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作品名稱　Natural resources utilization for the in-house production of fluorescence lipid nanoparticles

得獎獎項　四等獎

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Project Detail Form

Natural resources utilization for the in-house production of fluorescence lipid nanoparticles

SECTION 1: INTRODUCTION

Nanotechnology, a transformative force, has steadily gained traction across multiple scientific disciplines, including physics, chemistry, engineering, and biology. It offers unprecedented capabilities, especially in the realm of nanoscale particles, ushering in new paradigms in various applications.

One of the most revolutionary applications of nanotechnology is in the pharmaceutical sector. Here, nanoparticles have transformed drug and vaccine delivery systems, offering both efficacy and precision. Among these nanoparticles, lipid nanoparticles (LNPs) have stood out, especially for their role in delivering nucleic acid-based drugs and vaccines. These LNPs are intricate assemblies composed of lipids and nucleic acid complexes, offering an amalgamation of stability and deliverability. Such properties have rendered LNPs as invaluable tools in enhancing therapeutic efficacy while minimizing off-target side effects.

The myriad of nanoparticles available includes the likes of silver, gold, and lipid nanoparticles. However, the emphasis of this research lies with lipid nanoparticles, given their widespread success in the pharmaceutical arena. LNPs have showcased their potential in delivering drugs with low therapeutic indices, emphasizing their capability to act as versatile platforms for novel drug development. Recent advances have further expanded the horizons of LNPs, paving the way for novel antisense oligonucleotides, innovative vaccines, and complex lipid nanoparticle formations.

Characterizing these nanoparticles is paramount, not only for the development of novel drugs but also to comprehend their in vivo behavior. Their multifaceted nature, stemming from their unique excipients, core-bilayer design, and varying sizes, makes their characterization a critical step in the research and development pipeline.

To augment the utility and traceability of LNPs, there's a growing interest in rendering them fluorescent. Fluorescence is a phenomenon where chemical fluorophores like carotenoids or proteins absorb short-wave light and subsequently emit longer-wave light upon releasing the absorbed energy. This mechanism makes fluorescent nanoparticles, especially LNPs, indispensable in optical molecular imaging. Their potential is especially pronounced in theranostic applications, where they can be instrumental in both diagnostic processes, such as cancer detection, and therapeutic interventions.

Venturing into a more sustainable and innovative domain, this research delves into the utilization of natural resources for fluorescence. By leveraging vibrant dyes extracted from Curcumin and Mint powder, we aim to produce in-house KAIMRC-LNPs that are fluorescently labeled. Envisaged applications for our naturally labeled LNPs range from assessing dye encapsulation efficiency to tracking LNPs both in vitro and in vivo.

As we embark on this journey, we will explore the intricacies of LNPs, the significance of their fluorescent labeling, and the potential of natural resources in enhancing the capabilities of these nanoparticles for a myriad of therapeutic and diagnostic applications.

SECTION 2: Literature Review

Scientific Background :

1. Fluorescence Lipid Nanoparticles (FLNPs):

Fluorescent lipid nanoparticles are lipid-based nanostructures that exhibit fluorescence. Their core is usually composed of lipids, which can encapsulate both hydrophobic and hydrophilic substances, while their fluorescent properties arise from embedded fluorescent dyes or inherently fluorescent lipid derivatives.

2. Importance of FLNPs:

Due to their unique combination of lipid-based encapsulation and fluorescence, FLNPs have found applications in:

Biomedical Imaging: For tracking the delivery and release of drugs or other therapeutic agents.

Biosensing: Due to their ability to interact with specific biomolecules and produce a fluorescent signal.

Research Tools: As markers or tracers in cellular and molecular studies.

3. Traditional Production of FLNPs:

Traditionally, FLNPs are synthesized using synthetic or semi-synthetic lipids combined with fluorescent dyes. These materials can be expensive, not easily accessible, and sometimes not environmentally friendly.

4. Natural Resources in FLNP Production:

Utilizing natural resources in producing FLNPs offers the advantages of sustainability, potential biocompatibility, and reduced cost. Some of the natural resources explored include:

Vegetable Oils: Rich in triglycerides, they can serve as lipid sources. Common examples include soybean, olive, and sunflower oils.

Marine Lipids: Derived from fish or algae, these lipids often have unique fatty acid compositions that might confer specific benefits to the nanoparticles.

