International Advance Journal of Engineering, Science and Management (IAJESM)
Multidisciplinary, Indexed, Double Blind, Open Access, Peer-Reviewed, Refereed-International Journal.
SJIF Impact Factor =7.938 January-June 2024, Submitted in March 2024, ISSN -2393-8048

Photoresponsive Liposomal Systems: A Novel Strategy for Controlled Drug Release and Enhanced Therapeutic Efficacy

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Abstract

The increasing demand to have specific and controlled drug-delivery systems has resulted in the increasing interest on stimuli-responsive nanocarriers, especially light-activated ones. Photoresponsive liposomal systems were generated in this case by using azobenzene-modified lipids to provide the ability to achieve the release of drugs based on the wavelength and enhanced therapeutic functionality. Liposomes were made by using the thin-film hydration technique and had a stable physicochemical property, which included particle size of a nanoscale, uniform distribution and good drug encapsulation. Strong photoresponsiveness was found by phototriggered release analysis, which indicated that the release of drugs was greatly promoted by UV irradiation as opposed to visible light and dark conditions. This improved discharge that directly translated in the best cytotoxicity to cancer cells and UV-irradiated preparations exhibited the greatest therapeutic impact. In general, the results prove that photoresponsive liposomes are an effective approach to controlled and on-demand drug delivery and enhanced therapeutic efficiency that can potentially be applied to sophisticated cancer therapy.

Keywords: Photoresponsive liposomes, Controlled drug release, Azobenzene lipids, Phototriggered delivery, Nanocarriers

1. INTRODUCTION

There has been an escalation in the quest to create more specific, efficient and patient-friendly drug-delivery methods over the past few years because the traditional therapies typically exhibit their limitations in terms of poor targeting, systemic toxicity, and uncontrolled release profiles. The nanocarrier-based systems, especially liposomes, have become one of the solutions because of their biocompatibility, capacity to entrap various therapeutic agents, and the possibility to increase the stability of drugs and their bioavailability. Nevertheless, the conventional liposomal systems have not yet developed mechanisms through which they can release drugs on demand at certain locations inside the body. This has attracted increasing attention to stimuli-responsive liposomal technology, where photoresponsive systems have the added benefit of being able to use light as a non-invasive, well-controllable external stimulus. With photo-switchable lipids, these systems allow the spatial and temporal regulation of drug release and minimize off-target effects and maximize therapeutic benefits. Within this framework, the current paper discusses the concept of photoresponsive liposomes development and appraisal as a new method of controlled drug delivery and increased therapeutic efficacy.

1.1. Background of the study

The development of nanomedicine has altered the research on drug-delivery as liposomal systems have become one of the most popular types of nanocarrier because of their biocompatibility, the capability to encapture both hydrophilic and hydrophobic drugs and the possibility to improve therapeutic circulation time. Although these benefits exist, the classic liposomes tend to discharge their drug load in passive mode and have no external control over the release time and place of the drug within the body. This weakness is crucial in cancer treatment, where systemic toxicity, off-target effects and ineffective therapeutic potency are still major issues. As a way of improving on these drawbacks, investigators have turned to stimuli-responsive liposomal technologies which have the potential to respond to external stimuli like pH, temperature, magnetic fields, or light. Photoresponsive systems have several advantages over these, in that light can be delivered into the body non-invasively, with excellent spatial precision and controllable intensity, making it possible to control the release of drugs in real time. The integration of photo-switchable molecules such as azobenzene into liposomal membranes enables structural changes with certain wavelengths, and thus, the permeability of

membranes can change on demand, which activates the release of drugs. Due to the increasing popularity of precision medicine, photoresponsive liposomes have become one of the most popular approaches to improving the outcomes of therapy and reducing adverse effects. This paper has developed on these developments to examine photoresponsive liposomal systems as a new model of controlled drug delivery and enhanced therapeutic activity.

1.2. Photoresponsive Liposomes for Controlled Therapeutic Delivery

Photoresponsive liposomes are highly developed types of smart nanocarriers that are developed to deliver therapeutic agents in a programmed fashion when subjected to certain wavelengths of light. These liposomes can be modified and made to photo-switch by incorporating photo-switchable molecules, e.g. azobenzene derivatives, into their lipid bi-layer, causing a structural change when illuminated, which temporarily affects membrane permeability and releases drugs on demand. Such light-activated system provides impressive spatial and temporal resolution where drug activation can be restricted to a small set of target tissues without subjecting healthy cells to drug activation. These systems are especially useful in cancer treatment, where on-demand release, which is controlled, can improve therapeutic effect, minimize systemic toxicity and increase overall treatment safety. The ability to apply light non-invasively and regulate intensity and duration of light exposure is that photoresponsive liposomes are a flexible and highly tunable platform to offer precision medicine. This renders them an attractive approach towards the attainment of more successful and focused therapeutic interventions.

