OPTIMIZATION AND FORMULATION OF DOXORUBICIN (DOX) LOADED LIPOSOME WELL-USED IN CHEMOTHERAPY INVOLVING QUALITY BY DESIGN (QBD): A TRANSITORY RESEARCH

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Abstract:
Nanomedicine is a developing discipline that is constantly evolving and differentiating.Liposomal preparation has an important role in nanomedicine as a novel platform. QbD involves drug formulation and advancement of a pharmaceutical medicament, including understanding of product quality and formulation steps, processing, and implementing controlsto establish the product quality maintained by QbD. Primarily, drug and medicament governing bodies, including the USA and FDA, enhance product quality. Liposomal formulations and optimisation involve dependent and independent variables, requiring experience in optimisation. QbD is a risk-based approach used early in the pharmaceutical process to improveproduct quality and efficacy. Please shorten the given text so that it is more concise. QbD speeds up product development and ensures consistent, safe drug formulation in complex systems. In QbD, steps flow as adding variables related to CMAs, CPPs, and design places responsible for quality attributes for the final liposomal product preparation. QbD has recentlybeen proposed as a tool for obtaining higher-quality liposomal nanocarriers. The broader structure of this research discusses the involvement of QbD as recent approach including their different parameters. Overall, lastly, the current practices that employ QbD in the optimisation and formulation of doxorubicin (DOX), by the using thin film-hydration extrusion technique primarily. DOX is antitumor class drug with a brand (Doxil®) loaded liposomal with TNF receptor as nanocarrier optimisation and formulation.

Keywords: Quality by Design (QbD); Advance Drug delivery; nano-techniques; liposomes; chemotherapy; Optimization.

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1. Introduction:

The advanced approach to developing pharmaceuticals, known as Quality by Design (QbD), involves creating products and procedures based on a comprehensive comprehension of the product's intended use and crucial quality features (CQAs). QbD has been widely adopted in the pharmaceutical industry over the past few decades and has led to significant advancements in drug development. One of the key advancements in QbD has been the use of risk-based approaches to product and process design. QbD emphasises the importance of identifying and assessing risks throughout the development process, and using this information to inform decisions about design and control strategies [1]. This has led to the development of tools and methods for risk assessment and management, such as failure mode and effects analysis (FMEA) and hazard analysis and critical control points (HACCP). Another important advancement in QbD has been the use of process analytical technology (PAT) to monitor and control manufacturing processes in real time. PAT involves the use of sensors and other analytical tools to measure and control critical process parameters (CPPs) during manufacturing, with the goal of ensuring that the final product meets its intended quality attributes. This has led to increased process understanding, reduced variability, and improved product quality. QbD has been instrumental in improving the efficiency and effectiveness of pharmaceutical development and manufacturing, and has helped to maintain the quality, effectiveness, and stability of drug and medicament products [1, 2].

The advancement in the growing interest in the use of nanomedicine and therapeutics based on nanoparticles, both at the academic and industrial levels, QBD is a regulatory requirement in the pharmaceutical industry and is governed by guidelines issued by regulatory agencies such as the US Food and Drug Administration (FDA). Liposomes consist of a phospholipid bilayer

forming a spherical shape around a watery core [2]. Liposomes have been utilised as a vehicle for administering various types of nanomedicines.

Doxorubicin, an anticancer drug, is incorporated into liposomes to enhance therapeutic benefits and minimise negative side effects [4]. Furthermore, it has been extensively studied as a vehicle for nucleic acid-derived remedies like DNA and siRNA, enhancing targeted cell delivery, and safeguarding drugs from degradation [5]. It's noteworthy to mention that the initial COVID-19 mRNA vaccine has received FDA approval in 2020 and employs lipid-based nanocarriers as a means of delivery [6]. Liposomes pose challenges for production and development due to their complex formulations. The complexity of nanofabrication is due to intriguing material science and the techniques employed at the nanoscale. This involves numerous factors, which are required for comprehension and enhancement [7].

Nano preparations and manufacturing suffer from sensitivity and poor reproducibility due to a lack of understanding and optimisation. Undoubtedly, a beneficial technique for such systems is an innovative advancement that enables the recognition of significant factors and assists in comprehending their impact on the final product's attributes and excellence. In order to achieve this objective, different industries and regulatory organisations have suggested and endorsed quality by design (QbD) as a viable solution [8, 9]. The principle of quality by design (QbD) initially involves the recognition of the quality target product profile (QTPP). The QTPP can be comprehended as a concise summation of the quality attributes (QA) intrinsic to the end product. The objective of establishing the QTPP is to guarantee the effectiveness and safety of the product in question. Critical material attributes (CMA) and process parameters (CPP) influence quality assurance [10].

QbD identifies and optimises CMA and CPP, setting target specs for QA and QTPP of the final product [9, 11]. By applying appropriate experimental techniques, critical material attributes and CPP are correlated to quality assurance [8, 12], which then leads to the creation of precise requirements for materials, procedures, and the final output. Additionally, Quality by Design will facilitate a comprehensive evaluation of the collective consequences of various factors on the Quality Target Product Profile simultaneously. Risk management is utilised for the purpose of prioritising quality assurance measures [13]. Liposomes as drug delivery systems are in high demand for their clinical and preclinical applications, emphasising the need for their advancement. This would facilitate the enhanced therapeutic efficacy of loaded therapies.

Although there is literature on QbD involvement in liposomal drug delivery, we still need to expand our knowledge of QbD developments in

liposomal formulation. This knowledge ensures liposomal drug delivery with improved therapeutic effects and potential for industry use. As a result, this study focuses on the optimisation and formulations of doxorubicin-loaded liposomes, which are commonly employed in chemotherapy treatment.