Natural Dyes: Some plant-based dyes or pigments, like curcumin or anthocyanins, can be explored for their fluorescent properties.

5. In-House Production:

This refers to the production of FLNPs in smaller scales, typically in research labs or local settings, as opposed to large-scale industrial production. The benefits of in-house production include customization, rapid prototyping, and adaptability to specific research needs.

6. Advantages of Natural Resources:

Sustainability: Renewable and widely available, reducing the carbon footprint.

Biocompatibility: Reducing potential cytotoxicity, especially important for biomedical applications.

Cost-Effectiveness: Often cheaper than synthetic counterparts.

7. Challenges:

Standardization: Natural resources can have variability in composition.

Stability: Natural lipids may have different oxidation profiles or shelf-lives compared to synthetic ones.

Fluorescence Intensity: Natural dyes might not always match the intensity or stability of synthetic dyes.

8. Current Research Directions:

Optimization of Extraction Methods: To obtain pure and consistent lipid samples from natural resources.

Exploration of New Natural Fluorophores: To expand the range of fluorescence wavelengths and intensities available.

Integration with Other Nanotechnologies: Combining FLNPs with other nanoparticle types for multifunctional applications.

SECTION 3: Objectives

Research Questions:

What types of natural resources are most effective in the production of fluorescence lipid nanoparticles (FLNPs)?

How does the in-house production of FLNPs using natural resources compare in terms of efficiency, stability, and cost to traditional methods?

What are the biocompatibility and environmental impacts of FLNPs produced using natural resources?

Purpose:

Characterization: To characterize and identify the properties of FLNPs produced from various natural resources.

Optimization: To optimize the in-house production process of FLNPs to ensure maximum yield, stability, and fluorescence efficiency.

Comparison: To compare the performance and benefits of natural-resource-derived FLNPs to those produced using synthetic or semi-synthetic methods.

Novelty:

New Natural Sources: Explore previously unutilized or underutilized natural resources for FLNP production.

Innovative Processes: Develop novel extraction and production methodologies tailored for in-house setups.

Unique Applications: Identify any unique applications or functionalities of natural-resource-derived FLNPs that may not be possible with traditionally produced FLNPs.

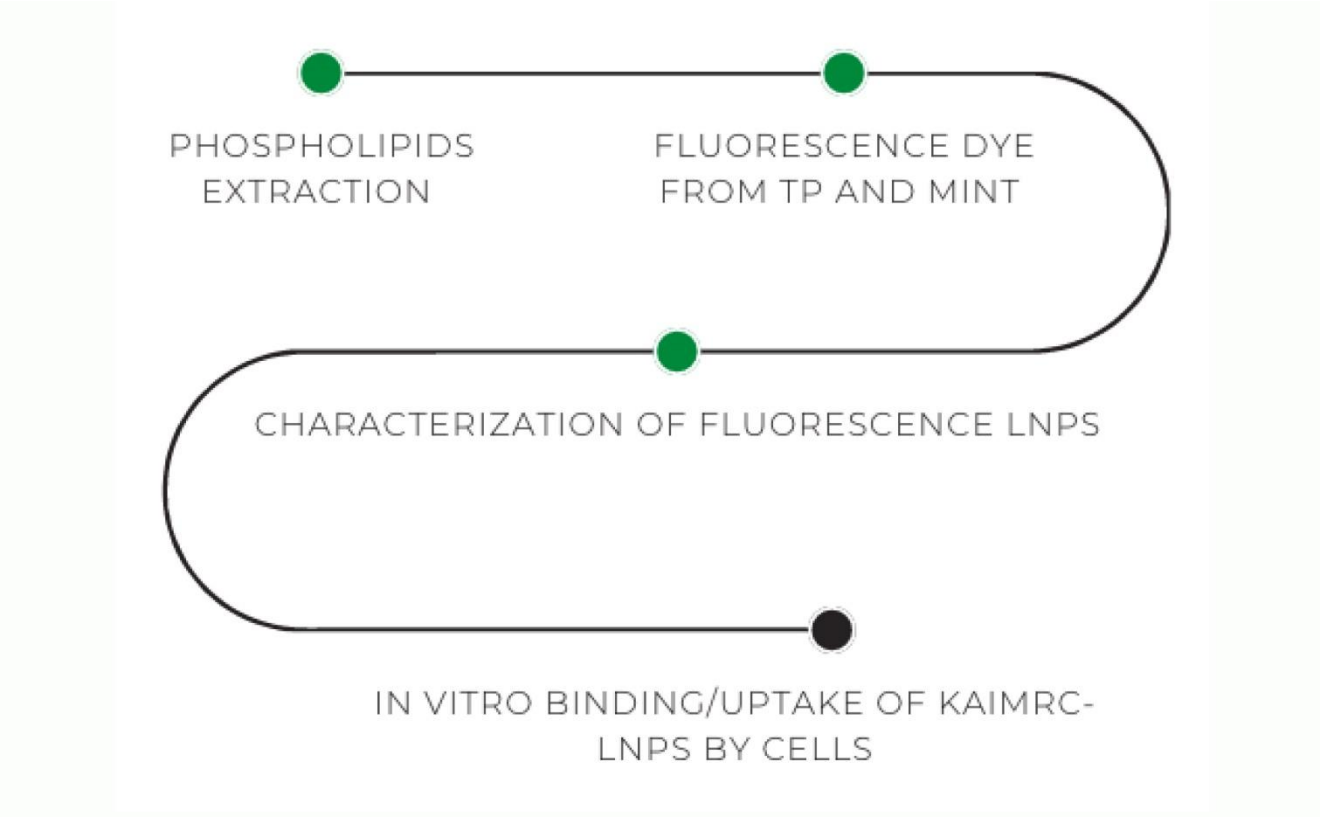
Hypothesis:

