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# Synthesis and characterization of stimuli-responsive niosomes functionalized with chitosan incorporating folic acid and hyaluronic acid for anticancer drug delivery

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## INTRODUCTION & AIM

Breast cancer is the most common cancer in women and conventional treatments often cause toxicity, low selectivity and drug resistance. Nanotechnology enables targeted drug delivery with better bioavailability and fewer side effects. Lipid nanoparticles (Nps) like niosomes,, offer good biocompatibility and can encapsulate both hydrophilic and hydrophobic drugs. Chitosan (CS), a biocompatible and pH-sensitive polymer, can be modified with folic acid (FA) and hyaluronic acid (HA) to target breast cancer cells overexpressing FR-α and CD44 receptors. Curcumin (Cur) and ascorbic acid (Asc) are natural anticancer agents whose efficacy improves when co-encapsulated in niosomes. This study aims to develop a CS-FA-HA coated noisome system for dual-targeted, pH-responsive co-delivery of Cur and Asc to enhance anticancer activity against MDA-MB-231 cells while minimizing toxicity to healthy cells

## METHOD

**CS–HA–FA composition:**

- CS-HA (hydroxyl bond), after protection of –$NH_2$ with benzaldehyde
- CS-HA deprotected with HCl, purified by dissolution.
- CS-FA via EDC coupling and purified by dissolution
- CS-HA and CS-FA in a 1:1 ratio (w/w) → CS–HA–FA with free $NH_2$

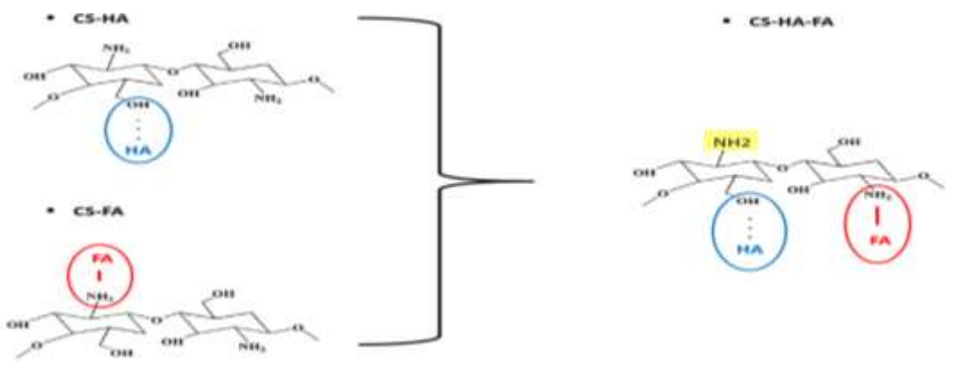


**Figure 1.** Modification of CS with HA and FA

**Preparation of nanoparticles and drug loading:**

- Thin film hydration with optimized cholesterol/Span60/Tween60 ratio
- Curcumin (10 mg/mL) dissolved in chloroform (organic phase)
- Ascorbic acid (10 mg/mL) dissolved in PBS (aqueous phase)
- Hydration → sonication → centrifugation → 0.22 μm filtration

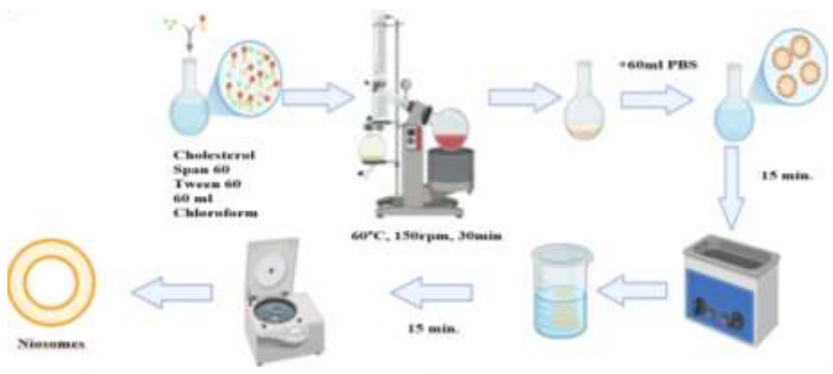


**Figure 2.** Preparation of niosomes with Thin film hydration method

**Surface coating:**

- Nanoparticles mixed with CS–HA–FA (1:1 v/v)
- Stabilization with TPP, stirring, centrifugation and filtration

**Characterization:**

- Size and charge: DLS, NTA
- Morphology: SEM
- Chemical confirmation: FT-IR (4000–550 $cm^{-1}$)

**Cytotoxicity test:**

- Tested on MDA-MB-231 and MCF-10A cells
- Cell viability via WST-1 assay (440 nm)

## RESULTS & DISCUSSION

The optical niosomes, with size 101 nm and low PDI, were formed at a cholesterol/surfactant ration of 0.2:1:1. The nps averaged 174.5 nm after being co-loaded with Cur and Asc (10 mg/ml each) and coated with CS-FA-HA. The dual-loaded niosomes showed stabilizing interactions between the two substances since the were smaller than those containing only Cur or only Asc.

**Table 1.** Size of Nps

| Sample | Size Nps (mean) |
|---|---|
| Blank niosomes 0.2 chol: 1 span 60: 1 tween 60 | 101.0 nm |
| Nps Cur 10mg/ml | 264.6 nm |
| Nps Asc 10mg/ml | 323.6 nm |
| Nps CS-FA-HA Cur-Asc 10mg/ml | 174.5 nm |

The stable shape and effective encapsulation of the nps were confirmed by SEM examination, which revealed that they were mostly spherical and had smooth surfaces.