2. Quality by Design (QbD):

2.1. Quality by Design Involve in Medical Commodities:

The primary objective of the medical industry is to produce high-quality medical commodities [14]. All aspects that can affect the health of patients and their prescribed products must be considered in maintaining the quality of pharmaceutical products. Earlier, the quality by testing process (QbT) was the same process to ensure the standard of the output. Quality by testing is dependent upon process checking of inputmatter (excipients), intermediaries, and final output [15]. The medical industry seeks a new approach that guarantees quality in advance of production, while still upholding essential QbT control testing. Pharmaceutical companies and regulatory bodies are now adopting the QbD approach, which guarantees the advancement and manufacturing of pharmaceutical products according to predetermined quality testing parameters. This is anticipated to reduce extensive testing during or after production, and enhance consistency, production efficiency, effectiveness, and safety [16]. The quality development of pharmaceutical products is used in QbD in pharmaceutical industries such as (**Fig. 1**) below.

Figure. 01 Quality designing in pharmaceutical products using QbD

QbD can be described as a forward-looking approach to enhance the quality of a product [17]. The key elements of QbD have been extensively outlined by *ICH, US FDA, and* some other government body ensure the steadfastness of superior pharmaceutical products (**Figure. 01**), Different regulatory bodies from around the world have consistently shown interest in the implementation of Quality by Design (QbD) [16,18].

2.2. *Various Appliances and Key aspects involving in QbD*: Normally, the key aspects of the QbD are divided into 4 parts: The various elements such as QTPP, CQAs, CMAs,and CPPs are all interrelated and essential components in ensuring the quality of a product [19,20]. All the elements in (**Fig. 2**) as follows:

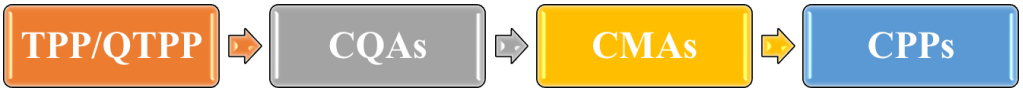


Figure. 02 The various elements involving in QbD Designing of API

All elements which collaborate to establish QbD framework. There are lots of key factors needed for experimental design and numerical inspection (**Fig. 2**) [16,21].

ICH guideline mainly defined QTP such involving "a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficiency of the drug product" [18,19]. Additionally, producer must evaluate complicated shifter such as drug pharmacokinetic characteristics, commodity steadiness, sterility, and medicament liberation

while identifying QTPPs and defining the needed effects of the output [20-22]. CQAs are defined by ICH as attributes that must be within appropriate limits to ensure desired product quality.

Based on this explanation, the CQAs are determined through the QTPP, governmental mandates, or existing composition-based comprehension. As a result, the selection of the drug product's dosage form, excipients, and manufacturing process is based on its critical quality attributes (CQA) and QTPP [23]. The combinations of ICH Guidelines in the formulation and optimisation of liposomes, which are doxorubicin (DOX)-loaded liposomes, with the following QbD steps all are briefly described in the (**Fig. 3**) in below section:

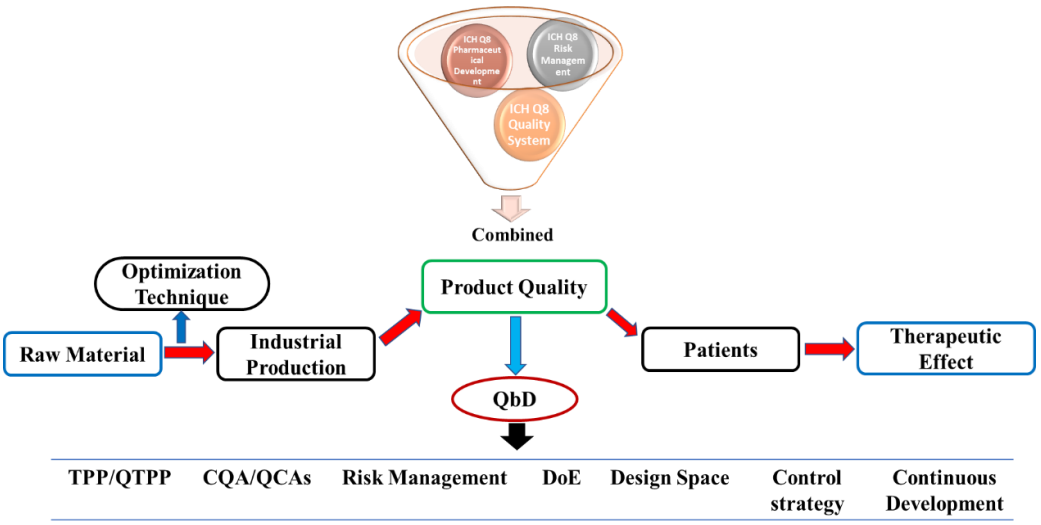


Figure. 03. The pharmaceutical development using of QbD for high-quality pharmaceutical products development under follows ICH, US FDA and EMA

Critical process parameters process involving magnitude which substantially influence the Quality target product profile [16]. Further, identification of CPPs, involving CMAs and CPPs to CQAs guarantee quality products will be obtained [24]. The particular function of each step involving in the QbD designing defined in the (**Fig. 4**) for the explanation of designing involving in QbD.

TPP/QTPP	CQA/QCAs	Risk Management	DoE	Design Space	Control strategy	Continuous Development
Identify the Target product profile (**TPP**) and Quality Target Product Profile (**QTPP**).	Determine the potential critical quality attributes (**CQAs**).	Link raw material attributes **CMAs** and process parameters **CPP** with CQAs.	Develop mathematical models. Multifactorial analysis.	**Design Space**, Real time release testing.	All the limitation involving.	Development of the API Designing as continuous/ drug life cycle.

Figure. 04 Steps of QbD in drug designing with their functions

Additionally, CPPs and critical material attributes are mainly detailed in the act as "a material or process whose variability has an impact on a critical quality attribute and should be monitored or controlled to ensure the desired drug product quality" [21, 24]. CMAs cover input materials (excipients, in-process material), while CQAs only focus on the product's quality. It is crucial to conduct risk management after analysing the impact of certain traits on critical quality attributes in order to pinpoint any formulations, substances, or procedure limitations that can vary CQAs. Moreover, qualitative as well as quantitative gauges have been employed in the evaluation of risk management level, with every determined factor pertaining to the preferred CQAs.

