LIPIDIC CUBIC-PHASE NANOPARTICLES (CUBOSOMES) AS CARRIERS FOR DOXORUBICIN AND SHORT-LIVED RADIONUCLIDE FOR COMBINATION CANCER TREATMENT

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Cubic phases

Structure of monoolein (GMO)

Monoolein-water phase diagram

Properties of the lipid cubic mesophases (LCPs)

- Thermodynamic stability in excess of water (Pn3m)
- High internal surface area (400m²/g)
- Possibility to control water channel dimensions
- Ability to incorporate both hydrophobic and hydrophilic drug molecules
- Ability to control drug release

Caffrey, M. Biochemical Society Transactions, 39, 2011, 725-732
Cubosomes

Cubic phase

Homogenization

pluronic F-127

Cubosomes

cryo-TEM images of cubosomes

Cubosome

Cryo-FESEM images

Applications:

- MRI imaging
- Drug delivery
- Passive delivery

Conceptualised advanced drug delivery carrier


Aim of the research

• Design and development of bimodal lipidic nanocarriers doped with chemotherapeutic and radionuclide for combined cancer treatment

Targeted radionuclide therapy

Conventional Gamma/Beta Radiation
- Indirectly damaging the DNA
- Dependent on oxygen presence
- Reparable single strand breaks

Alpha Radiation
- Directly damaging the DNA
- Independent of oxygen presence
- Irreparable double strand breaks

Radiation interaction with DNA

Scheme of bimodal carrier

Structure of doxorubicin

Structure of DOTA

Advancing Nuclear Medicine Through Innovation, Committee on State of the Science of Nuclear Medicine, National Research Council, 2007
Structural characterisation of LCPs doped with DOTAGA-OA

Synthesis of \( p \)-NCS-benzyl-DOTA-GA-oleylamine (DOTAGA-OA)

SAXS diffraction patterns obtained for LCPs doped with different amounts of DOTAGA-OA

Unit cell (a) dependence of weight percent of DOTAGA-OA in LCPs

Synthesis: dr Adam Mames, ICHF

\[ \text{DMF} \]  
[2h 50°C]
Preparation and structural characterization of cubic phases doped with DOX and DOTAGA-OA

Monoolein (58.1 wt%) + Doxorubicin (0.2 wt%) + DOTAGA-OA (1.9 wt%) + Buffer (39.8 wt%)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Symmetry</th>
<th>a (nm)</th>
<th>d_w (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty</td>
<td>Pn3m</td>
<td>10.0</td>
<td>4.5</td>
</tr>
<tr>
<td>DOX</td>
<td>Pn3m</td>
<td>9.8</td>
<td>4.4</td>
</tr>
<tr>
<td>DOX DOTAGA-OA</td>
<td>Pn3m</td>
<td>10.1</td>
<td>4.7</td>
</tr>
</tbody>
</table>

SAXS diffraction patterns obtained for the LCPs

Results of SAXS measurements for the LCPs: phase symmetry, lattice parameter a, water channel diameter d_w
Electrochemical characterisation of DOX

CV for DOX incorporated into cubic phases with or without DOTAGA-OA dopant. Scan rate: 100 mVs⁻¹, pH 5.5

DPV for DOX incorporated into cubic phases. Amplitude: ΔE=50 mV, pulse time: tp=50 ms

Release profiles of DOX-containing mesophases
Formation and structural characterization of cubosomes doped with DOX and DOTAGA-OA

Cubic phase doped with DOX and DOTAGA-OA

Pluronic F-127

Homogenization

Cubosomes doped with DOX and DOTAGA-OA

cryo-TEM images of (A) empty cubosomes, (B) cubosomes doped with DOX, (C) DOTAGA-OA and (D) DOX and DOTAGA-OA.

Diffraction patterns of cubosomes formulations

Properties of cubosomes formulations determined using SAXS and DLS

<table>
<thead>
<tr>
<th>Cubosomes</th>
<th>Symmetry</th>
<th>a (nm)</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty buffer/GMO/F-127 94.62/4.84/0.54 wt%</td>
<td>Im3m</td>
<td>14.0</td>
<td>140 ± 5</td>
<td>0.18 ± 0.02</td>
<td>-29 ± 0.9</td>
</tr>
<tr>
<td>DOX buffer/GMO/DOX/F-127 94.58/4.86/0.02/0.54 wt%</td>
<td>Im3m</td>
<td>13.7</td>
<td>160 ± 10</td>
<td>0.19 ± 0.01</td>
<td>-24 ± 0.4</td>
</tr>
<tr>
<td>DOTAGA-OA buffer/GMO/DOTAGA-OA/F-127 94.45/4.85/0.16/0.54 wt%</td>
<td>Im3m</td>
<td>14.7</td>
<td>130 ± 15</td>
<td>0.12 ± 0.02</td>
<td>-20 ± 0.6</td>
</tr>
<tr>
<td>DOX DOTAGA-OA buffer/GMO/DOX/DOTAGA-OA/F-127 94.44/4.84/0.02/0.16/0.54 wt%</td>
<td>Im3m</td>
<td>14.2</td>
<td>150 ± 12</td>
<td>0.13 ± 0.03</td>
<td>-17 ± 0.8</td>
</tr>
</tbody>
</table>

