Static and Dynamic Analysis of Contrast Agents – Parameter Estimates and the Effect of Constitutive Law

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Funding: "HERACLEITUS II" Program, Greek Ministry of Education

European Union
European Social Fund
OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS, CULTURE & SPORTS
MANAGING AUTHORITY
Co-financed by Greece and the European Union
NSRF 2007-2013
programme for development
EUROPEAN SOCIAL FUND

9th EUROMECH Fluid Mechanics Conference, Rome 2012

Microbubbles (Contrast Agents)

- Bubbles surrounded by an elastic membrane for stability
- Low density internal gas that is soluble in blood
- Diameter 1 to 10 μm
- Polymer, lipid or protein (e.g. albumin) monolayer shell of thickness 1-30 nm

Motivation

- Contrast perfusion imaging $\Rightarrow$ check the circulatory system by means of contrast enhancers in the presence of ultrasound (Sboros et al. 2002; Frinking & de Jong, Postema et al., Ultrasound Med. Bio. 1998, 2004)

- Sonoporation $\Rightarrow$ reinforcement of drug delivery to nearby cells that stretch open by oscillating contrast agents (Marmottant & Hilgenfeldt, Nature 2003)

- Micro-bubbles act as vectors for drug or gene delivery to targeted cells (Klibanov et al, Adv. Drug Delivery Rev., 1999; Ferrara et al. Annu. Rev. Biomed. 2007)

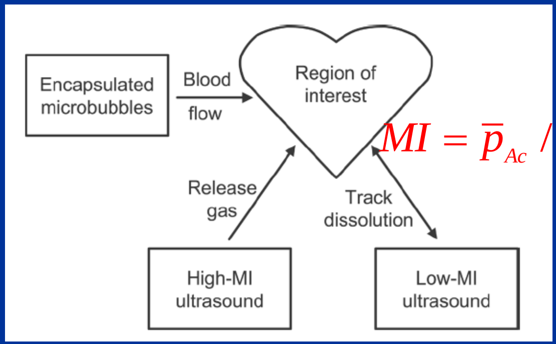
- **Need for specially designed contrast agents**

  Controlled pulsation and break-up for imaging and perfusion measurements

- **Chemical shell treatment for controlled wall adhesion for targeted drug delivery**

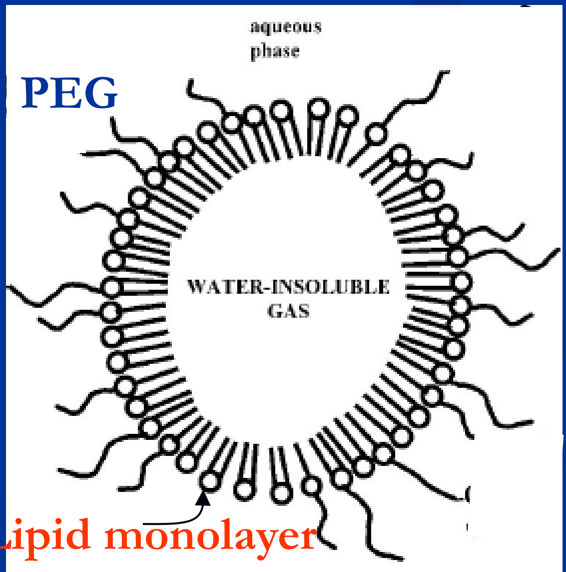
- **Need for models covering a wider range of CA behavior (nonlinear material behavior, shape deformation, buckling, interfacial mass transport etc, compression vs expansion only behavior, nonlinear resonance frequency- thresholding)**

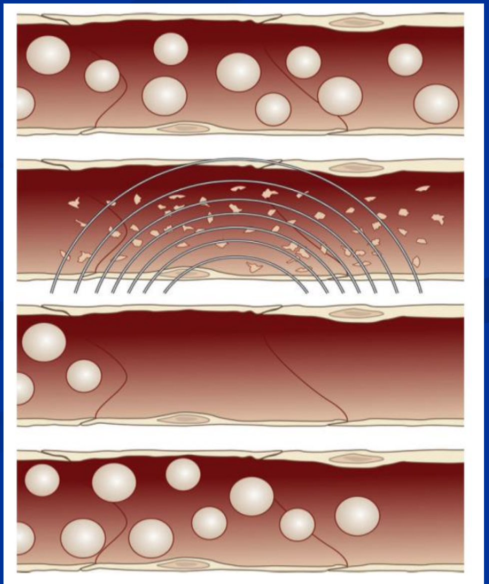
- **Need to understand experimental observations and standardize measurements in order to characterize CA's**



$$MI = \bar{p}_{Ac} / \sqrt{\nu_f}$$

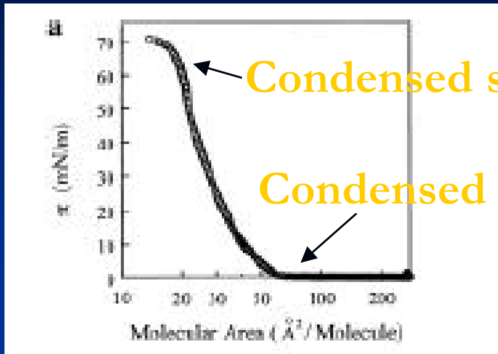
Contrast enhanced perfusion imaging, via a sequence of low and high Mechanical Index (MI) ultrasound pulses





- Shell is important for designing and controlling the behavior of contrast agents

Phase Diagram



- Phase transitions occur as the available area per lipid molecule decreases, $\pi=\gamma_0-\gamma$

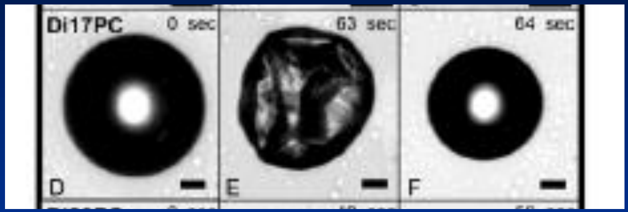
π-A isotherms in a Langmuir trough

γ_0: surface tension of substrate

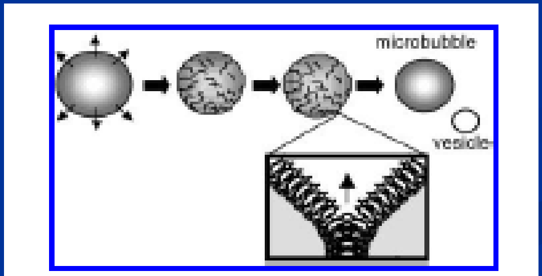
γ: surface tension of lipid surfactant monolayer

Wang et al. J. Phys. Chem. 1996

Static Response



- They buckle when gas diffuses through the shell (Borden & Longo, Langmuir 2002)