As a result, a risk management scale mainly based upon instability or predictability involving

severity and impact on efficacy and safety must be developed for products. The identification of CQAs can be accomplished through the use of sequel inspection and the defect in the system. Subsequent to undertaking the chance assessment procedure, certain factors emerge as potentially pivotal for the critical material attributes (CMAs), necessitating particular characteristics and a selection within a tolerable scope to safeguard the critical quality attributes (CQAs) of the final product [25, 26]. The processes in the QbD employed in optimisation and formulation are depicted in (**Fig. 5**).

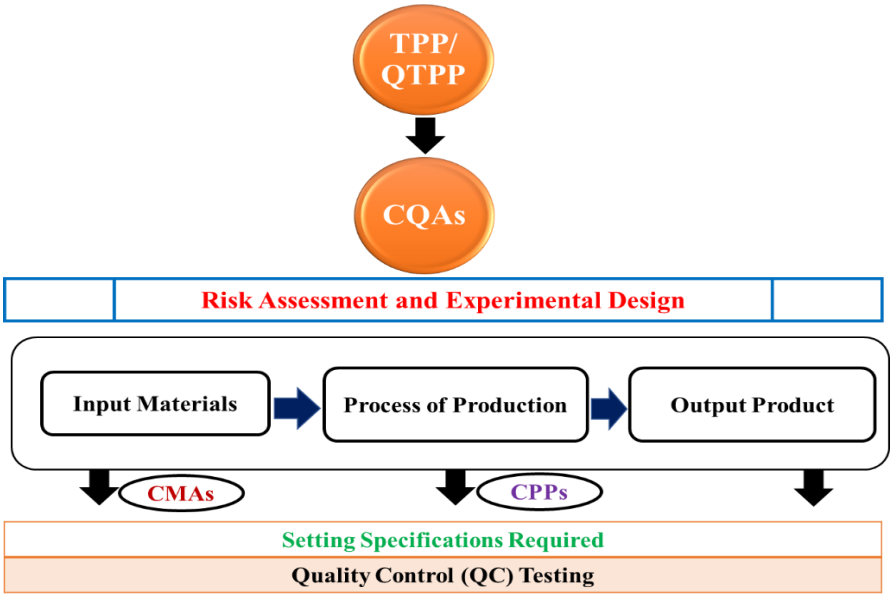


Fig. 5. Key aspects of the QbD roadmap, as well as the QTPP and CQAs

2.3. QbD in Liposomal Formulation:

The contents, preparation, characteristics, and manufacturing variables all have an impact on the quality of liposomal pharmaceutical products. Hence, QbD includes planning the ultimate liposomal items by improving input fabric and fabricating forms to obtain a medicinal item alongside predominant standards [27]. Furthermore, QbD categorizes and convey vital metrics and prime shifter in order to develop a elevated-standards medication matching a high standards [28]. In fact, as summarized in, various liposomal products have been produced using the QbD technique (**Table. 01**).

Table 1. The various examples of liposomes formulation by using QbD

Drug	QTTPs	CMAs/CPPs/CQAs	Refs.
Cefoperazone	Dry powder, pulmonary routeof administration, particle size,PDI, entrapment efficiency.	CQAs: Particle size, PDI, entrapment efficiency (EE%). CPPs: Hydration time, sonication time	[30]
Pravastatin	Systemic administration,tumour accumulation,enhanced stability and efficient process.	CQAs: Average particle size, encapsulated solute retention, zeta- potential, residual moisture content and kinetic stability.	[32-34]
Azacitidine (Azadine-O®)	Particle shape and size and % entrapment efficiency (%EE).	CPPs: Concentration of Lipids (mg), concentration of cholesterol (mg) and sonication time (min).	[35]
Salbutamol	Cholesterol concentration, Phospholipid concentration and Hydration time.	CPPs: Drug-lipid ratio, drug entrapment efficiency (%EE), sonication time and hydration time. CQAs: Vesicle size, zeta potential and drug encapsulation efficiency.	[36]
Doxorubicin* Curcumin	Decreasing doxorubicin (DOX) toxicity, enhancing curcumin (CUR) solubility, stability improvement.	CQAs: The size, surface charge/ morphology, drug loading, %EE and zeta potential. CPPs: pH and temperature, phospholipid concentration, the phospholipids to cholesterol ratio and the extrusion temperature.	[37]

The selection of QTPP is essential to guarantee that the final pharmaceutical product meets the desired quality standards [27]. Typically, QTPP is established through the correlation of scientific research data and significant in-vivo testing [25].

In order to identify the quality attributes that may affect the liposomal product's critical process parameters (CPPs) and Quality Target Product Profile (QTPP), it is imperative to initially enhance and recognize the common CQAs such as particle shape and size, distribution, zeta potential, drug content involving, In-Vivo stability, and drug release In-Vitro among others [25,38].

Although including the numerous advantages to applied QbD to the preparation of liposomal-contained products, there are numerous obstacles which prevent this. Benefits and difficulties are listed in (**Table 2**).

Table 2. The advantages and difficulties of implementing QbD principles in the production of liposomal products.

Functional Benefits: A Recent QbD Approach
▪ Improving the knowledge of how the compatibility of liposomes' constituents affects the production process.
▪ Delivering an improved liposomal product model with minimal challenges encountered during formulation and manufacturing.
▪ Facilitating progressive enhancements in the development and production processes of liposomal formulations.
▪ Consistent liposomal formulations can be achieved by comprehending the related risks.
▪ Making decisions based on efficient designs rather than relying on observational evidence.
▪ Integrating clinical testing with design by linking liposomal formulations and manufacturing.
▪ The FDA's approval process can be expedited by reducing the number of post-approval modifications, thus minimizing the overall expense of developing a liposomal formulation.

There are parcels of challenges with the QbD planning of burrowed medicaments counting parts of challenges within the given (**Table 3**) as taking after underneath portrayal:

Table 3. List of different challenges in QbD for drug designing

Various Challenges Involving in QbD
➢ The cost and duration of research and development have risen.
➢ The considerable expense associated with initiating the production, analysis, and design of liposomal products.
➢ Difficulties in maintaining consistent medication forms and concerns regarding adherence toregulations and technical specifications.
➢ The number of experiments has risen as a result of an increase in the characterization factorsof liposomes.
➢ The challenge lies in determining the impact of variables that may obscure or influence theresults.

