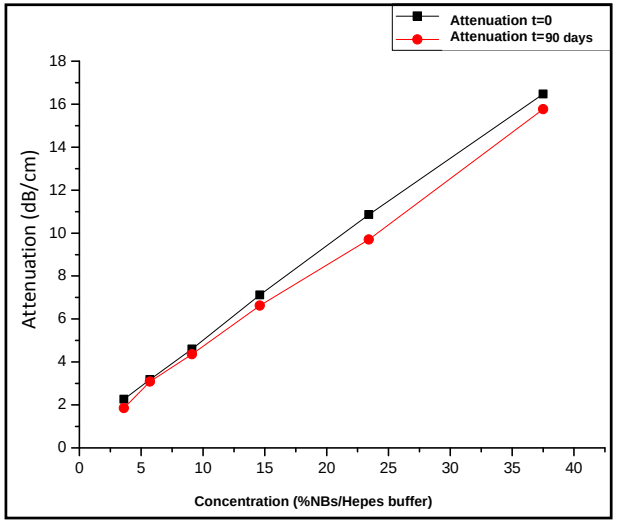
DLS



SAXS

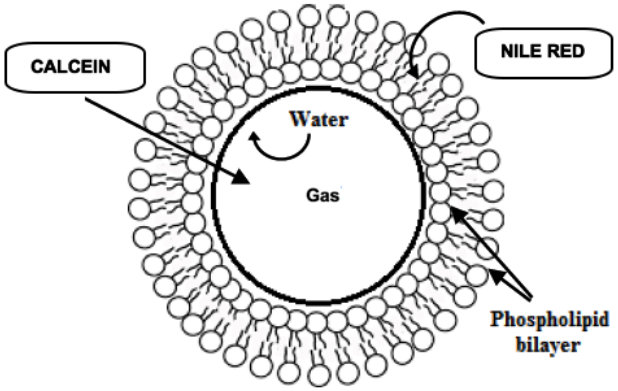


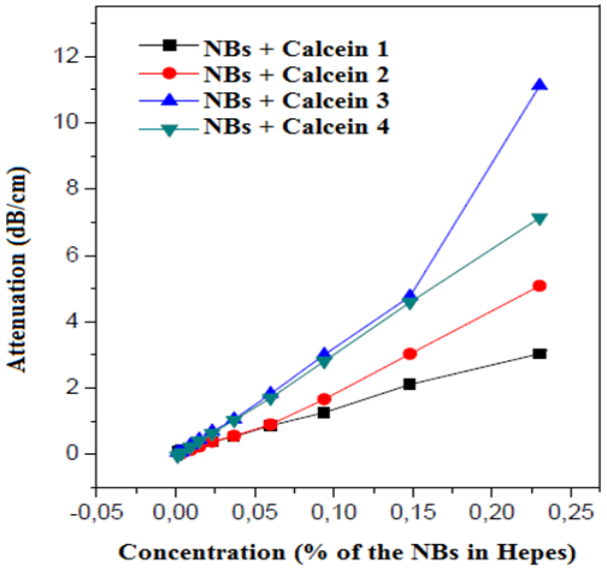
Pulse-echo



- ✓ **The vesicles are stable during the period of storage**
- ✓ **The entrapment efficiency of the gas in the NBs is the same after three months**
- ✓ **The morphology of the NBs do not change after one month**

Bubblesomes® are theranostic systems



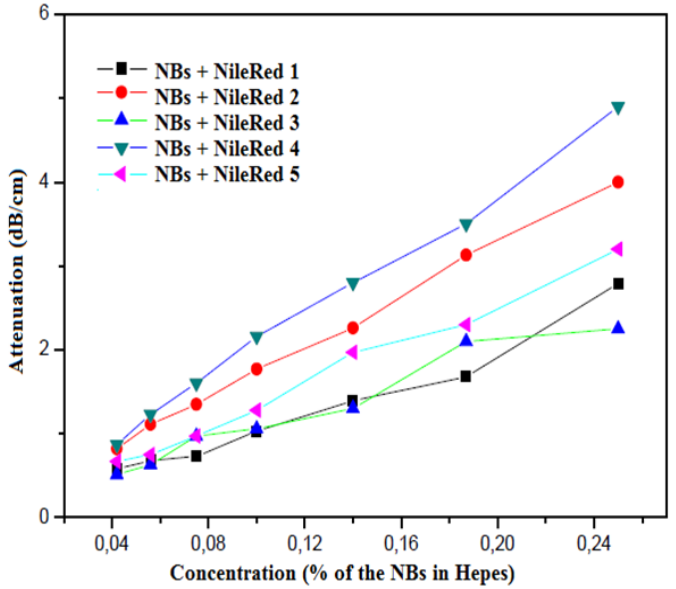


Vesicles + with or without gas +
Calcein (fluorescence) or Nile Red (absorbance)

Fluorescence and absorbance analyses: The gas does not affect the entrapment efficiency of probes