- They regain sphericity via a zippering mechanism that binds the hydrophobic tails of lipid monolayer

- Borden & Longo, Langmuir (2006→Shedding of excess lipids (**lipid shedding** or budding)
- Lee et al., Annu. Rev. Phys . Chem (2008)→ **Formation of bilayers** (reversibility)

• Dynamic Response

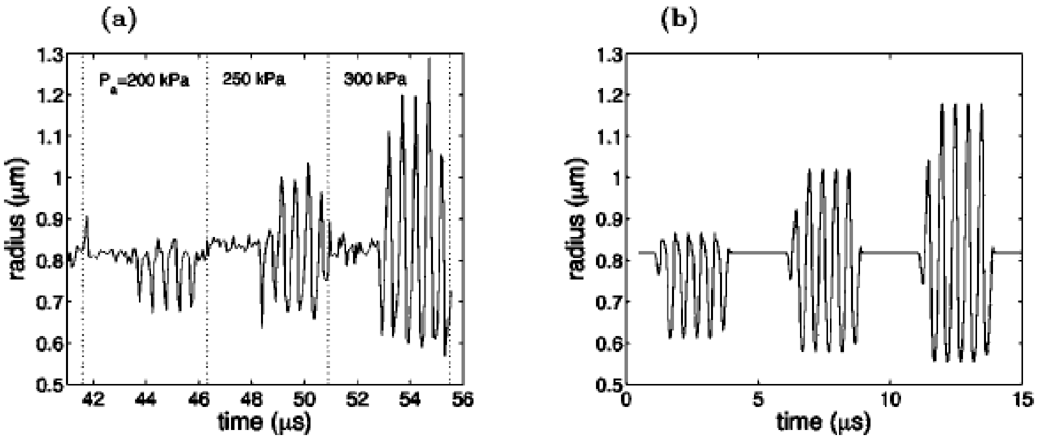
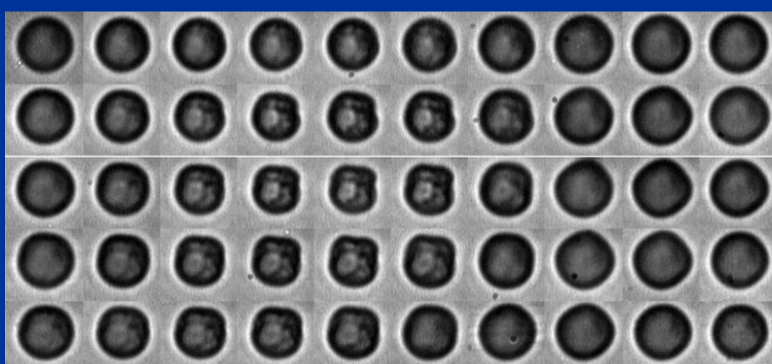
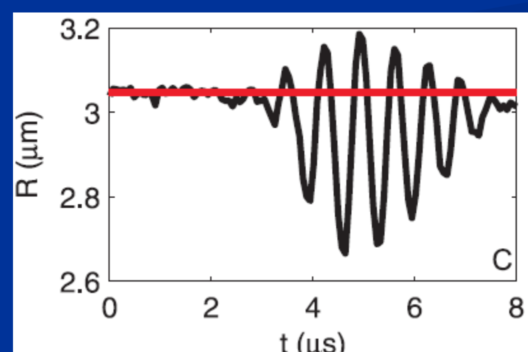


FIG. 8. (a) Experimental recordings of a BR14 bubble response to repeated 2 MHz pulses separated by 60 ms, with an increasing acoustic pressure. (b) Simulation with the same acoustic pressures. The fitted shell parameters are $R_{buckling}=R_0=0.82\ \mu m$, $\chi=1$ N/m, $\kappa_s=7.2\times10^{-9}$ N, while the critical break-up is $\sigma_{break\text{-}up}=0.13$ N/m.

- **Compression only behavior at low amplitudes followed by expansion only at large amplitudes, Marmottant et al. JASA 2005**





M. Overvelde,
Ph. D Thesis (2010)
Univ. Twente

$P_{Ac} = 40\text{kPa}, \quad \varepsilon = 0.4$

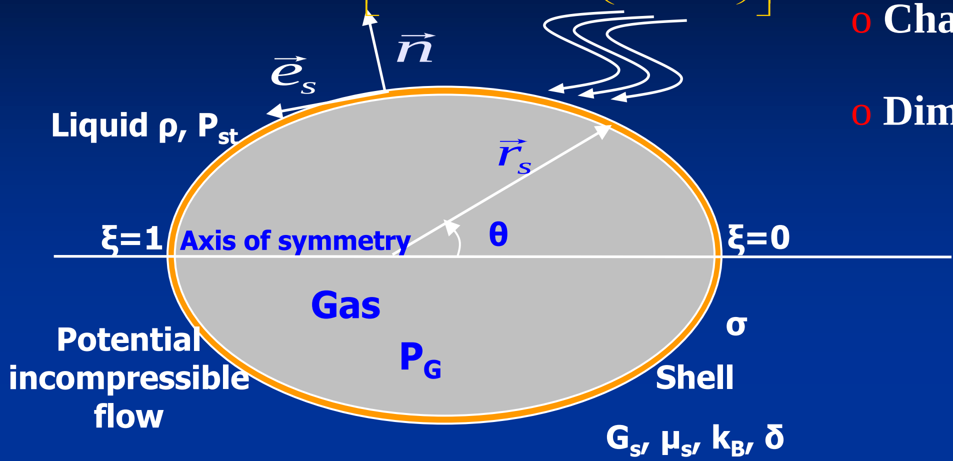
BR – 14

- **During “compression only” behavior, the microbubble is mainly deformed during compression**

Axisymmetric Pulsations

$$P'_\infty(t') = P'_{st}\left[1+\varepsilon\cos\left(\omega_f t'\right)\right],\quad \omega_f = 2\pi\nu_f$$

$G_{S,2d} = \delta G_S$



- Characteristic space and time scales: $R_{Eq},\ \sqrt{\rho R_{Eq}^3 / G_{S.2d}}$
- Dimensionless parameters:

