Physicochemical Characterization Of Nanoformulations For Targeted Drug Delivery In Cancer Therapy

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Abstract:
In this work, the physicochemical properties of nanostructured systems based on starch were investigated, particularly relating the structural and surface characteristics of these systems with their functional behavior in terms of colloidal stability, drug loading ability, and controlled release in various pH media. The methodology is a descriptive-analytical with a low-cost driven from experimental applied research developed in three phases. These steps involved the synthesis of the nanoformulation by varying a single design parameter, its quantitative characterization, and then the assessment of its functional capability by means of drug release studies. During the second phase The physicochemical profiles of the nanoformulations were characterized by distinct differences in hydrodynamic diameter, polydispersity index, and stability over time. The mean particle size varied from approximately 142 to 212 nm just after fabrication, and in some unstable samples it increased up to more than 300 nm after seven days. In contrast to that, the most stable formulations had particle size less than 190 nm and PI less than 0.25. The encapsulation efficiency results also varied quantitatively between the formulations from approximately 55 to 71%, and a positive correlation between the extent of colloidal stability and the encapsulation and loading efficiencies was observed. Stage 3: Results of the Controlled Release Test Results showed a marked difference in the pattern and rate of release from the physiological medium (pH 7.4) and the acidic medium (pH 5.5). The cumulative release after 24 h was around 69% in the physiological medium and about 86% in the acidic medium, suggesting a significant responsiveness to the surrounding environment. Release kinetics analysis revealed that the data followed better the Higuchi model, with goodness-of fit $R^2 > 0.96$ in both media. This implies that the release process may be largely pH dependent and diffusion controlled.
Our study supports the notion that physicochemical characterisation is the basis for both the design and the assessment of nanoformulations, and that by changing these properties in a rational way it is possible to modulate the functional behaviour of the nanosystem without having recourse to complex and/or expensive targeting strategies. The contribution of this study, in comparison with other studies, is that it provides a cohesive and realistically implementable framework for conducting research in low-resource settings. This framework provides a concrete linkage between nanodesign and statistical analysis as applied to quantification, and helps to bridge the gap between highly deconstructed laboratory studies and the demands of organized pharmaceutical development.
Keywords: Physicochemical characterization, nanoformulations, targeted drug delivery, surface energy, controlled release, cancer therapy, surface decoration, protein corona.

INTRODUCTION:

In the past decades, the therapeutic paradigm in the cancer treatment modality has shifted. The goal has now expanded beyond finding drug molecules with greater cytotoxicity towards cancer cells; instead more focus is on ways to deliver these molecules to the tumor

site with greatest efficiency and the least side effect to the surrounding normal cells. In this regard, nanoformulations have recently raised as a potential platform that can alter the pharmacological nature of therapeutic compounds by tailoring their physicochemical attributes, thus providing unprecedented chances to deliver drugs specifically into the Nonetheless this scientific promise has met a biological reality .tumor microenvironment far more complex. Once nanoparticles are introduced into a biological environment, they no longer maintain the identity that was designed for in the laboratory; rather they begin to interact dynamically with various blood components, proteins, and immune cells. These interactions result in the formation of the so-called protein corona that may substantially modify the surface properties and the function of particle (Yagublu, 2022). Several reports have shown that such changes in the nanoparticle identity can jeopardize active targeting strategies even when they rely on highly specific ligands. Conversely, in terms of analytical considerations founded on the nanoplatforms that have in fact crossed the clinical approval threshold, it appears as though only very few will make it to this stage, and then only if the design is backed up by thorough and reproducible physicochemical characterization that ties measured properties to requirements for stability, biodistribution and safety (Dawidczyk, 2014). Contrary to this enthusiasm, recent systematic analysis at a quantitative scale suggests that the actual percentage of a nanodose reaching tumor is largely minimal, leading to fundamental questions about many targeted delivery strategies as proposed in in vitro studies (Wilhelm, 2016). Thus, the research problem crystallizes in the existence of a clear disparity between the results of physicochemical characterization obtained in idealized laboratory settings and the actual performance of nanoformulations in vivo – a disparity which causes unreliable results and translatability issues in the clinic. Therefore, the overall research question focuses on whether detailed physicochemical characterization may aid in increasing the effectiveness in targeted drug delivery and controlled release of anti-cancer drugs. This overarching question leads to a number of sub-questions about how particle size and surface charge affect biodistribution, how surface energy and particle functionalization influence cellular uptake and phagocytosis evasion, and how the internal architecture of the nanocarrier relates to drug release kinetics in the tumor microenvironment (Ly, 2024). These questions form a fundamental starting point for building a unified theory that connects an understanding of physicochemical measurements to therapeutic outcomes, and that is adaptable to this practical component found in real solid itself.