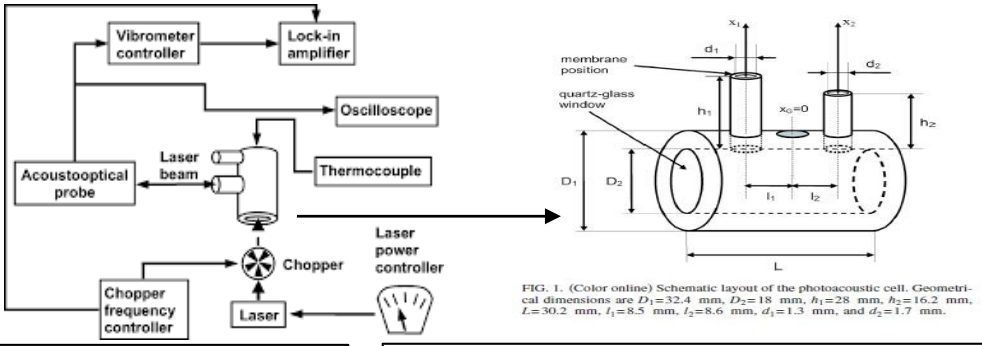
Pulse-echo measurements: The probes do not affect the acoustic efficiency of NBs

NBs show the ability to retain their acoustic efficiency in presence of probes/drugs



Bubblesomes® and Sonovue®

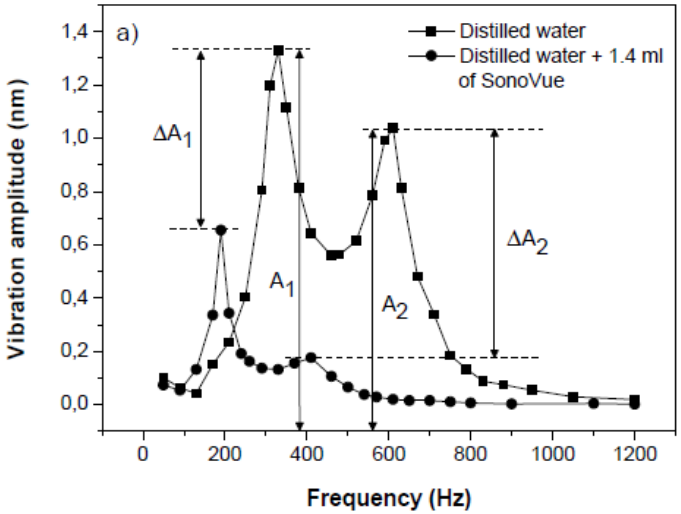
To evaluate acoustic behaviour of both micro- or nano-bubbles.

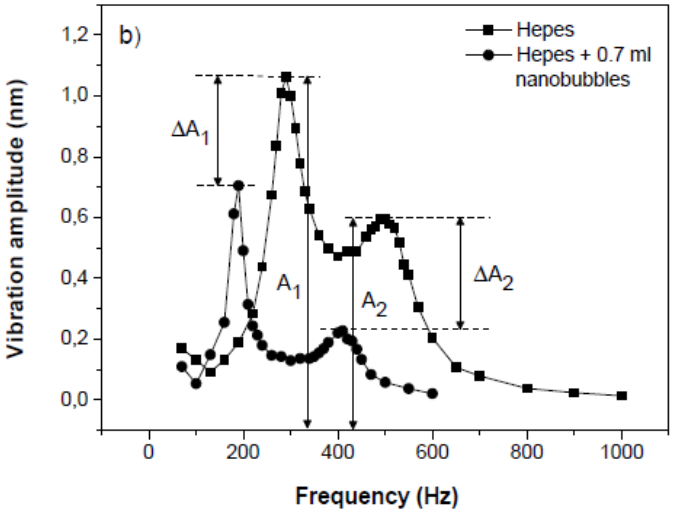


Photoacoustic measurement: is detecting the vibrational amplitude (nm) of a membrane on top of the cell

1.4 ml of SonoVue® diluted in 7.68 ml of distilled water
$(\Delta A_1/A_1)_{SV} \simeq 0.77$ and $(\Delta A_2/A_2)_{SV} \simeq 0.48$

0,7 ml of Bubblesomes ® diluted in 7.68 ml of Hepes buffer
$(\Delta A_1/A_1)_{NB} \simeq 0.54$ and $(\Delta A_2/A_2)_{NB} \simeq 0.33$





ΔA=the variation of vibration amplitude
A_1=1st resonant frequency (200-300 Hz)
A_2=2th resonant frequency (400-600 Hz)

At the same volume of bubbles diluted in a medium, Bubblesomes® show a greater acoustical efficiency (30%) than the SonoVue®

…Following

- **Bioavailability studies**

- **Blood-Brain barrier opening and closing (in vivo)**
- **Model molecules brain localization**
- **Acoustical characterization by using a preclinical ultrasound scanner**

At the College of Medicine and Veterinary Medicine (University of Edinburgh)

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Summary and Conclusions

The Bubblesomes® show:

- **Nanometric size;**
- **Good physico-chemical properties in terms of size, ζ-potential, microviscosity, polarity and fluidity;**
- **Stability for three months;**
- **Stability in the biological fluids (data not shown);**
- **Ability to entrap and release several drugs/probes (data not shown);**
- **Ability to maintain their acoustic efficiency in presence of drugs/probes;**
- **Acoustic efficiency equal or greater than the commercially UCAs.**

The characterized Bubblesomes might be considered a promising system for diagnostic imaging and therapeutic delivery
(Patent Pending).

Acknowledgement

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Thank You for Your Attention