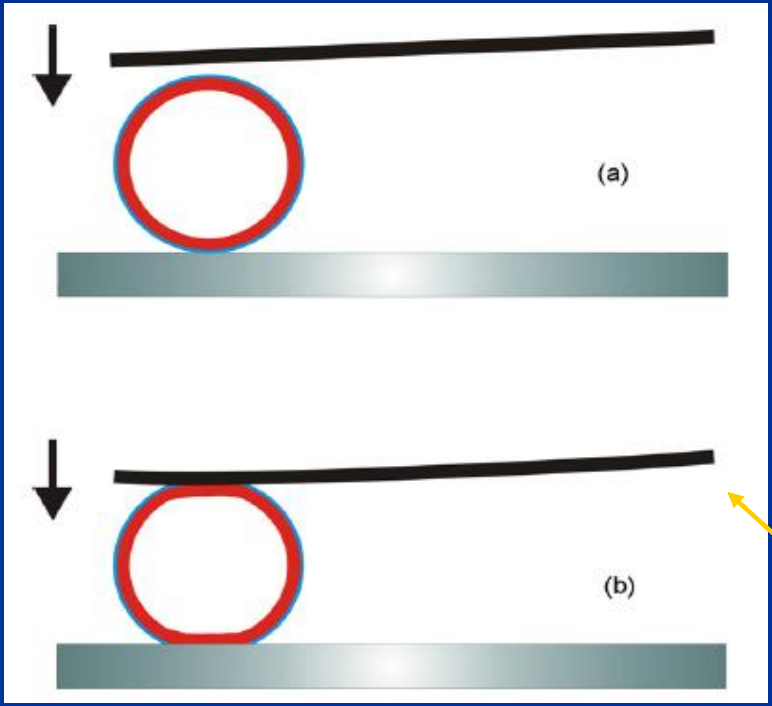
$$\omega_f = \frac{\omega'_f}{\sqrt{G_{S,2d}/\left(\rho R_{Eq}^3\right)}}\qquad P = \frac{P_{St}}{\rho G_{S,2d}^2 / R_{Eq}^2},\qquad We = \frac{G_{S,2d}}{\sigma}$$

$$Re_l = \sqrt{\frac{\rho G_{S,2d} R_{Eq}}{\mu_l^2}}\qquad B = \frac{k_B}{G_{S,2d} R_{Eq}^2}\qquad Re_s = \sqrt{\frac{\rho G_{S,2d} R_{Eq}^3}{\mu_S^2}}$$

- Axisymmetry
- Ideal, irrotational flow
- Incompressible surrounding fluid with a sinusoidal pressure change in the far field
- Ideal gas in the microbubble undergoing adiabatic pulsations
- Very thin viscoelastic shell, often a phospho-lipid monolayer, whose behavior is characterized by the constitutive law, e.g. Hookes's, Mooney-Rivlin or Skalak law
- The shell exhibits bending modulus that determines bending stresses along with curvature variations
- The shell may be pre-stressed but is always at equilibrium
- The shell parameters are: area dilatation modulus, $\chi = 3G_s\delta$, shear viscosity, μ_S, degree of softness or area compressibility, b or C, for strain softening or strain hardening shells and the bending modulus, k_B

- Shell viscosity dominates liquid viscosity, $Re_s << Re_l$ and we can drop viscous stresses on the liquid side.
- Therefore the tangential force balance is satisfied on the shell with the viscous and elastic stresses in the shell balancing each other.

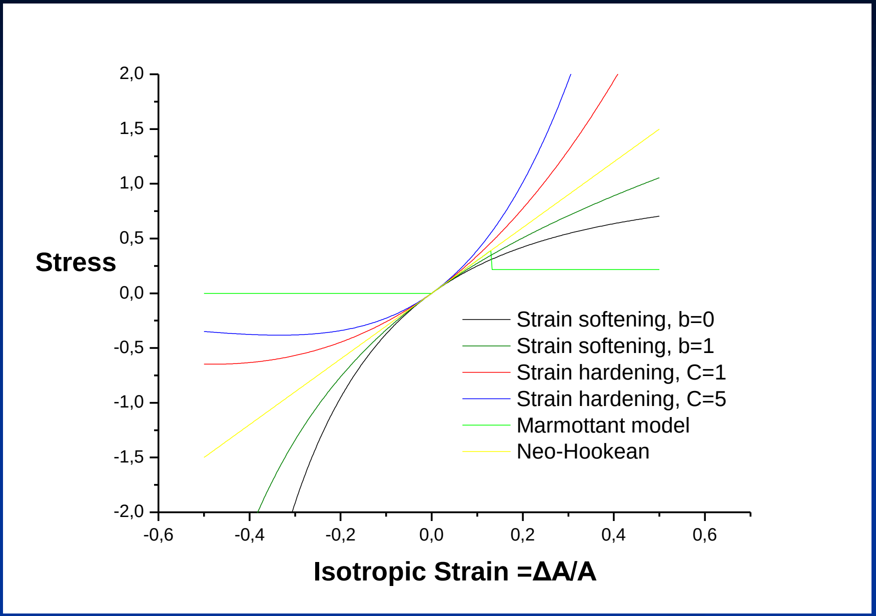
Axisymmetric Static Deformation



- Obtain estimates of bending and stretching elasticities based on AFM measurements
- Solution of the normal and tangential force balance along with the torque balance for a shell of small but finite thickness
- Point or distributed loads are considered
- The effect of gas compressibility is also considered

Schematic of an AFM setup for compression of single hollow microsphere with a cantilever

Shell Constitutive Laws-Isotropic Tension



- Linear behavior ⟶ Hooke's law
  Kelvin-Voigt law with viscous stresses

$$T_1^H = G_S \frac{1+\nu_s}{1-\nu_s}\left[\lambda^2 - 1\right] = K\left(\lambda^2 - 1\right) = K\frac{\Delta A}{A}$$

K: area dilatation modulus,

G_S: shear modulus

ν_s: surface Poisson ratio

ΔA/A: relative area change

- Strain softening material (e.g. lipid monolayer) 2D Mooney-Rivlin law

$$T_1^{MR} = \frac{G_{MR}\left(\lambda^4 + \lambda^2 + 1\right)}{\lambda^6}\left[\lambda^2 - 1\right]\left[\Psi + \lambda^2\left(1-\Psi\right)\right], \quad 0 \le \Psi \le 1$$

$\Psi = 1-b$: degree of smoothness, unlimited area dilatation

- Strain hardening material (e.g. red blood-cell membrane that consists of a lipid bilayer) Skalak law