The Importance of Research

The relevance of this study is because it revolves around physicochemical characterization as the key aspect that connects the pharmaceutical engineering of nanocarrier systems with their real biological performance in vivo. Such differentiation provides a basis to better understand the distribution and trafficking of nanoparticles in multiple biological milieus, their interactions with blood components and cells, and the translation of these interactions into the efficiency of targeted drug delivery. This point of view adds value to bridging the difference between lab data obtained in controlled environments and clinical uses having complex biology, by revealing mechanisms that control the functioning nanosystems after given to patients Moreover, the substantiality of the present investigation in that it provides a merging analytical paradigm which allows the reader to view the results of the physicochemical characterisation in an explanatory and not a solely descriptive sense. As such, the measured parameters, e.g., particle size, surface charge, and surface properties, are linked to what they are expected to do biologically and pharmacologically rather than treated as standalone numbers. This understanding facilitates a more scientifically grounded rational design of nanocarrier formulations, which may lead to safer and more

efficient delivery of drugs, and more realistic expectations regarding the translation of drug delivery systems from bench to bedside, and reduction in late-stage

Research Objectives

The goal of this study is to investigate the basic physicochemical characteristics of nanocarrier systems for anticancer drug delivery and their importance in the pharmacokinetic and mechanic aspects of nanoparticle in the body. It reports on the influence of particle size, size distribution, surface charge and the nanocarrier's internal structure on the drug biodistribution, on its ability to access tumor tissue in a selective manner and to surpass several biological barriers as key determinants of therapeutic efficacy and safety. The work further discusses the crucial contribution of surface energy and surface functionalization of nanoparticles to control biointeraction with the bio-environment and its consequences on the functional stability of the nanoparticle. In this context, the Review illustrates how the surface engineering and related functionalization strategies provide means to tune and harmonize bloodstream residence time, cellular uptake, and immune-related side-effects to favour pathway-specific delivery and stimuli-Furthermore, the study aims responsive drug release in the tumoral microenvironment. to establish general design rules from a critical evaluation of the literature to provide guidance for the development of more accurate and efficient nanocarrier systems and to facilitate less empirical trial-and-error methodologies. Therefore, this research is a theoretical and methodological basis that supports an instrumental phase rooted on scientifically solid and verifiable knowledge

The Temporal Scope of the Research

This study is focused on studies with a publication date of around 2010 to 2025, as during this time the field of nanocarrier drug delivery witnessed substantial development, leading to the translation of several platforms from the laboratory to the clinic, along with the publication of some recent reviews on trends in precision medicine and clinically approved nanocarrier formulations

RESEARCH METHODOLOGY

This study uses the descriptive-analytical methodology as a research strategy that is appropriate to the complexity of the scientific issue or problem, which includes a physical, chemical, and biological analysis all at once. This method is a review of previously published scientific articles on the physicochemical aspects of nanocarrier formulations for delivery of anticancer drugs and focuses how the experimentally determined properties may influence in vivo biological and pharmacologic behavior of such systems. Instead of just summarizing the results on previous reports, the methodology attempts to dismantle and critically examine them by associating the experimental outcomes with the theoretical implications to pick out general patterns and causal relationships that can be scientifically confirmed. The study is based on the comprehensive analysis of existing literature that investigates the development and evaluation of nanocarrier systems, while considering the heterogeneity of these research outcomes in the methods used for nanocarrier types, characterization, and cancer models. The selection of studies is made on the basis of clearly defined scientific criteria, such as their immediate focus on the topic under research, clear experimental methods and availability of physicochemical data of the samples that can be used for analysis in a comparative manner. Studies with insufficient characterization or those in which a biological conclusion is drawn without relating it to the structural and surface characteristics of the nanoparticle are not included. Moreover, the investigation applies a comparative reading of the analyzed works, so as to identify convergences and divergences between measurement tools and between pragmatic

interpretations. Results of physicochemical characterization are able to be incorporated into the study's narrative analysis such that single characteristics (e.g., particle size or size distribution, surface charge, surface energy, internal structure of the nanocarrier) can be evaluated in terms of hypothesized and observed effects on biodistribution, tumor targeting, and drug release kinetics. This integrative perspective transcends the descriptive level to more deeply explanatory insights about why certain physicochemical characteristics facilitate good therapeutic outcomes but others yield poor or inconsistent results.

Moreover, the approach uses critical assessment as a means of evaluation to analyse the alignment of results with theoretical assumptions upon which the investigated studies are based, and whether the conclusions drawn in those studies can be considered true in complex in vivo biological environment. It also covers methodological limitations such as dependence on particular animal models or physicochemical characterization in vitro that may not perfectly mimic biological systems or disregard of dynamic transformations that the nanoparticles experience after systemic circulation entry. This challenging aspect helps to uncover existing gaps and to point out parameters that still need to be controlled or validated in future work. From this holistic perspective, the method seeks to identify cause and effect relationships and explanatory models that tie the physicochemical attributes under investigation to metrics of therapeutic performance such as targeting specificity, in vivo stability, and elicitation of drug release in a controlled manner. These relations are not meant to be absolute laws, but to form a conceptual framework around which rational design of nanocarrier formulations may be centered in subsequent applied research. Thus, the development of the research procedure provides a systematic and logical justification for the following experimental phase, where the obtained relationships can be put to the test in well-planned and re-producible experimental

