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Doxorubicin-containing gold nanoparticles-anchored liposomes as delivery carriers: A quality by design strategy to optimize the surface functionalization

Nanopartículas de oro ancladas a liposomas que contienen doxorubicina, como sistemas transportadores: estrategia de calidad por diseño para optimizar la funcionalización de la superficie

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1. Background information

Liposomes (Lip) exhibit high biocompatibility and are attractive carrier systems for delivering anticancer drugs. They can be functionalized with gold nanoparticles (AuNPs) and are useful in cancer nanomedicine for diagnosis and treatment. Thermosensitive Lip constitute an interesting alternative as nanosystems capable of responding to thermal stimulus. Thermo-responsive Lip can be obtained by using temperature-sensitive phospholipids as well as anchoring their surface with AuNPs because of their light-induced heating response.

Surface functionalization of Lip with AuNPs can affect their interfacial properties and their ability to carry and deliver the payload.

2. Purpose

The aim of this study was to apply a quality by design (QbD) strategy to rationalize the experimental design to anchor doxorubicin (Dox)-loaded Lip with AuNPs and evaluate their performance as carrier systems.

3. Methods

Thin-film hydration and transmembrane pH-gradient methods were used to prepare the Lip and for Dox loading, respectively. Selected variables: Lip-Dox:AuNPs ratio (3:8, 8:8 and 8:3 (v/v)), stirring time (1, 2 and 3 min), temperature (4, 25 and 42 °C) and time of anchoring (0, 24 and 48 h post-functionalization) were subjected to experimental verification through variable-response correlation, using a Taguchi matrix design. Interfacial properties: hydrodynamic diameter (dH), polydispersity index (PDI) and electrokinetic potential (Z); and Dox loading efficiency (EE %) were evaluated. The data obtained were mathematically and statistically analyzed, using the DOE pack© 2000 software. Variables that presented adequate levels of statistical significance ($p<0.001$) were considered critical. Drug release studies toward different media (pH 7.4 and 5.1 at physiological and hyperthermal temperatures) were carried out for optimized liposomal dispersions.

4. Results

Non-functionalized AuNPs Dox-loaded Lip exhibited nano-scale sizes (355 nm), acceptable PDI values (<0,46), and positive Z-potential (14 mV, after drug loading) because of the presence of dimethyldioctadecylammonium bromide in the bilayer. This cationic surface of Lip allowed the anchoring with negative charged AuNPs by electrostatic interactions. LipDox:AuNP ratio, stirring time and temperature significantly affected the interfacial properties of AuNP-Lip-Dox. Nanometric sizes (516 nm), acceptable PDI values (<0.4), positive Z-potential (9,85 mV) and EE % equal to (80 ± 2) % were only achieved at 8:3 Lip-Dox:AuNPs ratio, 3 min of stirring time and 42 °C. When comparing the dH of empty Lip (287 nm), Dox-loaded Lip andAuNPs-Lip-Dox, it can be observed that the higher the system complexity, the higher the sizes.

Although the anchoring can destabilize the Lip bilayer, Dox kept well-encapsulated and non leakage was observed after functionalization ((78 ± 2) % and (80 ± 2) % for Lip-Dox and AuNPsLip-Dox, respectively). The release of Dox toward both receptor media was controlled, and as expected, its release was triggered under hyperthermia conditions (light-induced heating), confirming the thermo-responsive behavior of liposomal dispersions. Acid medium also promoted Dox release, thus demonstrating AuNPs-Lip-Dox presented pH-sensitive responsiveness.

5. Conclusions

The QbD was a useful strategy to evaluate different parameters for surface functionalization of Lip with AuNPs that can affect their performance. Optimized conditions for the anchoring process were achieved, allowing the preparation of liposomal dispersions with thermos-responsiveness that exhibit promising properties for cancer nanomedicine and hyperthermia therapy.