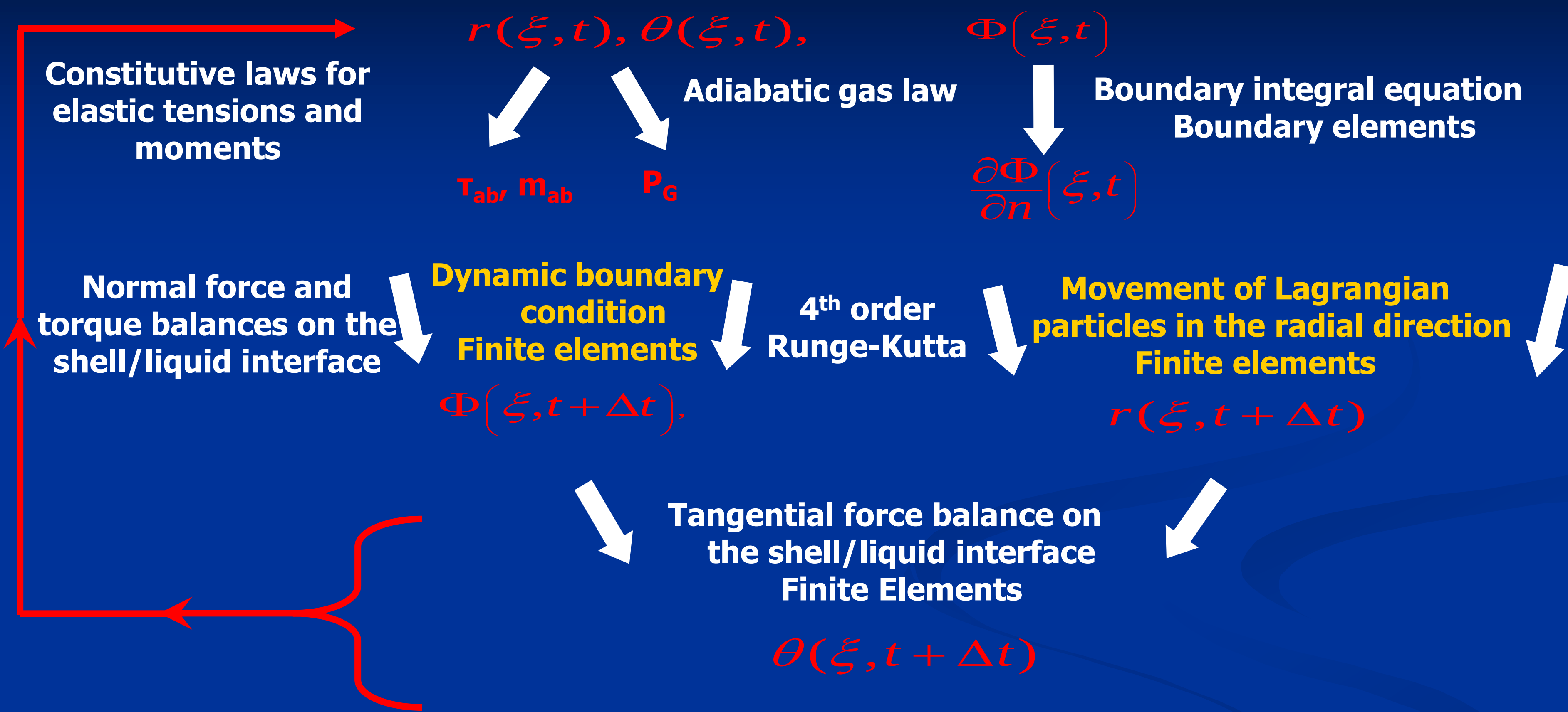
$$T_1^{SK} = G_{SK}\left[\lambda^2 - 1\right]\left[1 + C\lambda^2\left(1+\lambda^2\right)\right], \quad 1 \le C,$$ C: degree of area compressibility

In the limit of small deformations, $||e_i|| << 1$, all hyperelastic laws reduce to Hooke's law

$$MR: G_{MR} \to G_S \text{ when } \nu_S = 1/2, \quad SK: G_{SK} \to G_S \text{ when } \nu_S = C/(1+C),$$

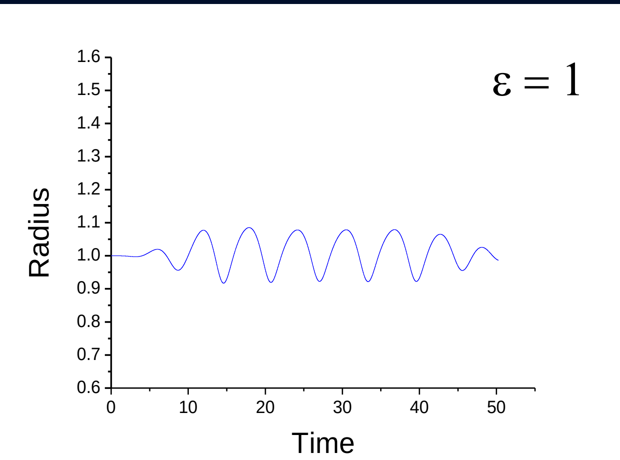
Numerical Methodology

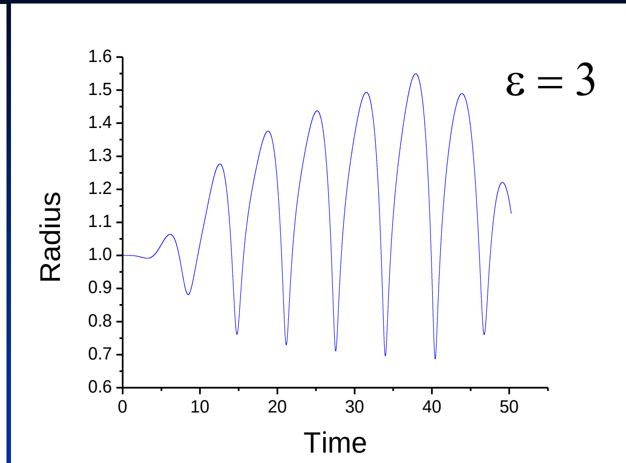
Algorithm

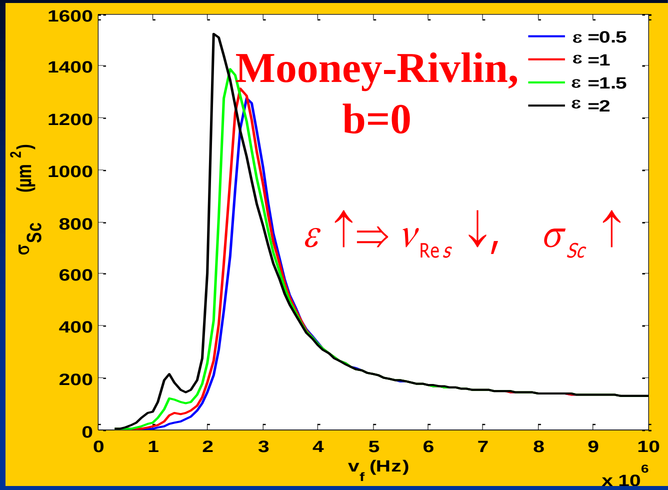


- Stability analysis is also performed on radially pulsating microbubbles, in order to identify
  - ♦ the eigenfrequencies of axisymmetric shape modes of coated microbubbles
  - ♦ the phase diagram, i.e. amplitude and frequency of the acoustic disturbance as a function of bubble radius, for parametric shape mode excitation and dynamic buckling