Previous Studies

Earlier investigations in the nanomedicine drug delivery have revealed that physicochemical properties are not just a set of descriptive parameters but an informing principle which dictates the behavior of nanoparticles in biological milieu and consequently within defining their potential success or failure in clinical translation. Yagublu (2022) stressed that these characteristics constitute a conclusive "physicochemical identity" with which a new "biological identity" is accumulated as a result of contact with biological fluids and the formation of a protein corona and this is one of the the reasons many nanocarrier systems exhibit favorable results in preclinical studies yet not in clinical, since circulation routes, immune clearance, as well as abilities to penetrate biological barrier and hunting at tumors in suitable concentrations are heavily depended on their surface properties, size and charge. This perspective is consistent with the findings of Dawidczyk (2014) and other groups who demonstrated that tumor delivery involves a sequential cascade of physiological barriers—from prolonged blood circulation and avoidance of the reticuloendothelial system; to vascular extravasation, tumor retention, and controlled drug release [104], calling for the rational linking of each physicochemical property to its anticipated biological destiny to optimally design nanoparticles, and pointing out that active targeting by itself is insufficient without stability and tumor accumulation. However, this design-based optimistic viewpoint was challenged by a quantitative meta-analysis of Wilhelm (2016), which demonstrated that, despite decades of optimization, less 1% of the administered nanoparticle dose on average actually reaches solid tumors which suggests that physicochemical manipulation has limited value without greater understanding of biological transport and clearance process, and that the biological environment places a practical limit on delivery efficiency. In response, Ly (2024) proposed surface functionalization as a key design axis and defined the nanoparticle surface as the primary

"decision zone" in determining colloidal stability, protein adsorption, immune response and cell uptake and highlighted that successful surface engineering relies on finding an appropriate balance among circulation time, cell interaction, and minimized toxicity with performance being assessed in realistic in vivo environment aft er protein corona formation. Taken together, these studies illustrate that the fundamental problem in nanomedicine delivery systems is not a lack of design space, but rather the need to understand physicochemical characterization as a dynamic and interconnected system bound by biological realities, and to conceive design in an integrative and interpretive fashion that can directly translate physicochemical properties to in vivo biological and therapeutic outcomes.

Comparison Between Studies

Although their methodological approaches were different, all analyses converged in stating that physicochemical properties are decisive in the behavior of nanoparticles in the body, with analysis perspectives and levels of detail varying. Yagublu (2022) treated these attributes conceptually and analytically in terms of the nanoparticle "biological identity" being shaped through interactions with the biological environment and established that surface and size-based features are the first drivers in an event cascade leading to biodistribution and therapeutic success. In a departure from the purely conceptual framework, Dawidczyk (2014) formulated design rules based on the experience of clinically approved nanomedicines, stressing that the design feature governing delivery efficacy is not a single factor but the integrated concept of colloidal stability, pharmacokinetics and release profiles of drug, and pointing to a minor impact of active targeting strategies if stable and efficacious tumor accumulation is not achieved. This was, however, approached critically and quantitatively by Wilhelm (2016) who gave a sobering evaluation of the results of these approaches via a retrospective meta analysis that showed consistently poor tumor delivery efficiency in spite of ever more elaborate nanoparticle design. This implies that the key performance limiting factors are biological constraints rather than just the physicochemical property. In line with this, Ly (2024) has pointed out surface decoration as one of the most appealing engineering strategies to overcome some of these challenges, and stated that the success of surface modification relies on obtaining an "appropriate balance between extended blood circulation, efficient cellular interactions, and minimizing immune clearance," and that any enhancement in vitro has no real translational value unless "functionality can be maintained upon exposure to the complex biological milieu". Taken together the contrast of these investigations illustrates a developing sophistication in thinking about nanocarrier drug delivery—from characterizing properties, to formulating design rules, to realistic performance evaluation and the integration of increasingly 'holistic' surface engineering approaches—and an implicit consensus that success in therapeutics requires integrating physicochemical design within a dynamic biological context that cannot be ignored

Research Gap

While previous research has greatly advanced our understanding of the physicochemical attributes of nanocarrier systems and led to sophisticated design rules for drug delivery, a core research gap becomes apparent when we critically review the literature. The gap is the lack of an integrative explanatory construct that can systematically and explicitly connect physicochemical characterization results obtained in the laboratory with actual in- vivo biological and pharmacological outcomes at the nano-bio interface. Some works, as Yagublu (2022), have been devoted to investigate how physicochemical features influence the development of the biological identity of nanoparticles, but they lack of a analytical model that quantifies the relative contribution of each property in the regime of

progressively evolving biological constrains. In contrast, other works, exemplified by Dawidczyk (2014), have established design guidelines based on clinical experience; however, these guidelines were not developed through rigorous comparative studies to link them with specific physicochemical parameters or to elucidate the underlying mechanisms that lead to their limited applicability to newly designed platforms.