2.4. QbD involving key specification for Liposomal based Products formulations:

2.4.1. Lipid Category and Content:

The lipid composition is a significant determinant of the solidity and integrity of liposomes. Lipids containing unsaturated fatty acids are prone to degradation through either hydrolysis oroxidation, whereas those containing saturated fatty acids are more stable and possess a greater transition temperature (Tm) [39]. Additionally, the lipid type and the liposome consist of lipidic compositions that have an impact on the fluidity, permeability, and surface charge of liposomes [40]. Such as cholesterol and phospholipids, classically alleviate liposome steadiness but should be improved and should not surpass 50% [41].

In general, the carbon series extent of prepared lipids may influence drug encapsulation efficiency for hydrophilic as well as hydrophobic medicines [40].

At instance, short fatty acid lipids can be used to create a big aqueous core, which enables an elevated inner volume in water-loving (hydrophilic) medicines.

In comparison, large carbon series lipids are highly capable to entrap the water-hating (hydrophobic) medicine inside the water-hating (hydrophobic) lipid bi-layer [42,43]. The composition of DOX medicament loaded liposomes is primarily illustrated using the followingrepresentation diagram (**Fig. 6**):

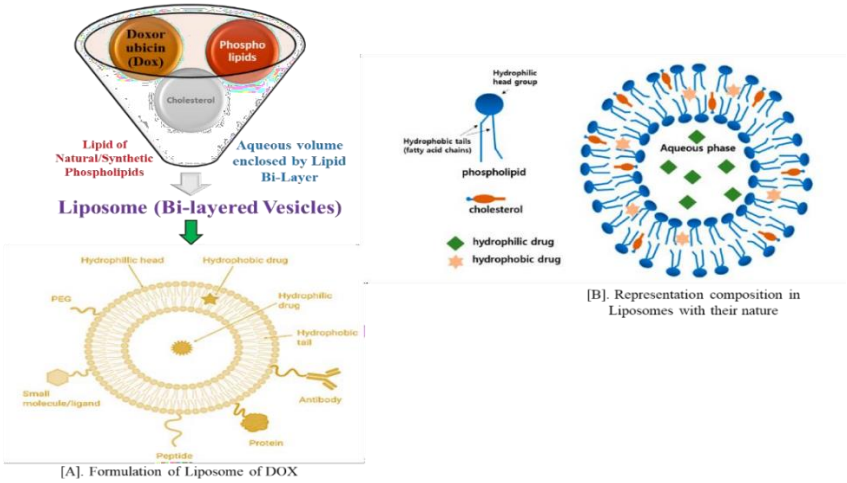


Figure. 06 Representation of DOX loaded liposome and their composition [A]. Formulation of Liposome of DOX; [B]. Composition of Liposome

Moreover, the morphology of the API particles is significantly affected by the composition of the material used in the formulations. The structure of lipidic-based particles transforms from multilamellar to electron-dense morphology with the variation in nucleic acid concentration during design [44].

Liposomes have been utilised for specifically integrating foreign RNA into cells since 1978 [45]. Numerous liposomes have been refined and manufactured to enclose genetic material with minimal harm and maximum efficacy [46]. Yet, ionizable lipids, mostly cationic lipids, are more preferred in this situation [47, 48]. Sadly, cationic lipids cause changes in a variety of proteins and the cell, including protein kinase C and cytoplasm vacuoles, cell shrinkage, and a decrease in mitoses [49, 50]. In contrast to viral vectors utilised in gene delivery, cation- containing lipids are easier to create, have a basic structure, and are less likely to trigger an immune response [51].

Both hydrophobic and hydrophilic combinations of cationic lipids are dangerous, particularly if they include a quaternary amine that blocks protein kinase C [52]. An innovative method to reduce the impact of a positive charge involved dispersing it by relocating it into an imidazolium heterocyclic ring, thus achieving delocalization [53] and pyridinium [54, 55].

2.4.2. Manufacturing Process:

The method of producing liposomes that is widely used is the thin-film hydration technique (Fig. 7) [56–57]. Alternative methods, including backwards-phase evaporation, injection of ethanol, and emulsification agents, can also be utilised [57–59]. The process of thin-film hydration results in the formation of liposomes that possess multiple layers and have an average size measured in micrometres [42]. In order to achieve better medicament loading and encapsulation efficiency (EE%), liposomes need to be reduced in size to enhance their surface area to volume ratio, the particle size will be around 200 nm.

To enhance the uniformity of prepared liposomes, lots of methods, such as extrusion, sonication (either through a probe or a bath), and freeze-thaw cycling, have been applied to reduce their size distribution [59]. The manufacturing and formulation process of liposomes using the "Thin-layer Hydration Extrusion Method" as in (**Fig. 7**).

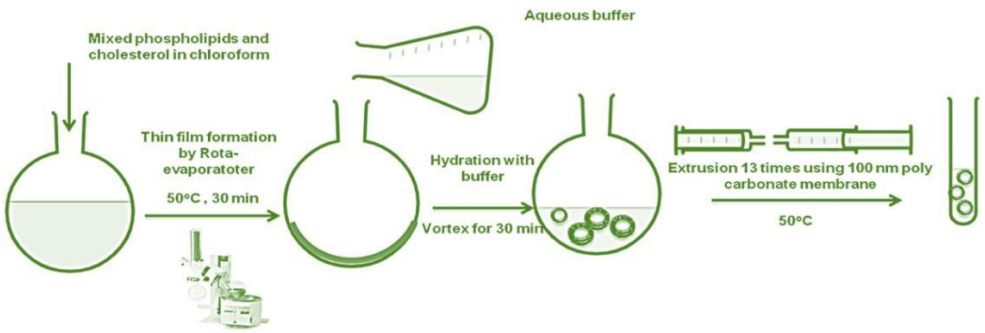


Figure. 07. The process of formulation of liposomes used of thin-film hydration extrusion technique[57]

The attainment of a standardized multilamellar thin film, along with appropriate size minimization, can be achieved by optimizing several different parameters [43]. Rotation speed, pressure reduction, and temperature on rota-evaporator can create well-encapsulated unilamellar liposomes [33,60].