Radial Pulsations - Effect of sound amplitude

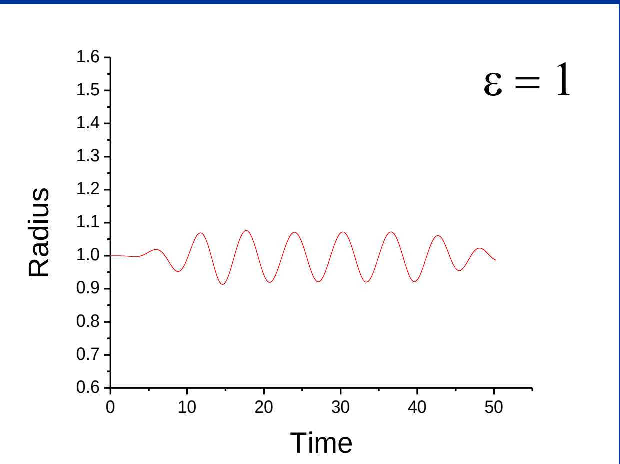


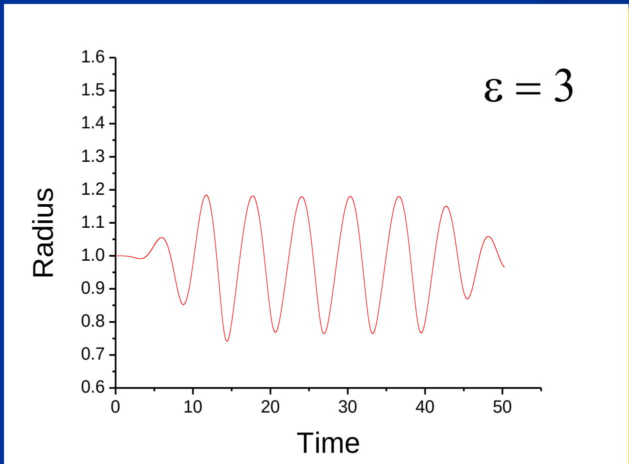


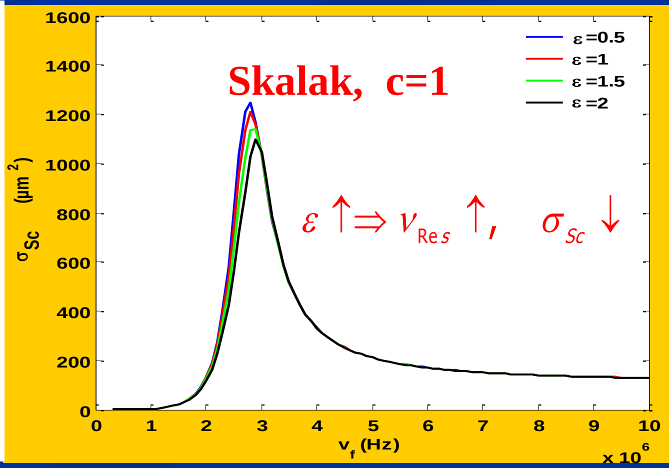


Strain softening shells (e.g. lipid monolayers prefer to be at expansion "expansion only behavior" – Their resonance frequency decreases with sound amplitude

ρ_l=998 kg/m^3, σ=0.045 kg/s^2, R =3*10^{-6} m, G_s=35 MPa, μ_S=0.6 kg m^{-1}s^{-1}, δ=15 nm







Strain hardening shells (e.g. lipid bilayers prefer to be at compression "compression only behavior" – Their resonance frequency increases with sound amplitude

Parametric Stability- Resonance-Dynamic Buckling

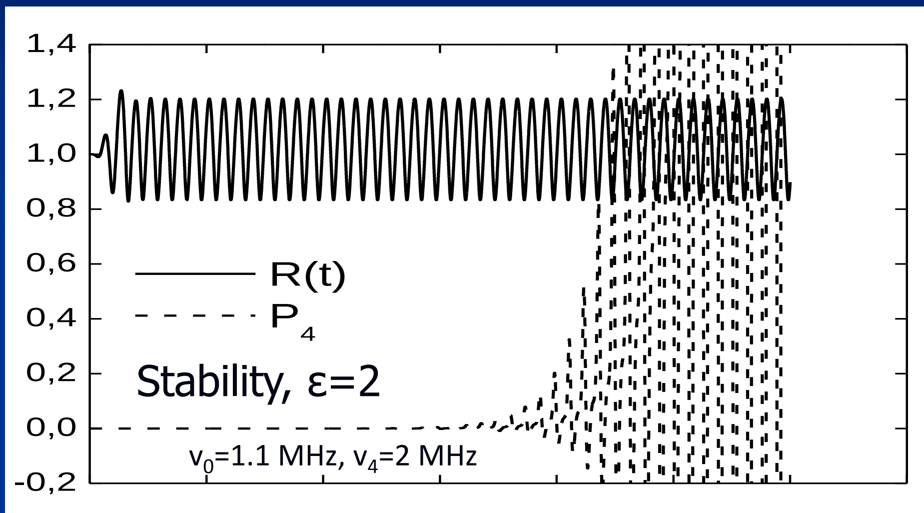
$$R_{eq} = 3.6\ \mu m,\ G_s = 80\ MPa,\ \delta = 1\ nm,\ \mu_s = 20\ Pa \cdot s,\ b = 0,\ \ \nu = 0.5,$$

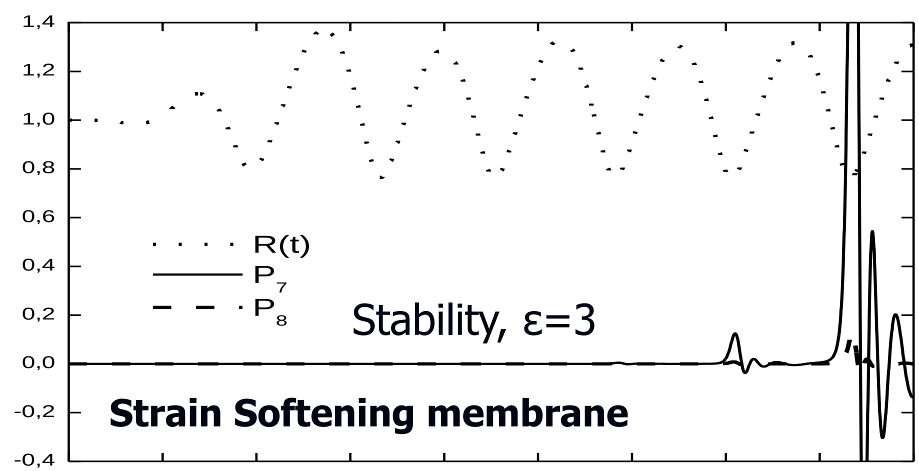
$$\rho_l = 998\ {kg}/{m^3},\ P'_{st} = 101325\ Pa,\ \gamma = 1.07,\ \nu_f = 1.7 MHz,\ K_{BD} = 3.0d - 14 Nm$$