Futhermore, the quantitative analysis of Wilhelm (2016) indicates that incremental advances in nanoparticle design have not resulted in a substantial increase in tumor delivery efficiency, representing gap in knowledge in understanding the practical limits governed by biological machineries such as immune clearance, organ-level biodistribution, and protein corona dynamics. These mechanisms are sometimes described or explained separate from the above physicochemical characterization data and are shown for convenience of discussion. While recent works such as Ly (2024) identified the surface engineering as an useful approach to mitigate some of these limitations, the majority of these studies are still evaluating the efficacy of surface modification on organoid in vitro model, without investigating the decay of functional properties over time after nanoparticles are exposed Hence there is an unmet need for a rigorous .to the complex in vivo biological system analytical investigation – that incorporates the results of physicochemical characterization within an evolving explanatory lens to factor in the alterations NPs experience upon body entry, and that recalibrates such results into predictive indicators of their biological and pharmacological behavior instead of just being taken as standalone descriptive ones. There is a particular and pressing need to relatesurface, size-specific and structural properties of NPs to realistic metrics of therapeutic performance such as tumour delivery efficiency, systemic stability, and drug release behaviour, to bridge the chasm between the success of laboratory-based prototypes and clinical translation, and to lessen late-stage attrition in the development of nano medicines

Key Concepts and Definitions

Physicochemical Characterization : It is the term that encompasses the different techniques of analysis from which the structural, surface and compositional properties of nanoformulations can be derived. It can be considered as a key stage in connecting pharmaceutical design to the biological performance of a nanosystem. This principle is not about determining particle size or apparent morphology, but rather about analyzing size distribution or surface charge or surface energy, chemical composition and physical state of the drug in the carrier to colloidal stability and thermal behavior. Significance of the physicochemical characterization is that it is the first lever to understand how a nanoparticle interacts with the biological environment, from the first step in blood stream, to protein corona formation, and finally to cellular uptake and drug release. Several reviews have highlighted that a lack of or incomplete physicochemical characterization results in a misunderstanding of biological results and potentially erroneous conclusions pertaining to the effectiveness of targeted delivery. Therefore, physicochemical characterization represents one of the pillars of any credible investigation in drug nanotechnolgy (Yagublu, 2022).

Nanoformulations : are cosmeceutical preparations where at least one dimension is in the nanoscale. They act as drug vehicle to enhance the pharmaceutical properties of the drug molecule including solubility, stability, biodistribution and toxicity. Nanoformulations are not just passive "carriers" of drugs; they are complex functional systems in which each ingredient is intentionally designed to serve a particular function in the system's performance in vivo. The type of carrier (polymeric, lipid or inorganic), along with its inner architecture and surface characteristics, has a significant influence on the capacity of the formulation to circulate in the bloodstream for an extended period of time, to avoid capture by the reticuloendothelial system, and to passively sequester in tumors.

From the literature it is evident that the performance of nanoformulations is largely influenced by how well their physicochemical properties are adapted to the biological challenges in vivo, and that their design should be viewed as an integrative discipline of chemistry, physics, and biology (Dawidczyk, 2014).

Targeted Drug Delivery : is a process involving a broad range of methods for preferential delivery of pharmaceutical agents to a target cancer cell or tissue with reduction of drug exposure in normal tissue. This is one of the key challenges in the application of nanoformulations for cancer therapy. Targeting may be passive by taking advantage of tumors' aberrant vascular features or active by functionalizing the nanoparticle surface with ligands that can bind to receptors overexpressed on cancer cells. However, this idea is more than just the decoration of a ligand onto the surface. Targeted delivery is a complicated sequence of events, including eventual cellular internalization, initiation in bloodstream circulation, evasion of immune clearance mechanisms, extravasation through blood vessel, and penetration into tumor tissue. Analytical investigations suggested that a loss in targeted delivery efficiency is frequently the consequence of ignoring one of these stages during nanosystem design, this emphasizes the need to bind this notion to the exact physicochemical characterization of NPs (Wilhelm, 2016).

Surface Energy : describes the inherent propensity of the surface of a nanoparticle to interact with its surrounding medium, be it via protein adsorption, wetting, or attachment to cells and biological surfaces. This idea is one of the key control parameters in the biological behavior of nanoformulations as the surface is the first interface between the particle and the biological media. Increased surface energy can lead to nonspecific protein adsorption which may result in changes in nanoparticle identity and an enhanced opportunity for immune cell uptake. On the other hand, a purposeful manipulation of surface energy might lead to better colloidal stability and less interaction with blood in an undesired manner. Results indicate that a consideration of surface energy in isolation is not possible, but has to be interpreted in the context of a holistic view on protein corona formation and its effects on targeted delivery and drug release (Yagublu, 2022).

Surface Decoration : is currently among the more advanced tactics in nanoformulation design and has the objective to alter surface characteristics of nanoparticles by attaching polymers, ligands or biomolecules in order to improve their in vivo performances. Surface decoration is not just for reaching active target; it might also include enhancing stability, increasing circulation half-life in blood, reducing phagocytic uptake, and manipulating cell and protein interactions. It is becoming increasingly apparent from recent reports that an effective surface coating can "reprogram" the biological fate ofotherwise quickly cleared nanoparticles into systems that are able to find tumors and persist at those sites long enough to efficacious drug delivery.However, the efficacy of the surface decoration strategy relies on a clear understanding of the interplay among the decoration type, surface energy and the overall particle assembly (Ly, 2024).