2.4.3. Average Particle Size and Nanoparticles Distribution:

The size of the particles plays a significant role in the subsequent in vivo use of liposomes. Typically, the size demanded falls between 20 and 250 nanometers. The key critical quality attributes (CQAs) for all nanoformulations are the distribution of nanoparticles and their average size. The parameters significantly affect the drug release, targeting ability, drug loading capacity, and in-vivo distribution of nanoparticles [62]. To optimise biodistribution via EPR effects, nanocarriers should be 10–100 nm to avoid RES elimination and kidney removal [63,64]. A small molecule's size implies a high surface area to volume proportion. The immediate release of sedatives occurs rapidly because the nanoparticles contain a larger concentration of drugs closer to their surface as compared to higher levels [65].

It's crucial to remember that to be effective in therapy, inhaled drug particles need to be less than 2 μm in size, making them best suited for settling in the pulmonary air sacs [66]. The size of liposomes affects their ability to be delivered through the skin. Liposomes smaller than 600 nm have easy penetration through the skin, while any liposomes exceeding 1000 nm are retained in the stratum corneum [67]. The PDI is a measure of the uniformity and distribution of nanoparticle dispersion. Liposomes that exhibit PDI values below 0.3 are indicative of uniformly distributed, stable, and dispersed structures [68, 69].

2.4.4. Zeta Potential (ZP):

ZP measures the stability of nano-dispersions, and it indicates that neutral nanoparticles exhibit reduced stability, resulting in their tendency to adherence [70]. If the electrical charge surpasses +30 or falls below -30 mV, it implies exceptional stability due to the intense electrostatic repulsion [71]. The nano-system's ZP has an impact on their distribution throughout the body, their ability to engage with bodily tissues, and recognition by cells.

Because of the negatively charged cell membrane, cationic liposomes have a higher cellular absorption than anionic liposomes [72]. Additionally, liposomes that have been charged possess the ability to effectively encapsulate drugs that possess charges that are opposite in polarity [73]. In order to attain the best possible stability, liposome formulations may incorporate fatty acids and hydrophilic polymers with varying characteristics [40].

In terms of final product control, the following characteristics of a liposome drug formulation are frequently determined: To effectively encapsulate drugs, certain parameters must be considered, such as average diameter of particles, the amount of drug in relation to lipid content, polydispersity index, phase transition, residual solvent levels, and drug release both in vitro and in vivo [71]. Fluctuations in these characteristics have the potential to impact the excellence of the liposomal medical preparations, which could also cause the discharge of the medication from the liposomes.

The QAs assessed for liposomes are listed in (**Table 4**), along with the techniques of analysis that are currently in use.

Table 4. The quality attributes monitored for liposomal products

The investigated property	Methods	Refs.
Morphology	UV-vis spectroscopy SpectrofluorimetryA combin ation of RMN, SAXS, and Freeze fracture technique followed by transmission electron microscopy is being employed.	[71, 72 & 73]
Net charge (ZetaPotential)	PALS & ELS (Phase analysis and electrophoretic light scattering).	[74]
Particle Size	Microscopy techniques (TEM, AFM, SEM)	[74, 75]
Encapsulation efficiency(EE%)	SpectroscopyLC	[76]
In-Vitro Release	SpectroscopyLC	[76]
In-Vivo Release	Radiolabeling, fluorescence labelling, MRI, CT, MS.	[76]

2.4.5. Drug Content:

Liposomal sedate substance may communicate in three ways: weight over volume (w/v); rate epitome proficiency (EE%, weight of medicate captured into the liposomes vs. the beginning sum of medicate utilised%); and medicate stacking

(DL%, the sum of sedate captured into the liposomes relative to the introductory weight of lipid utilised; drug-to-lipid proportion) [62, 74]. Liposomes with a higher EE% are capable of retaining elevated levels of the beneficial medicinal ingredient, leading to a reduction in production expenses. This improves pharmacokinetics and patient compliance [75].

Medicine's (lipidic) effectiveness can be influenced by factors such as drug-lipid ratio, phospholipid type, cholesterol proportion, and production process [76, 77]. As the lipid-to-drug ratio increases, so do the numerous nanovesicles that can entrap more hydrophilic medicines in their watery cores [78]. The presence of cholesterol and unsaturated lipids leads to the formation of additional crevices in the lipid bilayer, which results in the capture of a greater number of hydrophobic drugs [79, 80]. Repeated cycles of freezing and thawing have demonstrated an improvement in the energy efficiency percentage (EE%) [81]. Moreover, utilising remote loading methods has been demonstrated to enhance the encapsulation efficacy of ionizable medications as opposed to conventional passive loading approaches in liposomes[82, 83].

2.4.6. In-Vivo Stability:

The way in which the surface of liposomes behave towards water can impact how they interact with various elements in the bloodstream [84]. The endurance of liposomes in vivo is attributed to these interactions. The endurance of liposomes inside an organism result in the extended release of drugs and better localization of drugs in the desired tissue [42]. As an illustration, hydrophobic nanoparticles possess a strong inclination to attach themselves to blood proteins, leading to their swift elimination from the circulation system [38].

The In-Vivo ponder of DOX stacked liposomes such as depicted within the given (**Fig. 8**) in underneath depiction.