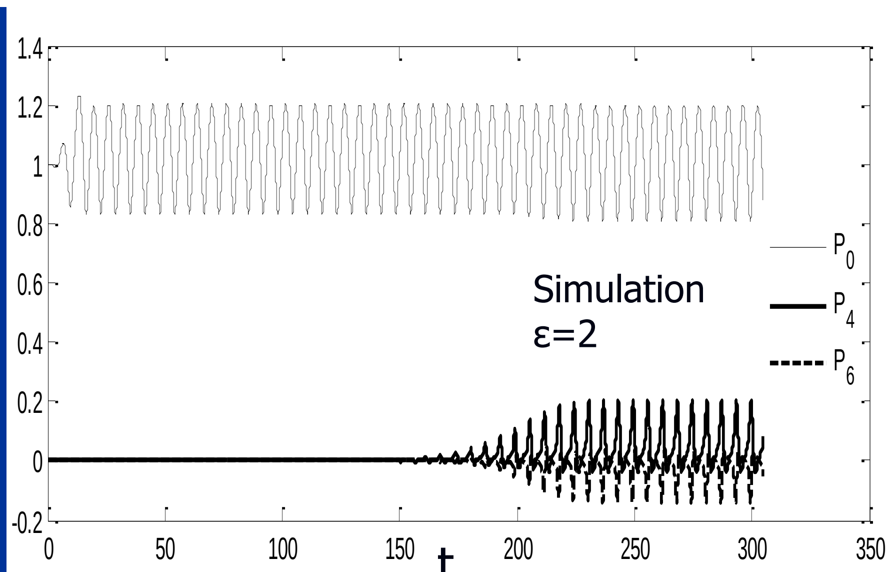
Saturation - Harmonic resonance

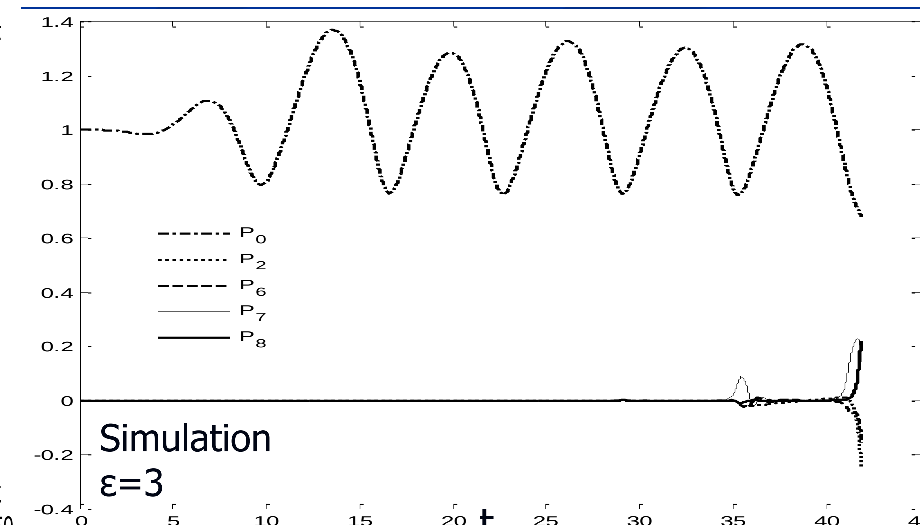
Transient Break-up

AMPLITUDE





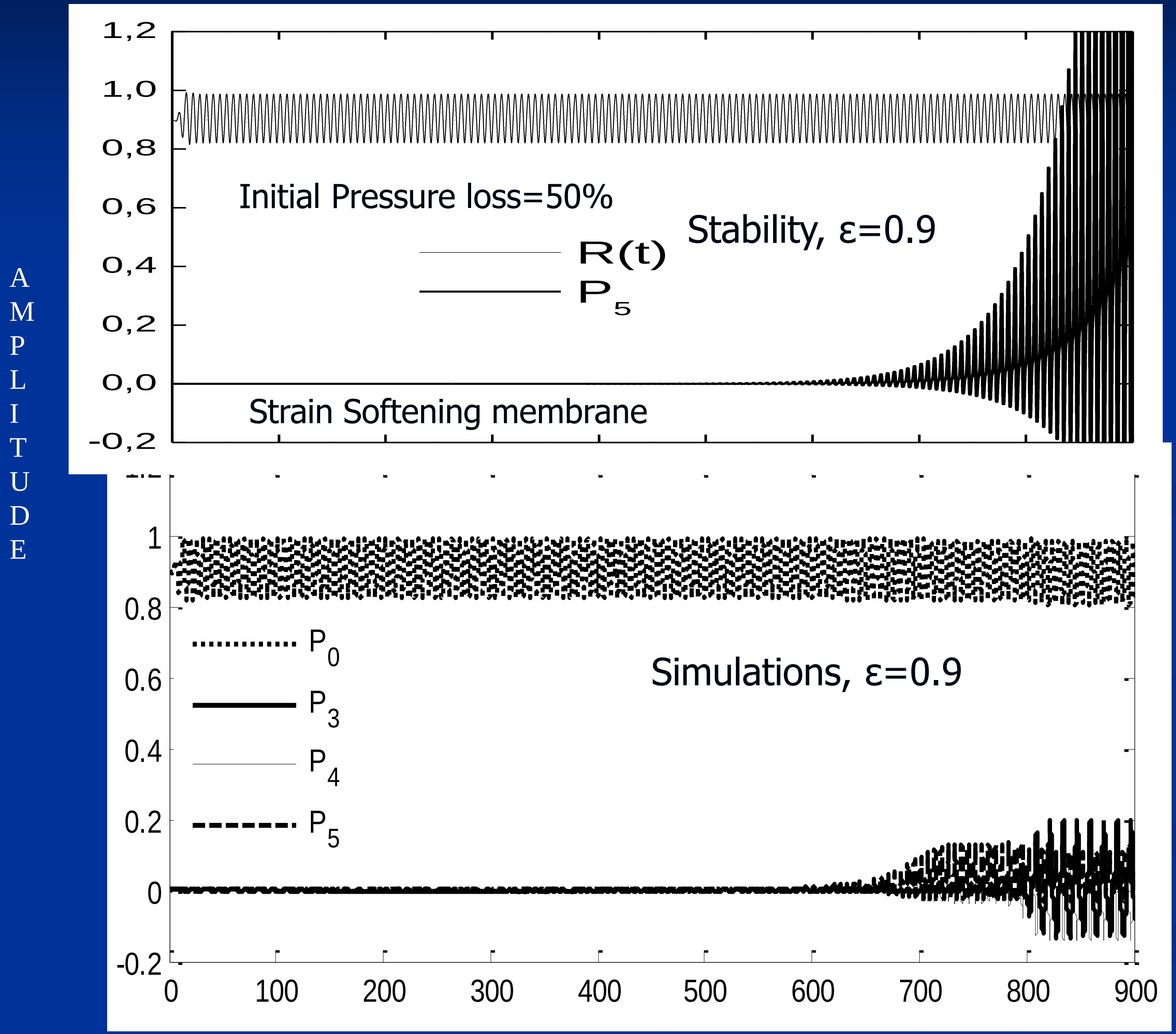




Parametric Stability- The effect of Residual stresses

$R_{eq} = 4.0\ \mu m,\ G_s = 80\ MPa,\ \delta = 1\ nm,\ \mu_s = 20\ Pa \cdot s,\ b = 0,\ \ \nu = 0.5,\ \mu_l = 0,\ C_l \to \infty,$

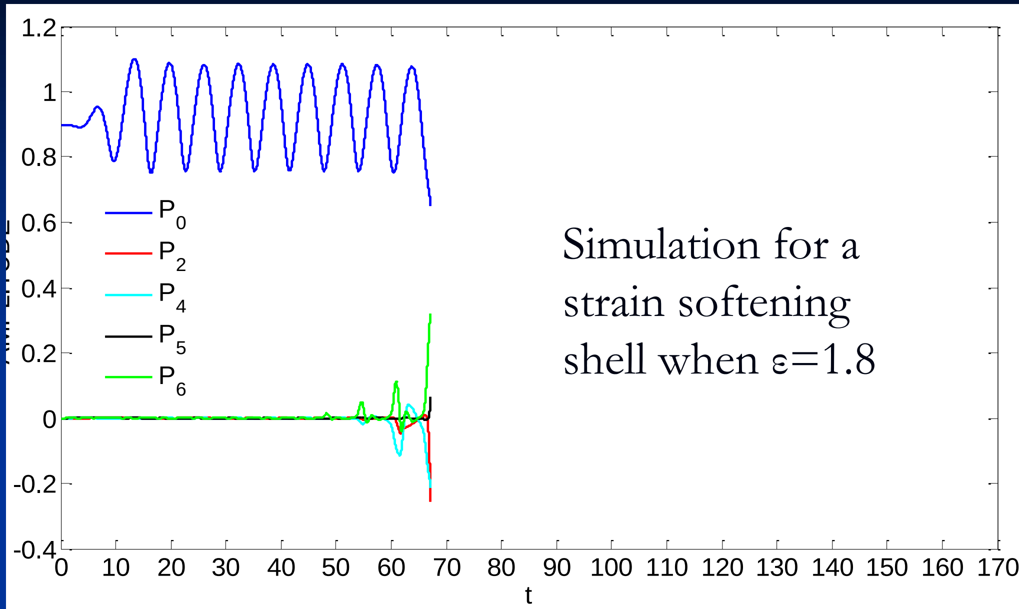
$\rho_l = 998\ {}^{kg}\!/\!{}_{m^3},\ P'_{st} = 101325\ Pa,\ \sigma = 0.051,\ \gamma = 1.07,\ \nu_f = 1.7 MHz,\ K_{BD} = 3.0d-14 Nm$

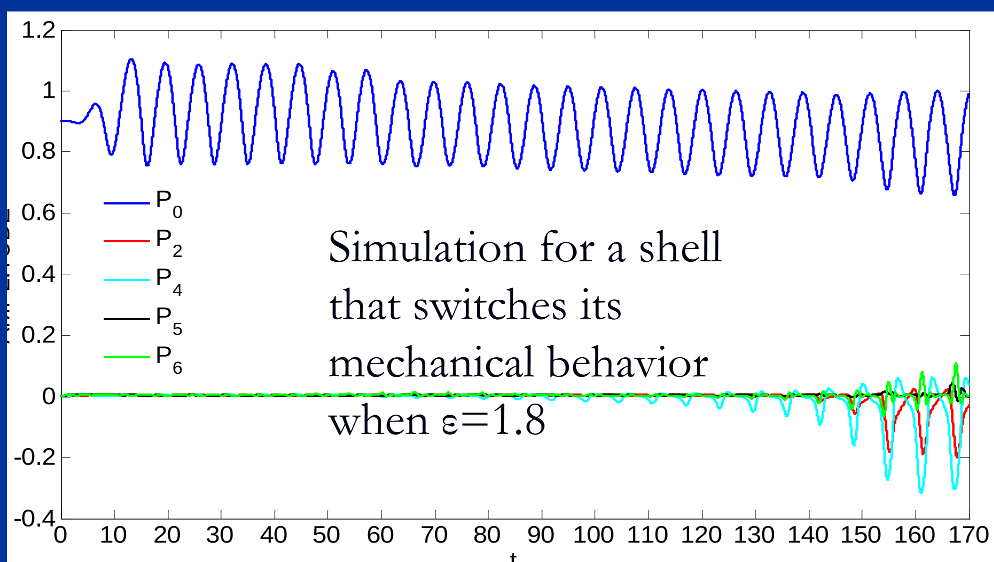


- Residual stresses significantly reduce the stability threshold in terms of sound amplitude