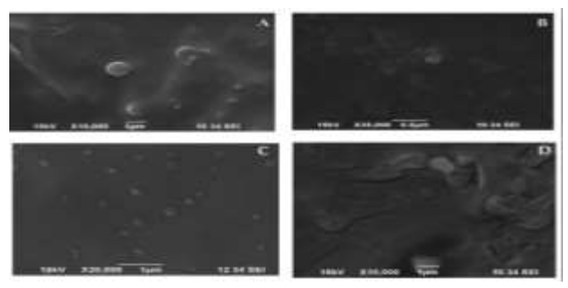

**Figure 3.** A) 0.2:1:1 ration of blank niosomes, B) Encapsulation of Cur 10mg/ml, C) Encapsulation of Asc 10mg/ml, D) Niosomes CS-HA-FA and co-encapsulation of Cur/Asc 10mg/ml

FTIR confirmed FA and HA in CS, successful coating and the preservation of free NH2. Cytotoxicity study shows that niosomes are suitable carriers. Cur or Asc loaded nps reduce cancer cell viability in a dose-dependent way. CS-FA-HA nps with Cur/Asc nearly eliminate cancer cells at high doses.

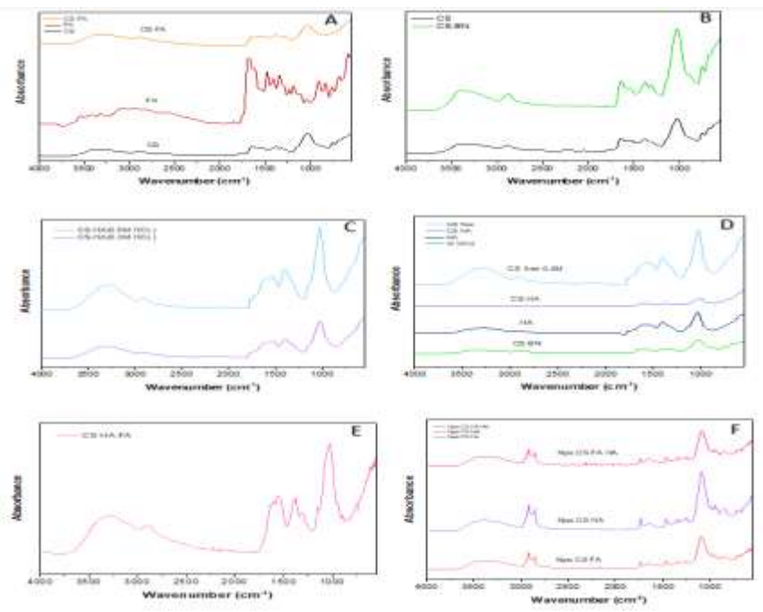


**Figure 4.** FTIR spectra A) CS-FA modification, B) protection of CS amino groups with BN, C) deprotection of CS with 0.5 M and 0.3 M HCl, D) modification of CS with HA and deprotection with 0.5 M HCl, E) modified CS with FA and HA, F) Nps coated with CS-HA, CS-FA and CS-HA-FA

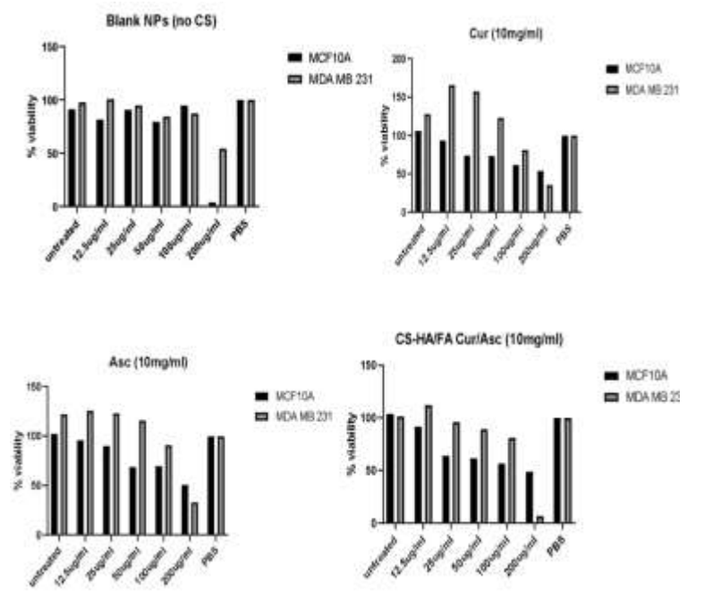


**Figure 5.** Effect of niosomes on the viability of normal and cancer cells A) blank, B) encapsulated Cur (10 mg/ml), C) encapsulated Asc (10 mg/ml), D) co-encapsulation Cur/Asc (10 mg/ml) and CS-HA-FA

## CONCLUSION

- Innovative system with triple anticancer action. Use of pH sensitivity and molecular targeting with FA and HA.
- CS-FA-HA niosomes loaded with Cur/Asc consistently produced nps of a suitable size
- Encapsulation of both hydrophobic and hydrophilic substances
- Co-encapsulation was synergistically stabilized.
- Strong and targeted anticancer activity that, at high doses, almost completely reduces cancer cells.

## FUTURE WORK / REFERENCES

For further system development, the anticancer drug Lapatinib will be encapsulated in the niosomes, serving as an additional targeting agent for breast cancer cells. This will create a quadruble targeted system. Cytotoxicity tests will be repeated to confirm the results.

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