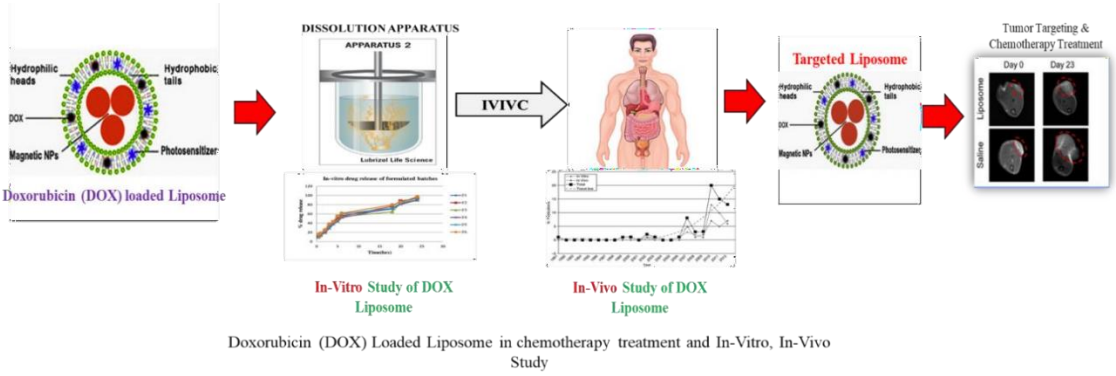


Figure. 08 Doxorubicin-loaded liposomes in chemotherapy/tumour treatment: In-Vitro and In-Vivo investigations

Furthermore, when stealth liposomes are coated with hydrophilic polymers, they exhibitincreased stability in the body and stay in circulation for a longer duration, thereby enhancing the effectiveness of the drug enclosed within [70].

2.4.7. Drug Release Kinetics:

To measure drug release from liposomes, dynamic dialysis monitors drug concentration in the recipient or originating solution. Initially, the medication is discharged from the liposomes and enters the donor solution within the dialysis bag. As a result, the medication is able to spread and move through the dialysis bag and ultimately reach the receiver solution [84, 85].

Another method for studying drug release kinetics is to use Franz cell diffusion between the donor and receptor compartments. Synthetic membranes mimic dynamic dialysis to monitor drug concentration during diffusion. The kinetics of medicaments that are released from liposomes are a vital characteristic for liposome formulation design and is regarded as a critical aspect in achieving optimal efficacy while minimising drug toxicity [85]. For optimal therapy, the drug delivery system must enter target cells via endocytosis or release the drug at the appropriate rate for a sufficient duration [86]. The liposome surface can be customised with targeting molecules to facilitate active drug delivery to specific areas [87].

These targeted ligands have the ability to specifically attach to particular receptors that are present in excess in malignant or unhealthy tissues. The various molecules, such as antibodies,

peptides, oligonucleotides, small carbohydrates, and small organic compounds, can act as ligands [88].

Liposomes can trigger drug release by including responsive ingredients in their makeup [89]. These additives create a disruptive impact on liposomes when they encounter certain stimuli, such as varying pH levels, light, temperature, or radiation [90, 91].

2.5. Liposomal product and process used in Design Space:

For the successful execution of Quality by Design in liposome detailing, QTPP ought to be tobegin with characterized, at that point to prepare and fabricating forms can be chosen and outlined to guarantee accomplishment of the pre-defined QTPP. The process of identifying CQAs and CPPs involves utilizing an experimental design that can effectively evaluate their impact on the CQAs [62]. The purpose of DS is to guarantee a superior product by exhibiting various formulation and/or process parameters [62,75]. DS includes product and process components. The product DS focuses on CQAs of the product, while the process DS centers on CPPs' related CQAs [92].

To prepare liposomes, one must carefully manage the compositions, substances, and production factors, thereby establishing a dependable procedure [32]. The DS methodology determined significant elements for lyophilized liposome critical quality attributes (CQAs), comprising the molar ratio of cholesterol, the proportion of PEG, the volume of cryoprotectant, and the quantity of extrusion cycles [32]. Particle size, drug entrapment, lyophilization, and phospholipid transition temperature affect QTPP. The effectiveness of using DS methodology was confirmed, indicating its significance in developing stable and top-notch lyophilized liposomes [32].

The DS approach was utilized on the extended-circulation liposomes containing prednisolone, through the thin-film hydration-extrusion process. The formulation parameters which were chosen for this study were the ratio of PEG and drug concentration within the bilayer membrane. Meanwhile, the process parameters that were examined were the temperature, number of extrusion cycles, and rotation speed [33]. The technique of DS strategy was applied in order to enclose tenofovir within liposomes that displayed a high EE% [62]. For achieving optimal outcomes, it is recommended to tune the drug/chitosan concentration and the organic phase-to-aqueous phase ratio while adopting ethanol injection to enhance the DS of chitosan-coated nanoliposomes [60]. The lots of Factors affect CQAs in DS strategy. Co-encapsulating drugs in liposome enhances attributes and variations may not improve product quality [37].

Liposome drying parameters are key CQAs to study for stable liposomes [93]. Control dryingto optimize drug content, particle size, ZP, and moisture level after lyophilization [94]. A new system was created to freeze-dry liposomes containing pravastatin, using the solidification rate and temperature of the drying rack during the initial phase. The product's critical quality attributes (CQAs) were significantly impacted by both of the processing components [34].

2.6. The Control Strategy:

The preparation processes and complex physicochemical properties of liposomes pose numerous objections for analytic and bioanalytical identifications despite their stability and effectiveness as a drug delivery system. The FDA guidelines specify that liposome drug products should have several critical quality attributes (CQAs) fully characterized (**Table 5**).

Table 5. Basic quality qualities (CQAs) required for full liposome medicate item characterization

CQAs	Measured Indicator(s)	Ref.
Lipid content and substituents	- Determine lipid assay - Composition determination	[95–101] [102,103]
Drug content Uniformity	- Assay Encapsulation efficiency	[99-112]
Liposome surface morphology, size & shape	- Determination of shape and size - Lamellarity - Average particle size andpolydispersity indices	[113–115] [116-119]
Liposome surface charge	- Zeta potential	[120–126]
Drug Release	- In-Vitro drug release	[130-135]

2.6.1. Lipid Content Identification and Quantification:

The quality of the extreme item is influenced by the source of lipids conjointly by the nature of the lipids: manufactured, semi-synthetic or normal. Liposomes are primarily composed of phospholipids as their main lipid constituent. The identification of lipids can be through nuclear

magnetic resonance (NMR) technology. The distinctive ^{31}P shifts in phospholipids enable ^{31}P-NMR to distinguish between various types of phospholipids [95]. The employment of ^{1}H and ^{13}C NMR aids in elucidating the chemical makeup of alkyl chains and polar head groups in lipids at a molecular level. Typically, NMR analysis necessitates the use of costly equipment [96].