H1: Natural resources provide a sustainable and cost-effective alternative to synthetic materials for the in-house production of FLNPs.

H2: FLNPs derived from natural resources demonstrate comparable, if not superior, biocompatibility for biomedical applications.

H3: The in-house production of FLNPs using natural resources can achieve similar fluorescence efficiency and stability as commercial production methods.

SECTION 4: METHODOLOGY



1.1: Phospholipids extraction :

First, we extract the egg yolk and mix it well, then we extracted the phospholipids (i.e. PC and PE) using several organic solvents such as chloroform, methanol and acetone. We purified our phospholipids using filters, then evaporate it until it dries under chemical hoods at room temperature. After it dries, store it in a tube In a refrigerator at a temperature of -29

1.2 : Fluorescence dye from TP and mint :

First, we took 50 mg of turmeric and mint powder and divided them into 12 samples, 6 of which were in 100% organic solvent, and the remaining samples were 99% water and 1% organic solvent at high temperatures for 5 to 30 minutes. After dye extraction, we then diluted each sample to different concentrations, which are as follows: 1/100, 1/1000, 1/10000, 1/100000, 1/1000000, and we put all the concentrations in Plates. Then we put it in the Tecan device, and we read it to give us the results

1.3: LNPs manufacture :

After the lipids dried well, we add about 5 mg of lipids in tubes then we add the dye (TP and Mint) in a volume of 0.5 ml , then we put the tubes in the water bath to dissolve the lipids in it. After that, we used the organic solvent direct injection method to create our fluorescently-labeled KAIMRC-LNPs. After that, we take the samples to the DLS to read its size and zeta. After reading, did put the LNPs into tubes with 10k filters and applied centrifugation to collect our LNPs. After centrifugation we wash the particles in a PBS , then we diluted the samples. After dilution, we measure the fluorescent intensity using the tecan device.

1.4: Transmission electron microscopy (TEM):

To visualize the morphology and size of the KAIMRC-LNPs at nanoscale, we employed Transmission Electron Microscopy (TEM). Initially, a small drop of the LNPs suspension was placed onto a carbon-coated copper grid. Excess liquid was gently blotted with filter paper. For negative staining, a drop of 2% uranyl acetate was added for 1 minute and then excess stain was blotted off. The grid was allowed to air-dry completely in a dust-free environment. The samples were examined under TEM at various magnifications. The size distribution and morphology of the nanoparticles were assessed from the TEM images.

1.5 :Experimental Procedure for In Vitro Uptake of Fluorescently-labeled KAIMRC-LNPs by Huh-7 Liver Cancer Cells:

1. Cell Culture:

- Culture Huh-7 liver cancer cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.
- Maintain the cells in a humidified incubator at 37°C with 5% CO2.

2. Cell Seeding:

- Seed 6 × 10^5 Huh-7 cells per well in a suitable multi-well plate.
- Allow the cells to adhere for approximately 20 hours in the incubator.