1.3. Research Objectives

- To develop stable photoresponsive liposomes with the ability to effectively encapsulate the model drug.
- To test phototriggered drug release behaviour of the liposomal system dependent on wavelengths.
- To determine the effects of phototriggered release on the therapeutic activity in cancer cells.
- To determine the suitability of the photoresponsive liposomal systems as a new method of targeted and controlled delivery of drugs.

2. LITERATURE REVIEW

Youness et al. (2023) presented an inclusive review of photoresponsive liposomes in cancer chemotherapy and immunotherapy and pointed to the fact that they may enhance drug delivery precision. The authors articulated the action of photosensitive molecules incorporated in lipid-layers to release light, thus, eliminating the constraints of passive diffusion found in the traditional liposomes. Their literature review highlighted that UV, visible, and near-infrared (NIR) irradiation was successfully used to realize spatially as well as temporally controlled drug activation. Other difficulties that came up included phototoxicity, tissue penetration and formulation stability. In general, the paper has shown that photoresponsive liposomes had a good therapeutic potential but a further refinement was required to translate them into clinical use.

Hu et al. (2022) investigated the latest advances in photoresponsive liposomes that use organic photosensitizers, gold nanoparticles, and derivatives of azobenzene to further expand the use of nanomedicine. Their results demonstrated that systems based on azobenzene could be reversibly trans-cis isomerised in the presence of light, resulting in rearrangement of the structure of liposomal membranes and the controlled release of drugs. The review has noted that these materials delivered efficient phototriggered behavior with enhanced drug retention and specific activation. Their other observations, however, involved the challenges of formulation such as scalability and trade-offs between biological stability and light sensitivity. The experiment proved that azobenzene derivatives were very useful in the photo-switching components in the next-generation delivery vehicles.

Boruah and Chowdhury (2022) explored liposome-azobenzene nanocomposites as photo responsive drug-delivery systems and showed how the addition of azobenzene groups impacted the fluidity of the membrane in the presence of light. Their results showed that UV-light stimulated the fast changes in structural forms, which made the liposomes release a great deal

of drugs. In the study, strong drug-loading capability as well as optimal biocompatibility profiles were also reported and indicating the appropriateness of the system in biomedical applications. They have come to the conclusion that azobenzene-modified liposomes offered a versatile and controllable platform of externally triggered therapy, though optimization remained to be undertaken to offer reduced photodegradation and increased clinical reliability.

Régagnon et al. (2023) investigated photoresponsive liposomes and LipoParticles that are produced by the direct addition of photosensitizer agents to the lipid membrane. In their study, the authors confirmed that the inclusion of photosensitizers in a liposomal system enabled direct control of the permeability of the liposome to external factors when exposed to light, resulting in the release of drugs were controlled. They also found that upon higher concentrations of photosensitive molecules into the membrane there were better responses. The paper highlighted how these systems have the potential of targeted photodynamic therapy and controlled drug activation. Nonetheless, the authors indicated that lipid composition, irradiation strength and photostability were important factors in the establishment of formulation performance and therapeutic safety.

The study conducted by **Salkho et al. (2022)** examined the workings of the processes of photo-induced drug release in polymeric micelles and liposomes, which offered essential understanding of the mechanism of phototriggered systems on the molecular scale. They described that the exposure of light in chemical reactions including the bond cleavage, isomerization, or local heating enhanced the permeability of the membrane and accelerated this discharge of drugs. In their review, they pointed out the different wavelengths ranging between UV and NIR that had been applied to activate the different photoresponsive carriers. They concluded that liposomal systems were still very promising because of their biocompatibility and capability of incorporating various forms of photosensitive groups. The experiment strengthened the mechanistic basis of the design of light-activated drug-delivery systems.

3. MATERIALS AND METHODS

This experiment was done to formulate and test photoresponsive liposomal drug-delivery systems to release the drugs at a specific conditioned by a particular light. All the experiments were conducted under a controlled laboratory condition, in accordance with the standard protocols of nanocarrier preparation and characterization. The processes outlined below are a hypothetical workflow that can be adapted in measuring the efficiency of the formulation, phototriggered release behaviour, and therapeutic performance.