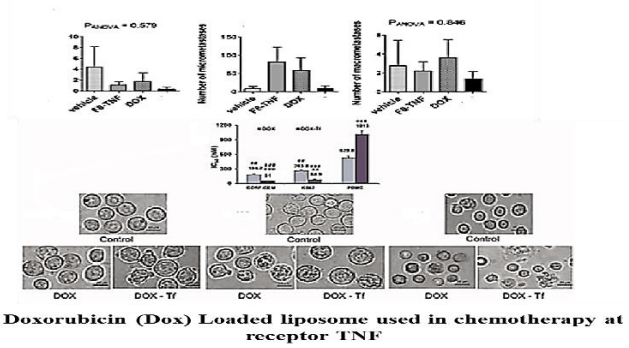
The combination of liquid chromatography and mass spectrometry is widely used to identify and profile lipids [134-136]. Sophisticated methods of ionization, such as electrospray ionization (ESI), can provide exact outcomes for lipid molecular weight determination with the assistance of MS, a powerful and efficient instrument [137]. Raman spectroscopy is a valuable tool for identifying and analyzing the vibrational movements of the carbon skeleton in lipids. The distinctive features of these substances are their vibrations in the C-C backbone range (1000-1150 cm^{-1}) as well as their stretching of C-H bonds (2700-2900 cm^{-1}) [137-139].

The utilization of liquid chromatography methodologies has been extensive in the precise measurement of lipid levels [139]. Initially, liposomes are required to undergo disruption using organic solvents, followed by chromatographic separation. There are various means to detect and measure lipids, which include UV, refractive index (RI), evaporative light scattering detector (ELSD), and charged aerosol detector [97, 98, 99]. Utilising RP-HPLC with UV and ELSD detectors, six different liposomal arrangements were assessed for their phospholipids and cholesterol content [100]. Lipid investigations have also been conducted using gas chromatography (GC) [102]. Before conducting GC analysis, it is necessary to convert lipid fatty acids into methyl esters that can easily evaporate [140]. In recent times, lipid analysis has been carried out using the supercritical fluid chromatography (SFC) technique as well [103, 141]. Numerous colorimetric techniques have been identified to assess phospholipid formation. The mixture of molybdate and phosphorus produces a shade in the blue range. The identification of bilayer membranes is often facilitated by using diphenylhexatriene (DPH).

Additionally, the utilization of DPH fluorescence detection has elevated the detection limits for phospholipid concentration [101]. Furthermore, various kits are available in the market that measures unsaturated phospholipids by utilizing the sulfo-phospho-vanillin reaction [102] or based on enzymatic assay [109,114].

2.6.2. Liposomes Magnitude and Morphological Identifications at their Receptor (TNF):

The analysis of particle size and shape can be conducted through the utilization of electron microscopy techniques, including SEM and TEM [113]. Cryo-TEM obviates the need for drying by instantaneously freezing the liquid specimen, resulting in negligible effects from the drying process. The development of Cryo-TEM aims to offer detailed structural information and high-resolution morphology concerning the encapsulation mechanisms and lipid layers (**Fig. 9**) [114,145]. The destructive preparation process makes SEM inappropriate for imaging liposomes, as it has the ability to pass through particle surfaces. Furthermore, the application of atomic fluorescent microscopy (AFM) has been employed to investigate the geometric characteristics of liposomes in a three-dimensional space [115].



Doxorubicin (Dox) Loaded liposome used in chemotherapy at receptor TNF

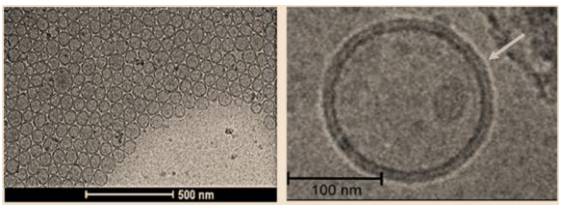


Figure. 09 Cryo-TEM figure of liposomes and attachment with TNF receptor [140-142]

The using ^{31}P-NMR, is mainly possible to assess the lamellarity of liposomes advancement [116]. Uni-liposomes show narrow spectral lines, while multi-liposomes have broader peaks due to

limited molecular movements across lipid layers [117]. Dynamic light scattering (DLS) has been utilized for determining the size distribution of nanoparticles. DLS is now the usual approach for quantitatively analyzing the size distributions of nanoparticles [118]. DLS measures fluctuations in scattered light from moving particles. Accurate hydrodynamic size measurement in DLS analysis requires predetermined temperature, viscosity, and refractive index values due to sample variation [119]. The Doxorubicin (DOX) loaded liposome mainly shows the treatment use into the chemotherapy by the attachment with TNF receptor widely for the treatment (**Fig. 09**) as below:

2.6.3. Nanoparticle Surface Charge (Zeta Potential, ZP):

The surface electric charges of liposomes are mainly influenced by the polar heads of the phospholipids, as well as by tertiary amines or carboxylate groups, which carry a negative charge [120,121]. One essential element necessary for strong liposome connection, adherence, and longevity of nanoparticles. The electrophoretic mobility of particles can be used to determine the value of ZP mainly including such as *phase analysis light scattering (PALS) orelectrophoretic light scattering (ELS) technique* [122] which shown in the given (**Fig. 10**) as below:

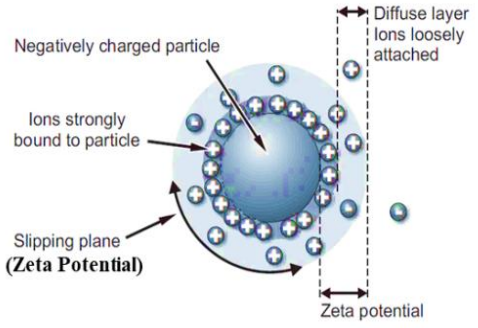


Figure. 10 The representation of zeta potential determination using PALS AND ELS Techniques

To measure accurately, property pre-establishment (i.e., phase characteristics, refractive index, viscosity) and temperature should be considered. Maintaining ZP values within the range of ±30 mV ensures the stability of nanosuspensions [123].