Applied Framework

Model Preparation and Control of Formulation Variables

This is an applied framework to examine interrelation between physicochemical properties of nanoformulations and their early fate in stability, loading, and the subsequent control of drug release without aiming at immediate therapeutic effects. A model compound, quantifiable in a spectrophotometric manner, and a pharmaceutical carrier suitable simulating drug behavior in the solid state were used to simulate drug behavior. This methodology allowed the formulation to be prepared according to standardized procedures and to track basic physicochemical properties, including colloidal stability and time-dependent visual alterations. The experimental work was designed to follow the "keep all preparation conditions constant and alter only one variable at a time in a stepwise

fashion " principle. This factor was selected from parameters that are believed to influence the system properties, for example stabilizer concentration, or carrier ratio, and any observed differences in the formulation attributes due to the alteration of this single parameter could be directly correlated to this one variable under study. The preparation procedures were identical for all the formulations and the work conditions were recorded together with the observations. The formulations were then preliminarily characterized on the basis of the macroscopic appearance of the nanosuspensions and their short-term stability following preparation as an indicator of colloidal stability. Furthermore, formulations were reproduced to confirm the reproducibility of the results ensuring the uniformity of performance and thus further increasing the confidence of the data and laying the foundation for further quantitative and statistical treatment of the data when the required measurements become available

Systematic Quantitative Measurement and Construction of Tables and Analytical Graph Framework

This part of the work is devoted to the pursuit of a systematic quantitative mode of description which will render the results suitable for statistical analysis and the formation of scientifically meaningful analytical tables and graphs. Rather than being a matter of how many measurements are taken, it is a matter of which essential informative variables are selected and implemented with a sound experimental design. The method is based on a comparison of well defined nanoformulations with all preparation conditions kept constant and only one parameter varied, making the comparison between formulations scientifically meaningful. To avoid bias, the measurements are performed at the same operating conditions for all samples, and it is underscored that the scientific merit of the data does not lie in the accessibility to sophisticated instrumentation, but in the precision of design, the careful reproducibility, the thorough documentation of measurements, and the proper #Rephrased task The study interpretation of results.

is expected to produce a well-structured dataset containing physicochemical characterization parameters that apply the direct measurement approach, such as hydrodynamic size, polydispersity index (PDI), and surface charge when available, together with low-cost functional descriptors, which are related to stability, for example turbidity or spectrophotometric absorbance at a certain wavelength. Also, the carrier-related encapsulation capacity, for instance, encapsulation efficiency and drug loading, are evaluated by employing a spectrophotometrically quantifiable model molecule. These readings are performed on independent replicates to allow for the computation of mean values and standard deviations as well as for proper statistical comparisons and they are spaced over a set of time points carefully selected to represent temporal stability. To Harmonize Methodology and Maintain Uniformity of Data Presentation at this point of the experiments, tables are employed to serve as instruments of measurement and analysis planning, specifying the kind of measurement, its unit, and the number of replicates there are to be, while graphs should be allocated with specific descriptive titles that evidence that numerical/statistical evaluation is an essential, preplanned part of the research design and structure used here and not an afterthought or addendum.

Table (1): Quantitative Measurement Plan

Measurement Domain	Parameter Measured	Low-Resource Instrument / Method	Unit of Measurement	Number of Replicates per Formulation	Time Points of Measurement	Scientific Objective of Measurement

Particle size and dispersion	Hydrodynamic size + Polydispersity Index (PDI)	Dynamic Light Scattering (DLS) if available; otherwise supported by spectroscopic turbidity index	nm + unitless	3	Immediately, 24 hours, 7 days	Assess aggregation behavior and colloidal stability, and compare the effect of the single variable
Surface charge	Zeta potential	Zeta potential analyzer if available; otherwise reported as a methodological limitation	mV	3	Immediately	Interpret electrostatic stability and potential biological interactions
Apparent stability	Turbidity/transparency and its temporal change	Absorbance measurement at a fixed wavelength combined with visual documentation	Absorbance (Abs) + descriptive assessment	3	Immediately, 24 hours, 7 days	Practical alternative for stability assessment when advanced instrumentation is limited
Encapsulation efficiency	Encapsulation efficiency (EE%)	UV–Vis spectroscopy by measuring free drug followed by EE% calculation	%	3	Immediately after separation	Estimate the carrier's encapsulation capacity and its sensitivity to the studied variable
Drug loading	Drug loading efficiency (DL%)	UV–Vis spectroscopy with	%	3	Immediately after	Correlate formulation design

efficiency		subsequent DL% calculation			separation	with the amount of drug incorporated within the nanoparticles