3. Preparation of LNPs:

- Prepare fluorescein-labeled KAIMRC-LNPs and non-fluorescein-labeled KAIMRC-LNPs using standard liposome preparation techniques, ensuring their sterility.

4. Incubation with LNPs:

- After the 20-hour incubation period, aspirate off the old medium from each well.
- Wash the cells once with sterile Phosphate-Buffered Saline (PBS).
- Add serum-free DMEM containing either fluorescein-labeled KAIMRC-LNPs or non-fluorescein-labeled KAIMRC-LNPs to the respective wells.
- Incubate the cells with the LNPs for 50 minutes in the incubator.

5. Washing and Lysis:

- After the incubation period, aspirate the medium off and wash the cells thrice with cold PBS to remove any unbound or uninternalized LNPs.
- Add appropriate cell lysis buffer to each well to lyse the cells. Ensure gentle mixing to achieve complete cell lysis.

6. Measurement of Fluorescence Intensity:

- Transfer the lysates to a suitable microplate for reading.
- Measure the fluorescence intensity of each sample using a fluorescence microplate reader. Set the excitation and emission wavelengths appropriate for fluorescein.
- Compare the fluorescence intensity of cells incubated with fluorescein-labeled KAIMRC-LNPs against those with non-fluorescein-labeled KAIMRC-LNPs to determine the uptake.

7. Data Analysis:

- Analyze the data for mean fluorescence intensity values, and calculate the percentage uptake by comparing it to a control (cells without LNPs).
- Plot the data and compute any statistical tests if necessary.

Given that the described experiment yielded an 11% uptake, future optimizations as you mentioned (like varying LNP size or zeta potential) would be pivotal for enhancing cellular uptake.

SECTION 5: RESULTS

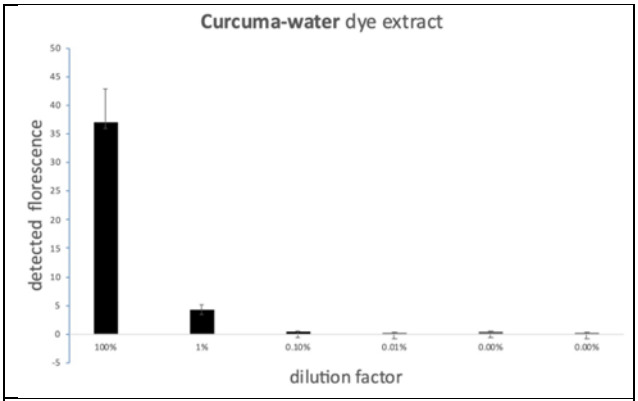


Figure 1.1: A curcuma-water dye extract was prepared by dissolving turmeric powder in distilled water at high temperatures for 5 to 30 minutes. The figure shows the dilution of the dye solution relative to the presence of fluorescence dye.

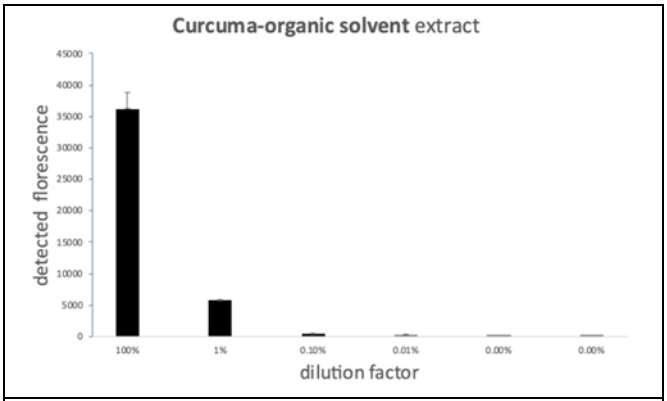


Figure 1.2: A curcuma-organic solvent dye extract was prepared by dissolving turmeric powder in an organic solvent at high temperatures for 5 to 30 minutes. The figure shows the dilution of the dye solution relative to the presence of fluorescence dye.