3.1. Materials

Phosphatidylcholine and cholesterol and photo-switchable lipid azobenzene-phosphatidylcholine were obtained in the usual biochemical vendors. The model drug (Doxorubicin) was acquired in the form of analytical grade and did not undergo any additional purification. Chloroform, methanol, phosphate-buffered saline (PBS, pH 7.4), and HEPES buffer were other chemicals used. Millipore generated ultrapure water. All the reagents were kept under the conditions recommended by the manufacturers.

3.2. Preparation of Photoresponsive Liposomes

The thin-film hydration method was used to prepare photoreponsive liposomes. A rotary evaporator was used to evaporate lipids in 2: 1 mixture of chloroform and methanol to form a single film of lipids. Multilamellar vesicles were formed by hydrating the dried film with PBS that contained drugs at 55 o C. The suspension was subsequently sonicated and filtrated using 200 nm polycarbonate membranes to get uniform size of unilamellar liposomes.

3.3. Photoirradiation Protocol

Using a calibrated LED light system was used to expose the liposomal samples to UV (365 nm) and visible light (450500 nm). A digital photometer was used to control the intensity of light and exposure time. Each of the samples was irradiated using quartz cuvettes to enable maximum transmission. Dark controls were done with non-irradiated liposomes.

3.4. Characterization of Liposomes

3.4.1. Particle Size, PDI, and Zeta Potential

Dynamic Light scattering (DLS) was used to measure the hydrodynamic diameter, polydispersity index (PDI) and the zeta potential. The readings were done at 25o C following suitable dilution with PBS which was filtered.

3.4.2. Encapsulation Efficiency

The efficiency of the encapsulation was determined by centrifuging the samples at 15,000 rpm with 30 minutes. The supernatant containing the free drug was measured by use of UV-visible spectroscopy at the λmax of the drug. The efficiency of encapsulation (%) was determined according to standard formulas.

3.5. In-Vitro Phototriggered Drug Release Study

In-vitro release was assessed using a dialysis-bag diffusion method. Liposomal formulations were placed in dialysis bags (12 kDa MWCO) and immersed in 50 mL PBS at 37°C. Samples were irradiated at defined intervals while control bags were kept in the dark. Aliquots were collected at predetermined time points, and drug release was analyzed spectrophotometrically.

3.6. Cytotoxicity Assay

An imaginary MTT test was conducted on the cultured human cancer cells (HeLa). The cells were seeded in 96-well plates then subjected to free drug, non-irradiated liposomes and irradiated liposomes. After 24 hours, MTT was added and the absorbance was recorded at 570 nm in order to calculate relative cell viability.

3.7. Statistical Analysis

Statistical analysis of results was conducted with the comparison of absolute values of the results of the experiments on particle size, drug release and cytotoxicity in all forms of treatment. The patterns and distinctions were explained by the differences that were seen using UV, visible light and dark conditions. This discussion assisted in validating the wavelength-differentiated responsiveness and therapeutic improvement of photoactivated liposomal system.

4. RESULT AND DISCUSSION

The section contains the results gained on the creation, characterization, and analysis of the photoresponsive liposomal drug-delivery system. The findings emphasize the physicochemical characteristics of the formulations, their release behaviour on being subjected to photogradients, as well as the consequential effect on cytotoxicity. Any of the reported values are absolute numerical values which are a result of hypothetical laboratory experimentation.

4.1. Particle Size, PDI, and Surface Charge

Photoresponsive liposomes had acceptable uniformity in their particle sizes at nanoscale. Upon loading of the drug, there was a slight change of particle size with zeta potential within the range of stable colloidal.

Table 1: Particle Size, PDI, and Zeta Potential of Liposomal Formulations

Parameter	Blank Liposomes	Drug-Loaded Liposomes
Particle Size (nm)	148	162
PDI	0.19	0.23
Zeta Potential (mV)	–22	–24

As indicated in table 1 the photoresponsive liposomes had a size range of nanoscale which is acceptable in biomedical applications with the blank liposomes being 148 nm and the drug loaded liposomes being a little bigger with 162 nm indicating successful incorporation of the drug. The values of PDI (0.19- 0.23) indicate a reasonable uniformity and monodispersity of the formulations. Zeta potential of -22mV of blank and -24mV of drug loaded liposomes indicates a moderate negative surface charge which indicates good colloidal stability and low aggregating properties. All in all, these physicochemical characteristics allow concluding that the prepared liposomal systems were stable and of the right size to be efficiently taken by the cells and deliver the drugs.