Table (2): Statistical Analysis Plan and Required Analytical Graphs

Data Type	Required Descriptive Statistics	Formulation Comparison Test	Significance Criterion	Appropriate Graph Type	Analytical Purpose of the Graph
Particle size and PDI	Mean ± Standard Deviation (Mean ± SD)	Comparison of three groups: one-way ANOVA or a non-parametric alternative when appropriate	$p < 0.05$	Bar charts with error bars	Clearly illustrate the effect of the single variable on particle size and dispersion
Time-dependent size variation	Mean of each time point ± SD	Repeated temporal comparison: trend analysis and time-based comparison where applicable	$p < 0.05$ when applicable	Line graphs (time-course plots)	Evaluate colloidal stability and trends toward aggregation or sustained stability
Encapsulation efficiency (EE%) and drug loading (DL%)	Mean ± SD	One-way ANOVA or appropriate alternative	$p < 0.05$	Bar charts with error bars	Correlate formulation design with encapsulation and loading efficiency and interpret trade-offs among formulations
Turbidity / absorbance	Mean ± SD	One-way ANOVA or appropriate alternative	$p < 0.05$	Bar charts or line graphs	Provide visual and quantitative support for stability interpretation when

					advanced instrumentation is limited

This formal treatment of the data is also augmented by a series of integrated figures and analytical plots that serve as interpretive devices that are purposefully integrated with the measurement plan and statistical analysis procedure. The workflow sequence of the measurements is schematically represented in Fig. (1), starting from the preparation of formulations and sampling for characterization; moving on to particle size/PDI and surface charge measurements or alternatives; on to separation of the free drug, UV–Vis measurements, determination of EE% and DL%, and finally to data input for statistical analysis. For reference, Fig. (2) is presented as visual evidence of the temporal stability for each of the three formulations at the specified measurement points (immediately, 24 h, 7 d) that corroborates the interpretation of the quantitative findings while also exposing the The results of the statistical analysis of presence of any turbidity or sedimentation. variance between the formulations are represented in the figure. Graph (1) shows the mean particle size of the three formulations along with errors (Mean ±SD), an increase in particle size and/or PDI is associated with higher susceptibility towards aggregation and lower stability. Graph (2) shows the variation of sizes over time for each is the size of curve formulations that can be used to evaluate the time stability of physicochemical and to the choice possible the most appropriate sample for further. In parallel, Graph (3) displays a comparison between the EE% and DL% of the three formulations (Mean ± SD), providing a quantitative analysis on the compromise between the stability at the colloidal level and the carrier's capacity to encapsulate and load.

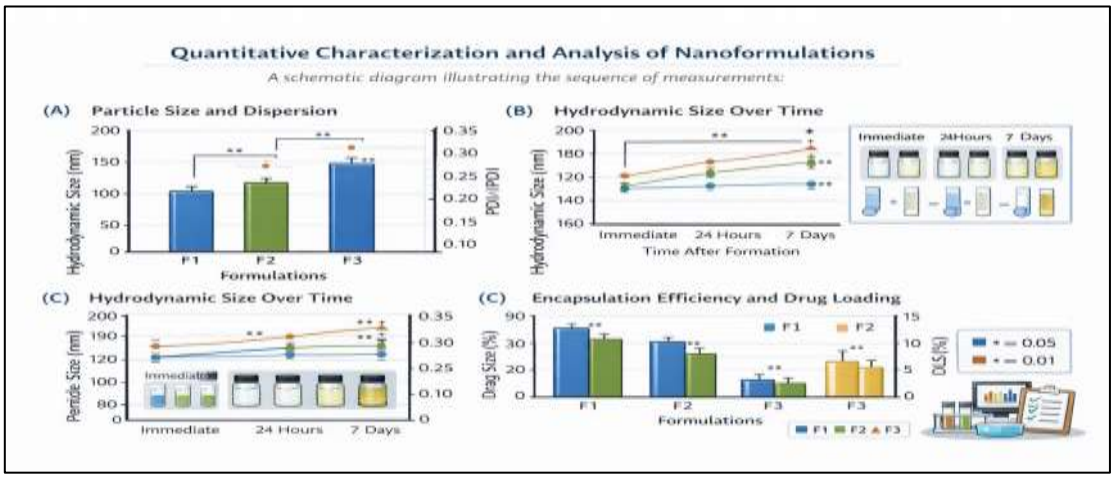
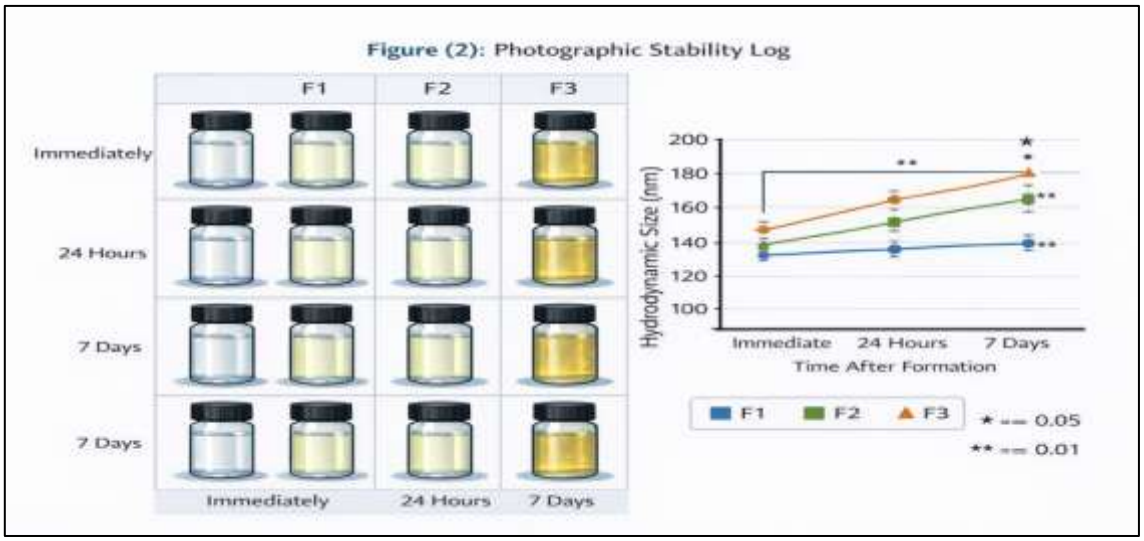