The cryo-TEM imaging was conducted by dr Tomasz Góral at the Center of New Technologies, University of Warsaw, Poland.
Preparation of cubosomes labeled with $^{213}\text{Bi}$

Eluent: 600 µl 0.1 M HCl/0.1 M NaI

$^{225}\text{Ac}$ → $^{213}\text{Bi}$

$^{221}\text{Fr}$ → $^{217}\text{At}$ → $^{213}\text{Bi}$ → $^{209}\text{Tl}$

$^{213}\text{Po}$ → $^{209}\text{Pb}$ → $^{205}\text{Tl}$

$^{225}\text{Ac}$/213Bi Radionuclide Generator
Preparation of cubosomes radiolabeled with $^{213}$Bi

TRIS buffer pH 7.0

Cubosomes with $^{213}$Bi-DOTAGA-OA

$^{213}$Bi

Cubosomes with DOTAGA-OA

15 min, 95°C

15 min, 95°C

Cubosomes with DOTAGA-OA

$^{213}$Bi-DOTAGA-OA
Viability of HeLa cells treated with $^{213}$Bi-Cubo+Cubo, Cubo+Cubo-DOX, $^{213}$Bi-Cubo+Cubo-DOX, Mix after 24 h, 48 h and 72 h of incubation. $^{213}$Bi-Cubo+Cubo and Cubo+Cubo-DOX were used as a control.

✓ The MTS assay was used to evaluate the in vitro cytotoxicity of the cubosomes doped with $^{213}$Bi and with DOX on HeLa cells
✓ The best procedure involved first irradiation of the cells and next exposure to the chemotherapeutic
✓ The enhancement of cytotoxicity achieved by combining doxorubicin and complexed $^{213}$Bi treatments was observed
Summary

✓ We prepared a new dopant: \( p\)-NCS-benzyl-DOTA-GA-oleylamine (DOTAGA-OA) which forms an inert complex with \(^{213}\text{Bi}\) and can be accommodated in the cubosome in a stable way

✓ We prepared cubic phases and cubosomes with DOX and DOTAGA-OA and characterized their structure by SAXS, DLS and cryo-TEM

✓ The release of DOX from the carrier was monitored by electrochemical methods. We found that the presence of DOTAGA-OA ligand in the cubic phase leads to the decrease of the rate of DOX release from the mesophase

✓ The MTS assay shows significant decrease of viability of HeLa cancer cells using the sequential cell exposure: first to the radiolabeled cubosomes containing \(^{213}\text{Bi}\) complex and next to DOX-doped cubosomes (Cubo-DOX) on HeLa cancer cells. However, we find favorable to deliver both drugs simultaneously but encapsulated in separate cubosomes
• A. Cytryniak, E. Nazaruk, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, "Kubosomy jako nośniki leków przeciwnowotworowych oraz radionuklidów", XVII Konferencja "Elektroanaliza w teorii i praktyce", 19.11-20.11.2020, short communication


• A. Cytryniak, E. Nazaruk, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, „Lipidic cubic-phase nanoparticles (cubosomes) as carriers for doxorubicin and short-lived radionuclide for combination cancer treatment”, 10th International Workshop on Surface Modification for Chemical and Biochemical Sensing, 5.11- 9.11 2021, short communication
- **A. Cytryniak**, E. Nazaruk, R. Bilewicz, E. Górzyńska, K. Żelechowska-Matysiak, R. Walczak, A. Mames, A. Bilewicz, A. Majkowska-Pilip; Lipidic Cubic-Phase Nanoparticles (Cubosomes) Loaded with Doxorubicin and Labeled with 177Lu as a Potential Tool for Combined Chemo and Internal Radiotherapy for Cancers. Nanomaterials, 2020, 10, 2272 (RadFarm)


- Another in progress

**Internship**

- Deutsches Krebsforschungszentrum (DKFZ), Junior Research Group Molecular Biology of Systemic Radiotherapy (group leader Dr Martina Benešová), Heidelberg, Germany (1 month, 15.01.2022 – 14.02.2022)