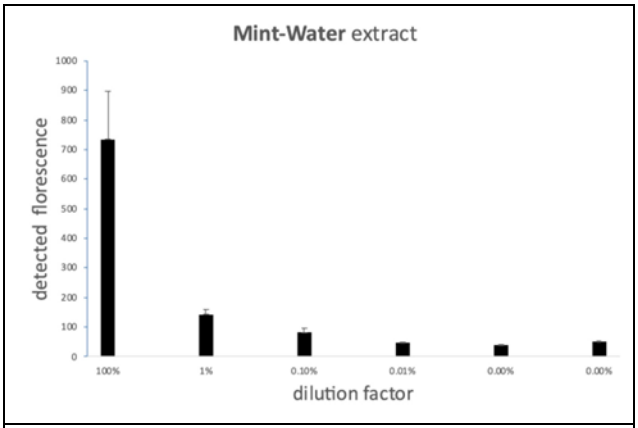


Figure 2.1: A mint-water dye extract was prepared by dissolving mint powder in distilled water at high temperatures for 5 to 30 minutes. The figure shows the dilution of the dye solution relative to the presence of fluorescence dye.

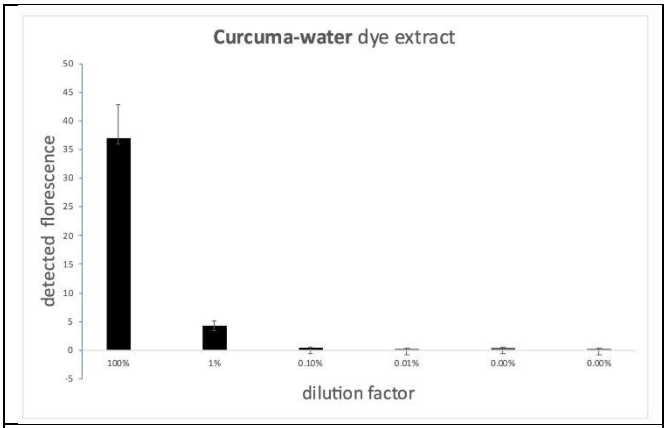


Figure 2.2: A mint- organic solvent dye extract was prepared by dissolving mint powder in an organic solvent at high temperatures for 5 to 30 minutes. The figure shows the dilution of the dye solution relative to the presence of fluorescence dye.

The reason why turmeric dissolved in organic solvent yields a better dye extraction is attributed to a number of properties of turmeric, including its **solubility**, turmeric is more soluble in organic solvents thanin water. **polarity**, turmeric is a non-polar molecule, This means that it does not have a positive or negative charge. Water is a polar molecule, which means that it has a positive and negative charge. The positive and negative charges of water molecules attract the opposite charges of turmeric molecules, whichhelps to dissolve turmeric in water. However, the non-polar molecules of turmeric are not attracted to thepolar molecules of water, so they do not dissolve as well in water as they do in organic solvents, and lastly **hydrophobicity** [4].

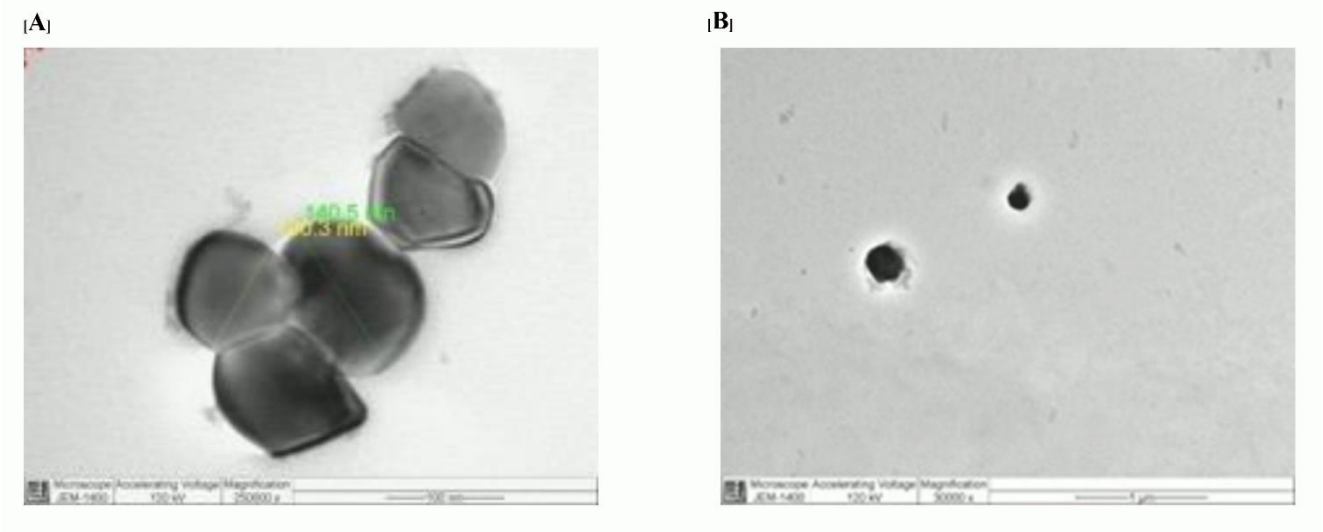
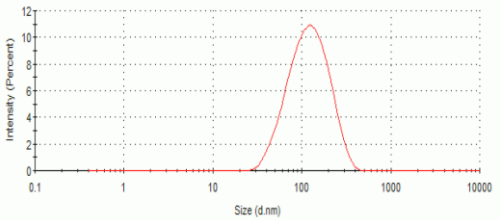