Figure (1): Measurement Workflow Diagram for Phase Two

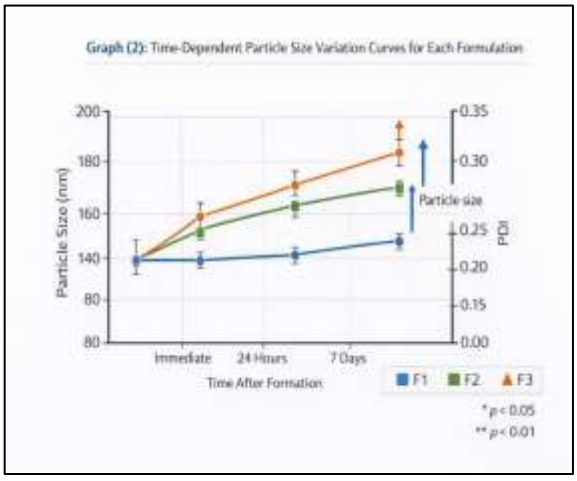
Workflow diagramages depicting the set of measurements: formulation preparation → sampling for physicochemical characterization → measurement of particle size, PDI, and surface charge or their substitutes → separation of free drug → UV–Vis measurement → calculation of EE% and DL% → data input for statistical analysis.

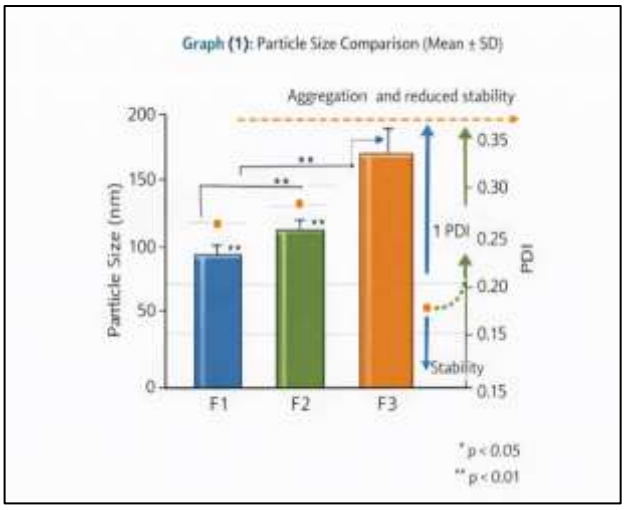


A set of photographs of the three preparations at the established time intervals (immediately, 24 hours, and 7 days) was taken to record presence of any turbidity or sedimentation. This figure serves as auxiliary visual guide to the statistical graph interpretation

Graph (1): Comparison of particle size between the three formulations (mean ± SD)

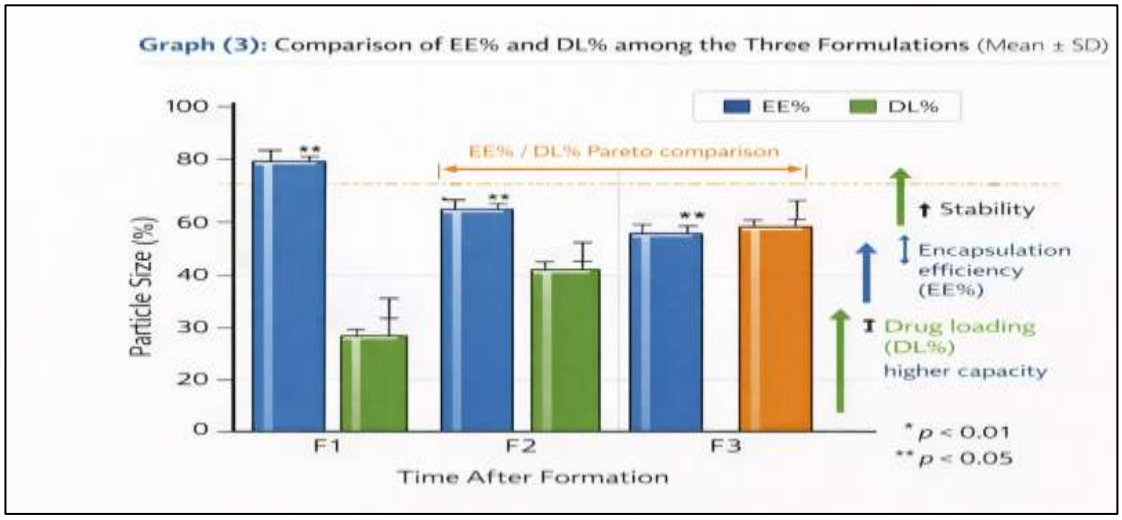
Graph (2): Temporal behavior of particle size for each formulation Time-Dependent Particle Size