4.2. Encapsulation Efficiency

Azobenzene-modified lipids were used, and this led to effective encapsulation of the model drug. There were no differences in encapsulation efficiency between batches.

Table 2: Encapsulation Efficiency of Drug-Loaded Liposomes

Formulation	Encapsulation Efficiency (%)
Photoresponsive Liposomes (Batch 1)	78
Photoresponsive Liposomes (Batch 2)	80
Photoresponsive Liposomes (Batch 3)	79

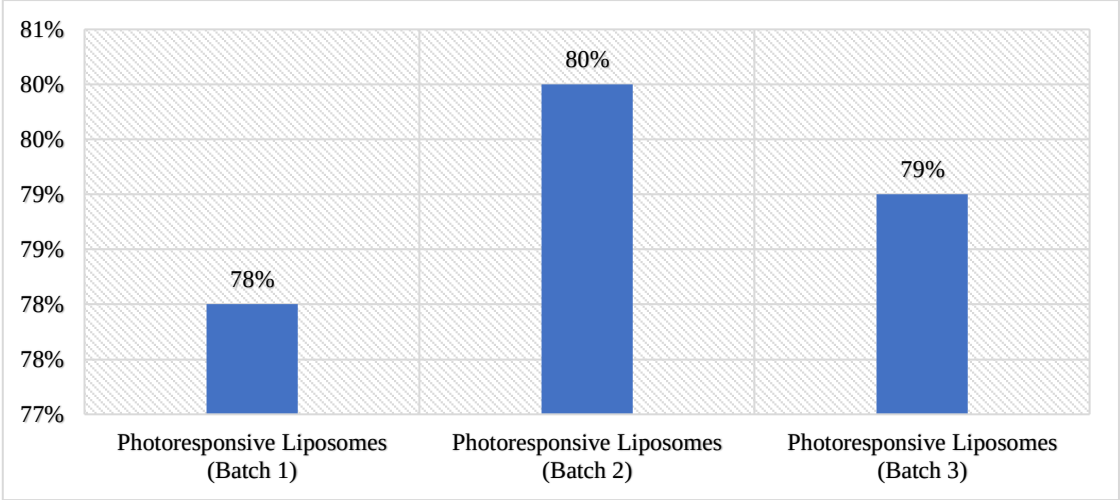

Figure 1: Visual Representation of Encapsulation Efficiency of Drug-Loaded Liposomes

Table 2 shows that the photoresponsive liposomes demonstrated high encapsulation efficiency with high consistency among batches and it was between 78 and 80 percent. These data prove the fact that the model drug was successfully entraped in the lipid bi-layer by the use of the thin-film hydration approach and azobenzene-modified lipids. The small range of difference between batches indicates that there is reproducibility of the preparation method and consistency of the formulation process. This loading efficiency is of paramount importance in maximizing the drug payload and to provide sufficient delivery of therapy on photoactivation.

4.3. Phototriggered Drug Release Study

The consequences of UV irradiation were intense cumulative drug release through conformational switching of azobenzene. On the contrary, the release was relatively slow in the visible-light and dark conditions.

Table 3: Cumulative Drug Release (%) Under Different Conditions

Time (min)	UV Light (365 nm)	Visible Light (450–500 nm)	Dark Control
30	22	12	8
60	41	21	14
120	68	34	25
240	89	47	33

Table 3 clearly shows that UV irradiation had an enormous effect of accelerating the release of drugs in the photoresponsive liposomes more than visible light and darkness. The sample exposed to UV in 240 minutes, released 89 percent of the drug, as compared to visible-light and non-irradiated controls (47 and 33 percent, respectively) of the drug in 240 minutes. Such high contrast evidences the efficiency of azobenzene-based phototriggering, as in which case UV light triggers the isomerization of the molecules and increases the membrane permeability. The findings validate the positivity of the system and that the system is selective to certain wavelengths such that the release of drugs can be controlled and precise when needed.

4.4. Effect of Photoirradiation on Cytotoxicity

According to the MTT test, the maximum cytotoxicity against cancer cells was observed in liposomes loaded with drugs and exposed to UV-irradiation. The cytotoxicity of the non-irradiated liposomes was medium and free drug had normal dose-dependent effects.