Figure 3.1: [A] Representative TEM images of KAIMRC-LNPs. [B] Representative TEM images of KAIMRC-LNPs loaded with curcuma dye.

Table 3.1: Size, Zeta Potential and encapsulation efficiency of Fluorescence Lipid Nanoparticles the average encapsulation efficiency of 100 nm LNPs is almost 98%, The negative zeta potential of the LNPs helps to prevent aggregation, which is important for maintaining a high encapsulation efficiency.

Type of LNPs	SIZE	PDI	ZETA POTENTIAL	ENCAPSULATION EFFICIENCY (%)
KAIMRC-LNPs				NA
Fluorescently-labeled KAIMRC-LNPs	**98.43 ± 9.29**	**0.2 ± 0.008**	**-13.56 ± 1.98**	**97.74% ± 0.52**



The encapsulation efficiency is a measure of how much of the drug is actually encapsulated in the LNPs. A high encapsulation efficiency is desirable because it means that more of the drug will be delivered to the target cells. The encapsulation efficiency of 98% is very good, and it suggests that the LNPs are likely to be effective at delivering curcumin to cells. The size of the LNPs is also important for drug delivery. LNPs that are too large will not be able to enter cells, while LNPs that are too small will be cleared from the body by the immune system. The size of 98.43 nm is a good compromise, and it suggests that the LNPs are likely to be able to enter cells without being cleared by the immune system. Polydispersity index (PDI): The PDI is a measure of the uniformity of the size of the LNPs. A low PDI indicates that the LNPs are relatively uniform in size, which is desirable because it means that the drug will be delivered more evenly to the tissues. The PDI of 0.2 is very low, and it suggests that the LNPs are very uniform in size [5].

In Virto Uptake and/or binding of Fluorescently-labeled KAIMRC-LNPs by cells

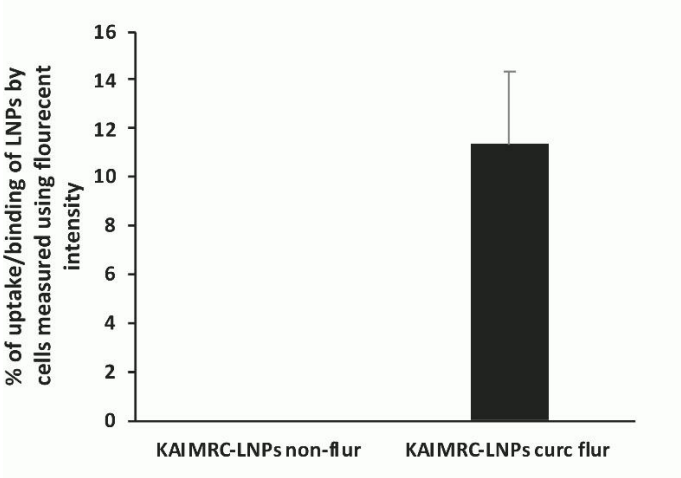


Figure 3.3: Uptake and/or binding of KAIMRC-LNPs by cells. Huh-7 liver cancer (6 × 105) were seeded. Twenty hours later, the medium was replaced with serum-free DMEM containing fluorescein-labeled KAIMRC-LNPs or non-fluorescein-labeled. The cells were washed after 50 min of incubation and lysed, and the fluorescence intensity was measured. Data are mean ± S.E.M. (n = 10).