- Shape mode growth occurs primarily during compression
- The amplitude window between saturation and transient break-up is condensed

The effect of Residual stresses – Change in the Constitutive Law

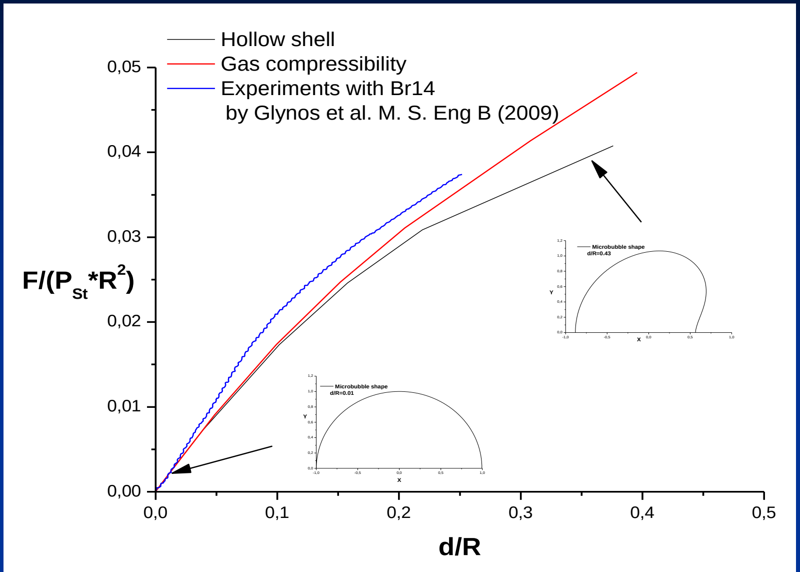
AMPLITUDE





- Increasing ε to 1.5 results in transient break- up of the microbubble via dynamic buckling
- Employing a change in the constitutive law from strain softening to strain hardening results in a compression only type behavior with viscous stabilization of microbubble pulsations
- This phase transition is employed when the amplitude of emerging shape modes grows beyond a certain level signifying the appearance of high curvature regions where lipid bilayers are formed