Various methods, such as the use of fluorescent markers, can be employed to ascertain the surface potential of liposomes [124], electron paramagnetic resonance (EPMR) [125] and harmonic generation from optical analyses [126].

2.6.4. Physical and Chemical Stability of Liposome:

To ensure excellent product quality, it is necessary to evaluate the durability (physical) and chemical composition of liposome formulations [147]. Sophisticated techniques like spectroscopy and dynamic light scattering (DLS) can be employed to evaluate liposome fusion and aggregation, respectively. Liposome disruption, on the other hand, can be ascertained using chromatographic methods outfitted with appropriate detectors [142].

The primary methods used to study liposomal fusion are differential scanning calorimetry (DSC) and assays that measure the mixing of lipids through fluorescence [127]. The phenomenon of liposome aggregation can be visualised through the use of microscopic techniques and measured through UV-visible spectroscopy or DLS analysis [128]. The rate of lipid degradation is subject to a variety of factors, including the nature of the lipids, the storage temperature, the type of buffering agents employed, and the prevailing pH levels. Various chromatographic methods can be utilised to isolate and gauge the precursor lipid types and their broken-down forms [129].

2.6.5. In-Vitro Drug Release of Drug Loaded Liposome:

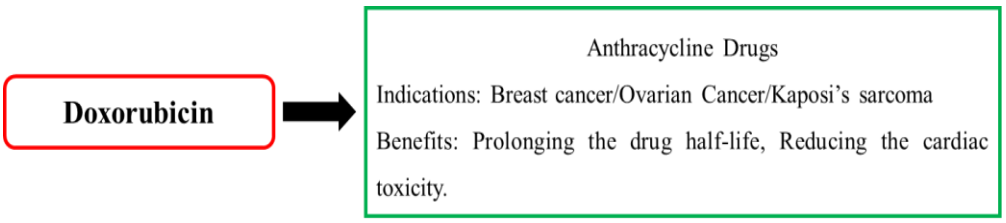
The various in vitro release analysis methods to predict in vivo behaviours of liposome formulations and their development [130]. The various techniques fall into distinct categorizations, including sampling and separation (SS), dialysis membrane (DM), and continuous flow (CF). The procedure employed by the SS method involves placing samples in release media, extracting the drug from liposomes

through either ultracentrifugation or filtration, and determining the drug's quantity through measurement [131]. The utilisation of this approach has led to the observation of suboptimal ultracentrifugation or filtration techniques in the separation of nanoparticles that are smaller than one micrometre. In vitro drug release for most nano formulations is often studied using DM. The principal DM procedures involve the use of either regular or tube dialysis, as well as reverse dialysis [132]. The nano-formulations are contained within the dialysis sac, enabling both release and separation to occur in tandem, with the added ability to measure the amount of drug that has been released. Consider the dialysis layer's characteristics and edge, the test and solvent volume, and the combining methods for applying this strategy [134]. The in-vitro study of liposomes in DOX-loaded loaded used in chemotherapy and diagnosis, as shown in Figure. 08)respectively.

2.6.6. Liposomes Safety and Toxicity:

Liposomes' biocompatibility, degradability and ease of fabrication have spurred their widespread adoption. Liposomes are employed as a viable technique for drug transportation may present safety issues that are linked to the concentration, type and charge of the lipid involved. Liposomes can stimulate the immune system of the patient, which may cause the drugs to accumulate in the mononuclear phagocytic system, thereby exerting an impact on theliver and spleen functions [128].

The numerous strategies mainly established to enhance drug security and cut down on the harmful effects of nanocarriers. One such method is to enhance drug encapsulation efficiency in liposomes, resulting in decreased lipid concentration needed for optimal therapeutic dosage [129]. The size, shape, lipid content, electrical charge, and percentage of cholesterol present in liposomes have a significant impact on their level of toxicity. Although liposomes are generally considered safe, their potential harm is dependent on factors such as type, dosage, presentationtime, and surface properties [134].



Liposome formulations reduce the toxic and side effects and improve patients compliance

Hence, a meticulous configuration of these variables will enhance the load-bearing potential of liposomes and lower their harmful effects [130]. Newly approved liposomes have reduced immune effects. The optimal design of a blend comprising of lipids and polymers needs to be developed. As a result, it is recommended to reduce the type and number of materials used for liposome functionalization [132-135].

3. Results:

The results study of formulation of doxorubicin (DOX) loaded liposome in the treatment of chemotherapy widely. Liposomes mainly formulated with the help of QbD an experiment (DoE) and the physical technique thin-film hydration extrusion. Some of the components in this chemotherapy treatment involves HLA or MHC antigens for humans, as well as elements such as cell-adhesion molecules, cytokine receptors, growth factor receptors, and Fas/Fas-ligand molecules. The using of QbD, (DoE) DOX drug loaded liposome widely attached with the receptors of well-known characteristics are CD95 (APO-1/Fas), TNF receptor 1 (TNFRI), TRAIL-R1, and TRAIL-R2. On the other hand, the function of DR3 is still not fully understood.

4. Conclusion and Current Challenges:

The involvement of QbD in the production of medicament formulations has emerged as a sophisticated method for the pharmaceutical sector to guarantee the potency and wellbeing of pharmaceutical goods, especially various dosage forms, including nanoparticles of high quality. Economical nanomedicines are a promising method for targeted drug delivery (TDD) with minimum side effects, but they cannot yet reach their full potential. Nano-medications are still in the preliminary stages of preparation and enlargement. Lately, nanomedicines have encountered numerous obstacles, primarily concerns with their structural stability and a limited comprehension of the manufacturing and

formulation procedures.

Liposomes have surfaced as drug delivery systems that are both environmentally friendlyand compatible with living organisms, and have exhibited significant efficacy in pharmaceutical applications in clinical settings. The various liposomal preparations mainly differ in morphology, size, materials, layout, and manufacturing methods. As a result, when compared to typical dosage forms, the use of a QbD technique in producing liposomes is crucial and complex. Consider using QbD to identify item properties and process parameters for quality liposomal products developed successfully. Implementing QbD is crucial in guaranteeing the formulated liposomes have their intended therapeutic and safety profiles, as well as achieving optimisation of their final product attributes.

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