The in house produced LNPs were able to be taken up by cells in the lab setting, which is a necessary step for drug delivery. Although promising, the 11% uptake is still relatively low. This suggests that there is room for improvement in the design of the LNPs. Future studies should focus on optimizing the properties of the LNPs to improve their uptake by cells. This could involve making the LNPs smaller, increasing their negative zeta potential, or targeting them to specific cell types.

SECTION 6: INTERPRETATION & CONCLUSIONS

The study of fluorescently-labeled KAIMRC-LNPs showed promising results in terms of their encapsulation efficiency, size, and polydispersity index. However, the in vitro uptake and/or binding of the LNPs by cells was relatively low, at 11%. This suggests that there is room for improvement in the design of the LNPs.

I recommend that future studies focus on the following areas: (i)Increasing the negative zeta potential of the LNPs: The negative zeta potential of the LNPs makes them attracted to cells. Increasing the negative zeta potential could help to improve the uptake of the LNPs by cells. (ii) Further purifying the curcuma dye used to make the LNPs: This could help to improve the efficacy of the LNPs by removing impurities that may interfere with their uptake by cells. I believe that these studies could help to make the in house produced fluorescently-labeled KAIMRC-LNPs a more effective drug delivery system.

SECTION 7: REFERENCES

[1] Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2019). The history of nanoscience and nanotechnology: from chemical- physical applications to nanomedicine. Molecules, 25(1), 112.

[2] Fan, Y., Marioli, M., & Zhang, K. (2021). Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. Journal of pharmaceutical and biomedical analysis, 192, 113642.

[3] Marshall, J., & Johnsen, S. (2017). Fluorescence as a means of colour signal enhancement. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1724),20160

[4] Sahne, F., Mohammadi, M., Najafpour, G.D., Moghadamnia, A. A. (2016). Extraction of bioactive compound curcumin from turmeric (Curcuma longa L.) via different routes: A comparative study. Pakistan Journal of Biotechnology, 13(3), 173-180.

[5] Wang, W., Chen, T., Xu, H., Ren, B., Cheng, X., Qi, R., … & Chen, C. (2018). Curcumin-loaded solid lipid nanoparticles enhanced anticancer efficiency in breast cancer. Molecules, 23(7), 1578.


good luck
to you

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1. Novelty and Significance:

   This science project demonstrates novelty and significance in its approach to developing fluorescently labeled lipid nanoparticles (LNPs) using natural resources. The use of vibrant dyes extracted from Curcumin to produce in-house KAIMRC-LNPs represents an innovative and sustainable approach to nanoparticle labeling. This research has potential implications for both diagnostic and therapeutic applications, particularly in the fields of drug delivery and optical molecular imaging. The project's focus on utilizing natural resources for fluorescent labeling aligns with current trends towards more sustainable and biocompatible materials in nanomedicine.

2. Strengths:

   The study's strengths lie in its comprehensive characterization of the developed LNPs. The researchers have successfully measured key parameters such as encapsulation efficiency, size, and polydispersity index, which are crucial for understanding the properties and potential applications of these nanoparticles. The use of transmission electron

microscopy (TEM) to characterize the morphology and size of the curcumin-labeled and mint dye-labeled LNPs demonstrates a rigorous approach to nanoparticle analysis. Additionally, the project's exploration of both in vitro and in vivo applications for these naturally labeled LNPs showcases the potential versatility of this approach in various biomedical contexts.

3. Weaknesses:

The project has several weaknesses that need to be addressed. Firstly, the lack of detailed information on the extraction methods used for obtaining fluorescent dyes from Turmeric Powder (TP) and mint limits the reproducibility of the study. The absence of data on the purity of these extracted dyes raises questions about the consistency and reliability of the labeling process. Furthermore, the discrepancy between using curcumin and mint dye-labeled LNPs for characterization but fluorescein-labeled KAIMRC-LNPs for cell assays is not adequately explained, potentially compromising the study's internal consistency. The project would benefit from a more detailed comparison with commercially produced or synthetic fluorescent LNPs to better contextualize the advantages and limitations of using natural resources. Lastly, while the

basic characterization is well-executed, further refinement is necessary to improve functional properties such as biocompatibility and uptake efficiency, which are crucial for the practical application of these nanoparticles in biomedical settings.