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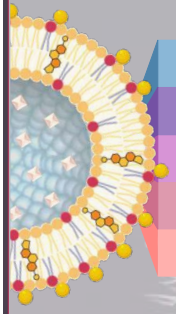
Introduction



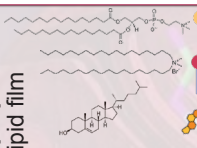
- ✓ High biocompatibility and low toxicity
- ✓ Attractive carrier systems for delivering anticancer drugs
- ✓ They can be functionalized with gold nanoparticles (AuNPs)
- ✓ Usefulness in cancer nanomedicine
- ✓ Thermosensitive liposomes (Lip) → interesting alternative as nanocarriers capable of responding to thermal stimulus
- ✓ Thermo-responsive Lip can be obtained by:
  - using temperature-sensitive phospholipids
  - anchoring their surface with AuNPs
- ✓ Surface functionalization of Lip with AuNPs can affect their interfacial properties and their ability to carry and deliver the payload

Aim

The aim of this study was to apply a **quality by design (QbD)** strategy **to rationalize** the experimental **design to anchor doxorubicin (Dox)-loaded Lip with AuNPs** and evaluate their performance as **carrier systems**.

Methods

Liposome preparation (Thin lipid film)



DPPC, DDAB, Cholesterol → **Dissolution** in chloroform → **Evaporation**. Formation of thin lipid film → **Rehydration** $(NH_4)_2SO_4$ solution Vortex 1 min + 42 °C bath (5 cycles)

MLV → **Extrusion** Polycarbonate membranes 0.8 µm and 0.2 µm, 42 °C bath, 5 cycles each → LUV → **Dialysis** Against phosphate buffer solution pH 7.4. Transmembrane ammonium sulfate gradient → **Liposome (Lip)**

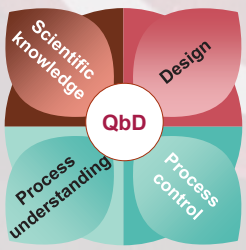
Dox encapsulation (Remote loading)

Liposomal dispersion → **Thermostatization** Bath at 42 °C, 5 min → **Addition of doxorubicin** (Dox) solution (small aliquots) → **Dox encapsulation** Vortex 1 min + 42 °C bath (alternated cycles)

Incubation Bath at 42 °C, 1 h → Overnight 4–8 °C → **Purification** Sephadex® G50 column → **Lip-Dox**

Functionalization with AuNPs

Lip / **Lip-Dox** → **Liposomal dispersions** → **Addition of AuNPs suspension** different ratio → **Stirring** → **AuNPs-Lip** / **AuNPs-Lip-Dox**



- Lip-Dox:AuNPs ratio (v/v): 3:8, 8:8 and 8:3
- Time: 1, 2 and 3 min
- Temperature: 4, 25 and 42 °C
- Time of anchoring post-functionalization: 0, 24 and 48 h

Variable-response correlation Taguchi L9 matrix

- **Interfacial properties:** hydrodynamic diameter (dH), polydispersity index (PDI) and Z-potential (Z-pot.)
- **Dox loading efficiency (EE %)**

Mathematical and statistical analysis *(ANOVA and ANOM). DOE pack® 2000 software*

Optimized liposomal dispersions

- **Drug release studies (Franz cells).** Conditions: phosphate and acetate buffer solutions (PBS pH 7.4 and ABS pH 5.1, respectively) at 37 °C and under IR irradiation.
- **Kinetic analysis** → Mathematical models: Zero-order, Higuchi and Korsmeyer-Peppas (K-P).

Results

QbD → Lip-Dox:AuNPs ratio, Stirring time, Stirring temperature → Influence (p<0.001) → dH, Z-potential, EE %

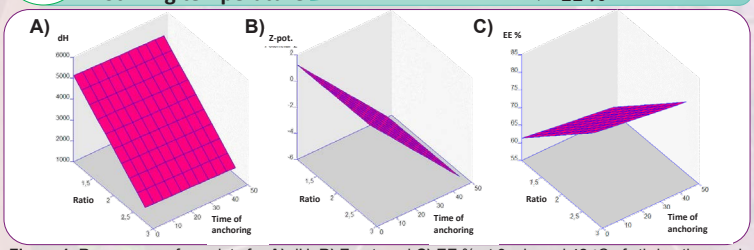


Figure 1. Response surface plots for **A)** dH, **B)** Z-pot. and **C)** EE % at 3 min and 42 °C of stirring time and temperature, respectively, obtained according to regression equations with DOE pack 2000 software.