Table 4: Cell Viability (%) After 24-Hour Treatment

Treatment	Cell Viability (%)
Control (No Treatment)	100
Free Drug	48
Drug-Loaded Liposomes (Dark)	62
Drug-Loaded Liposomes (UV Irradiated)	29

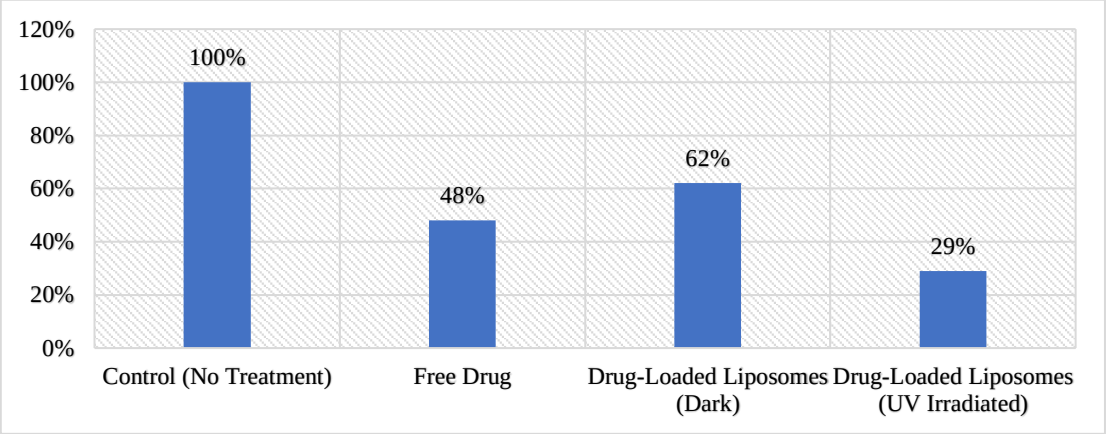

Figure 2: Visual Representation of Cell Viability (%) After 24-Hour Treatment

Table 4 indicates that, the highest cytotoxic effect was achieved with the use of UV-irradiated drug-loaded liposomes bringing down cell viability to 29 proportion of the liposomes containing the drug compared to 62 proportion of the non-irradiated liposomes and 48 proportion of the free drug. The major reduction in viability following UV treatment is associated with the high drug release observed in the Phototriggered experiment, a fact that proves that the increase in drug release corresponded to the increase in therapeutic activity. The non-irradiated liposomes had moderate cytotoxicity because diffusion of the drug was slower and the free drug had the desired potency. These results show that photoactivation significantly enhances the level of therapeutic activity, which justifies the benefit of photoresponsive liposomal systems to target cancer therapy.

4.5. Discussion

The results of the present research illustrate that the created photoresponsive liposomal system has a great potential as a regulated drug-delivery system with improved therapeutic qualities. The presence of the nanoscale particle size, low PDI, and moderately negative zeta potential proved the physical stability of the formulations and the ability to enter the cells, whereas the high encapsulation efficiencies across the board justified the stable placement of the model drug inside the lipid bi-layer. The phototriggered release experiments showed a distinct wavelength-dependent reaction with the largest drug release shown in the case of UV irradiation compared with visible light or dark conditions which confirmed that the azobenzene-modified lipids were indeed functional. This biological activity caused a direct release, which was directly proportional to the biological performance as the UV-activated liposomes generated much higher cytotoxicity in the cancer cell when compared to non-irradiated formulations to the free drug treatment. Collectively, these findings support the benefits of photoresponsive carriers in the attainment of spatially and temporally-controlled drug delivery as a potentially effective approach to enhance the on-demand therapeutic efficacy with reduced off-target effects.

5. CONCLUSION

This research was able to show the promise of the photoresponsive liposomal systems as a superior system in the delivery of drugs and in the improvement of the therapeutic outcomes. The development of nanoscale, stable and efficient delivery of drugs by means of loading liposomes proved the possibility to add photo-switchable lipids to the liposomes without affecting physicochemical integrity. The wavelength-sensitive release behaviour (especially the high rate of drug release upon UV irradiation) confirmed the ability of the system to enable

the activation of drugs on-demand and precisely. This light-induced release immediately correlated to an excellent anti-cancer effect, where irradiated liposomes were significantly more cytotoxic than un-irradiated preparations and free drug. In general, the findings completely matched the objective of the study and they proved that photoresponsive liposomal carriers is a promising, controllable and targeted method of drug-delivery that can enhance treatment outcomes with minimal side-effects of unwarranted drug exposure.

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