HPTLC as a useful tool for determining lipid composition of Archaeosomes, a new type of nanocarrier for gene and drug delivery

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Conventional liposomial formulations

- Bilayer lipidic preparations
- Extensively investigated as carriers for bioactive molecules
- Liposomial preparation already commercially available

However, their instability limits their field of applications

Archaeosomes : novel formulations based on saturated polar lipids

- Natural lipids from archaebacteria difficult to obtain in large quantities
- Synthetic tetraether lipids developed in the laboratory
- Mixture with conventional lipids (cholesterol, DOPE)

Increase of in vitro stability of formulations under conditions compatible with oral administration

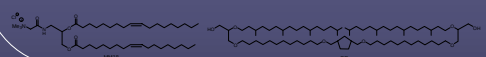
Quantitative determination of lipidic composition of various archaeosomes formulations using HPTLC

- Use of such nanocarriers as drug and gene delivery systems implies the perfect knowledge of their composition
- High Performance Thin Layer Chromatography as a tool for quantitative determination of lipidic composition

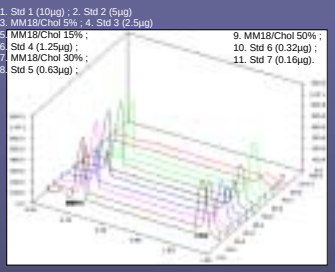
✓HPTLC conditions

Plate revelation : primuline ; Plate lecture : 366nm, fluorescence

	Eluant
Formulation 1 MM18/Chol	CH_2Cl_2/MeOH 80/20, 7% NH_4OH
Formulation 2 MM18/DOPE	CH_2Cl_2/MeOH 60/40, 7% NH_4OH
Formulation 3 MM18/GR	CH_2Cl_2/MeOH 80/20, 7% NH_4OH : MM18 CH_2Cl_2/MeOH 95/5 : GR



✓Chromatogram



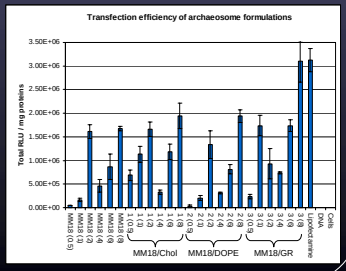
✓Lipidic composition

	Initial lipid composition	Archaeosomes lipid composition
Formulation 1 MM18/Chol	95/5 85/15 70/30 50/50	94.8/5.2 87.7/12.3 78.8/21.2 60.5/39.5
Formulation 2 MM18/DOPE	95/5 85/15 70/30 50/50	92.8/7.2 90.2/9.8 78/22 49.2/50.8
Formulation 3 MM18/GR	95/5 85/15 70/30 50/50	95.8/4.2 89.5/10.5 79.6/20.4 49/51

Archaeosomes were obtained by hydratation of lipidic film followed by sonication to reduce size and size distribution. The formulations have a diameter (measured by dynamic light scattering) between 100nm and 880nm with a polydispersity index between 0.3 and 1

Study of *in vitro* transfection efficiency

- Preparation of complexes between archaeosomes containing 5% of colipid (Chol, DOPE or GR) and DNA (4µg) coding for luciferase at various charge ratio (R = 0.5/1/2/6/8)
- Cell line : human alveolar epithelial cell line A549 (*in vitro* model for lung gene therapy)



Conclusions and perspectives

- Reproducible and reliable HPTLC method for determination lipidic compositions of archeaosomes
- HPTLC results show significant differences between lipidic composition of archaeosomes and initial lipid compositions
- HPTLC allows the correlation between exact archaeosomes composition and their *in vitro* transfection efficiency.
- **Improvement of HPTLC analysis using AMD**
  - Best tranfection efficiency for formulations containing 5% of neutral colipid and formulation 3(8), MM18/GR (R = 8), gives transfection efficiency similar to lipofectamine