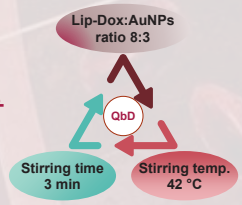
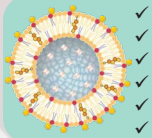


Table 1. Interfacial properties (dH, PDI and Z-pot.) and EE % of Lip and, Lip-Dox and AuNPs-Lip-Dox.

Sample	EE %	dH (nm)	PDI	Z-pot. (mV)
Lip	-	287 ± 29	0.22 ± 0.05	36 ± 2
Lip-Dox	78 ± 2	355 ± 17	0.37 ± 0.09	14 ± 2
AuNPs-Lip-Dox	80 ± 2	516 ± 19	0.27 ± 0.05	10 ± 3



- ✓ Nano-scale sizes
- ✓ Higher the system complexity → higher the sizes
- ✓ Low polydispersity
- ✓ Positive Z-pot. (DDAB in the bilayer → cationic surface)
- ✓ High Dox loading
- ✓ Anchoring with anionic AuNPs by electrostatic interaction

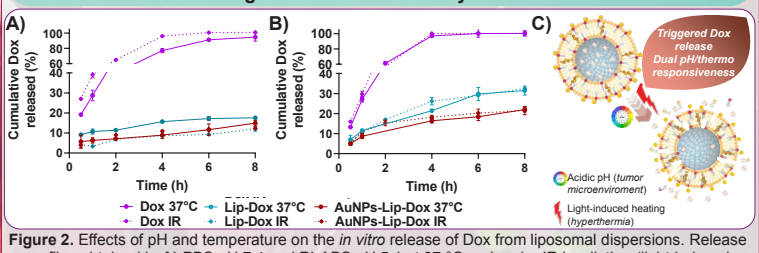
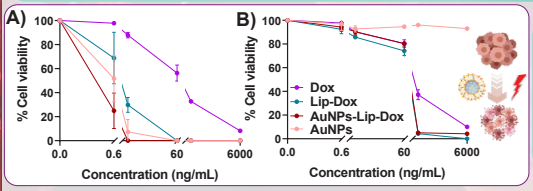


Figure 2. Effects of pH and temperature on the *in vitro* release of Dox from liposomal dispersions. Release profiles obtained in **A)** PBS pH 7.4 and **B)** ABS pH 5.1 at 37 °C and under IR irradiation (light-induced heating response). **C)** Schematic depiction of the Dox-loaded Lip anchored with AuNPs and their dual responsiveness to pH and temperature changes for triggering delivery of Dox.

- ✓ Controlled Dox release towards both receptor media
- ✓ Hyperthermia conditions → triggered release → thermo-responsive behavior
- ✓ Acidic medium → triggered release → pH-sensitive responsiveness
- ✓ Dual pH/thermo-responsive liposomal dispersions
- ✓ Diffusion as main release mechanism (K-P kinetic model with n ~ 0.5, R^2>0.95).



- ✓ Promising anticancer eficacy
- ✓ Concentration-dependent activity
- ✓ Higher cytotoxicity under IR irradiation

Figure 3. Preliminary results of cytotoxicity of free Dox, Lip-Dox, Lip-Dox-AuNPs and AuNPs against SK-OV-3 ovarian cancer cells **A)** with and **B)** without IR irradiation. Cells were treated for 6 h and allowed to grow for additional 66 h in drug-free medium. Cell viability was measured with the resazurin assay.

Conclusion

The **QbD** was a **useful strategy to evaluate different parameters for surface functionalization of Lip with AuNPs** that can affect their performance. **Optimized conditions** for the anchoring process were achieved, allowing the **preparation of liposomal dispersions with dual thermo/pH-responsiveness** that exhibit **promising** properties **for cancer nanomedicine and hyperthermia therapy.**

References

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