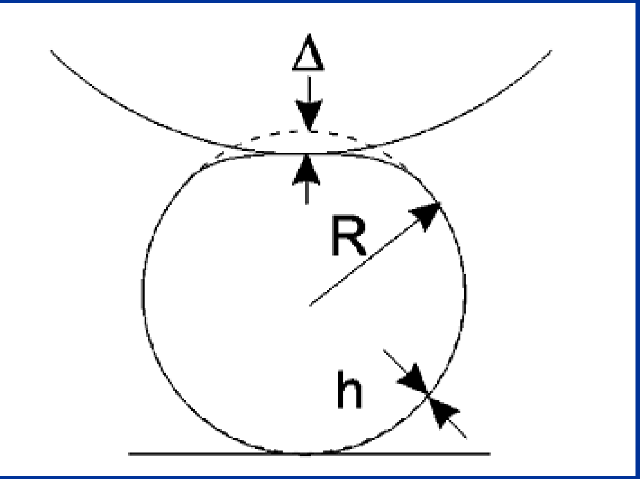
Static Simulations of Microbubble Response to a Point Load



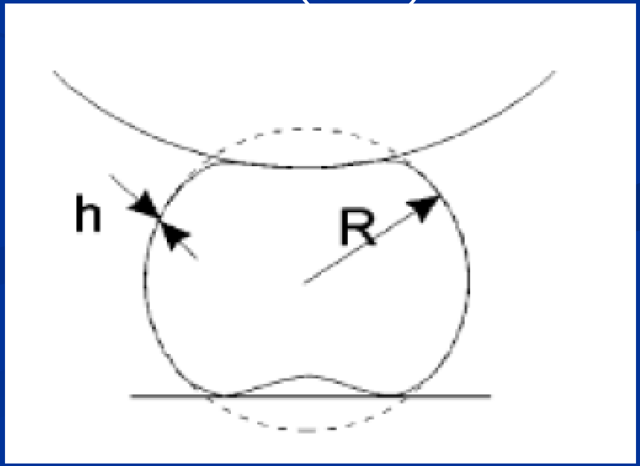
- Viscoelastic parameters of the shell are estimated based on asymptotic analysis of the experimental force displacement curve
- A linear regime is identified at very small displacements followed by a nonlinear regime (at this stage we ignore gas compressibility in the asymptotic analysis)
- Coupled of experimental data from the two regimes with asymptotic prediction provides estimates of $\chi=3G_sh$ and $k_b = \frac{3G_s h^3}{12(1-\nu^2)}$

Linear regime (Reissner): $F = \frac{8G_s h^2}{R} d$

Nonlinear regime (Pogorelov): $F = \frac{\sqrt{3.56 \cdot 3G_s h^{5/2}}}{(1-\nu^2)R} d^{0.5}$



For BR14 we obtain χ~0.1 N/m and k_b~0.5x10^{-14} N·m



Conclusions

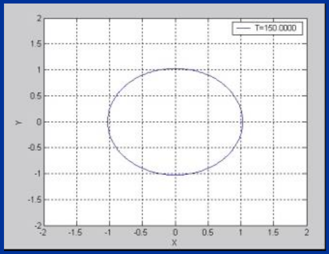
- Nonlinear shell properties, e.g strain softening vs. strain hardening membrane material, significantly affect contrast agent response
- Allowing for bending elasticity shape deformation and buckling are captured Bending elasticity is independent from area dilatation modulus due to non-isotropy of the membrane
- **Polymeric** shells follow a **neo-Hookean** behavior - **Lipid monolayer** shells exhibit a **strain softening behavior** (they become softer at expansion as the area density of the monolayer decreases) – **Lipid bilayer** shells exhibit **strain hardening** behavior (they become softer at compression)
- Static buckling occurs when the microbubble suffers a step increase in external pressure, or a sinusoidal change of very small frequency compared against its resonance frequency, of sufficiently large amplitude
- Static simulations of point load response reveal linear and nonlinear regimes that can be combined to provide estimates of χ and k_b

Shell thickness is not a relevant parameter for thin monolayer shells Compressibility effects will improve estimates for shells with stiffness that is comparable with stiffness due to gas compressibility, e.g. lipid monolayers

- Mode saturation is captured above the stability threshold (supercritical growth) for parametric excitation -- Growth of unstable modes occurs mostly during compression -- Part of the energy lost by the radial mode due to deformation is returned to it via nonlinear interaction with the emerging mode during compression $\rightarrow$ Preferential radial excursion at compression
- Strain softening shells exhibit this pattern more often and mostly for subharmonic ecxitation for which there is more time available for energy exchange – As the amplitude increases towards the threshold for dynamic buckling transient break-up takes place
- Dynamic buckling (equivalent to Rayleigh-Taylor instability for free bubbles) occurs exponentially fast for much larger sound amplitudes.
  Strain softening shells tend to exhibit this behavior at lower amplitudes than strain hardening ones due to viscous stabilization of the latter
- "Compression only" behavior is probably associated with bending at compression and subsequent formation of bilayer structures that introduce strain hardening behavior to the shell response $\rightarrow$ Results in significant radial excursion at compression and stabilization, against growth of shape modes and transient break-up, due to viscous damping
- For initially pre-stressed shells the amplitude thresholds for parametric mode excitation and transient break-up are significantly reduced and the window for saturated pulsations shrinks – "Compression only behavior" is a natural means to extend stability and cohesion of the microbubble

Future Work

- Develop a constitutive law for bending and stretching energy that is taylor-made for each contrast agent and accounts for monolayer to bilayer phase transitions
- Develop a set of static, e.g. via AFM, and dynamic measurements for contrast agent characterization
- Study Bubble-wall, bubble-cell interaction and the dynamic behavior of trapped microbubbles



Acknowledgements

This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

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Thanks for your attention

Questions