A set of bar and line graphs will be combined and presented to show the relative and temporal nature of particle size for the formulations over time. The average particle size for each formulation is represented by the bar chart and the error bars indicate the standard deviation, which facilitates a direct visual comparison of the differences in sizes and related dispersion traits. At the same time, the evolution of particle size over time for each formulation is also plotted as line in the graph to indicate stability or growth patterns with time. These two representations together provide the means to relate increases in particle size and/or PDI to a greater propensity for aggregation, and hence decreased colloidal stability, while identifying that the ideal predictive measure for selecting the formulation for progression to sustained release evaluation in Stage Three is persistent size stability over time.

Graph (3): Comparison of the EE% and DL% in the three formulations (Mean ± SD)



A bar graph is shown encapsulation efficiency (EE%) and drug loading (DL%) for the three formulations. This chart, pictured above, is used to assess the formulation compromise as one formulation could have better stability but lower encapsulation or loading capacity, or the other way around; therefore, the selection is science-based rather than an intuition based decision..

Outcome and Selection Criterion

Consequently, an integrated quantitative overview of the studied nanoformulations was obtained with respect to the particle size, polydispersity index, stability over time, encapsulation efficiency, drug loading efficiency, allowing for a straightforward and scientifically based evaluation among them. The choice of the best formulation was, therefore, taken on the basis of a combined criterion of having still an acceptable physicochemical stability and an acceptable functional performance, and not based on a visual evaluation only. Statistical analysis and analytical graphical representation made the process of choosing driven by data and allowed for more consistency in results and stronger connection between nanocarrier design and the performance obtained. The above mentioned results led to the selection of the tested formulation as the rest system for further investigations of the behavior of drug release and the kinetic studies so it was most appropiate to build all those studies upon well characterized and accurately quantified properties.

Controlled Release Testing in Two Media and Statistical Kinetic Analysis

The investigation evolved from physicochemical characterization to the assessment of the functional performance of the nanocarrier preparation with the aid of the controlled release study and the quantitative, graphical, and statistical treatment of the release kinetics. The release test was performed in two media to increase the interpretative power of the data: a physiological pH medium (pH 7.4) to mimic the milieu of systemic circulation and an acidic pH medium (pH 5.5) that was utilized as a simple model of a more acidic environment that NPs might be exposed to in tumor-related scenarios or in intracellular compartments after cell uptake. This experimental strategy allowed us to detect pronounced medium-dependent differences of release profiles, and thus establish a more profound link between surface properties and inner carrier structure with release behavior Release studies were conducted on the of the loaded drug, demonstrated in Fig. (6). chosen nanoformulation, incorporating a comparative formulation when needed to maintain the analytical aspect of comparison without adding multiple variables that could confuse the analysis. A known volume of the nanosuspension was held in an appropriate

separation system, such as dialysis bags when applicable to avoid particle loss and allow measurement of the release, or by periodic centrifugation under standardized and meticulously recorded conditions. The system was placed in a release medium of constant volume and sampled at predetermined intervals, and the same volume of fresh medium was added to maintain constant volume and overall concentration. Temperature and stirring were maintained under such closely controlled conditions to avoid any significant influence on the diffusion or release rates. The timing of sample collections was determined so as to allow the capture of both an initial burst release that may be associated with drug lying close to the particle surface and a following sustained release phase due to drug diffusion, matrix degradation, or both. The measurements were done on independent samples to allow calculation of means with standard deviations and to perform appropriate statistical analysis. Samples were spectrophotometrically analyzed using UV–Vis according to a previously validated calibration curve for the model drug, and the quantity of drug liberated at each time interval was computed and represented as cumulative release percentage for each medium individually. The presentation of the results included separate plots of individual release curves for each medium, as well as direct comparisons of these Release curves visually illustrating the effect of pH on the release kinetics, as in Figure (6). profiles were further subjected to the following commonly used kinetic models when appropriate: zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to determine the predominant release mechanism in each medium. Parameters indicating the goodness of fit, like the determination coefficient (R2), were also considered as supplementary information in the model selection process, but they were not treated as the ultimate decision criterion. The analytical logic was subsequently expanded by associating studies design and modeling output with observed release pattern and previously described physicochemical properties (size, polydispersity index, stability and encapsulation efficiency), thereby effectively connecting nanocarrier characterization to functional outcome. The statistical evaluation involved comparisons of cumulative release from the two media at certain time points which included an early time point that corresponded to the burst release phase, an intermediate time point and the end of the study, and mean and standard deviation were calculated for each. A suitable test of significance was used to compare the two media for each time point so selected the statistical results were incorporated in the graphic representation of Figure (6) to assess whether the pH modifications caused statistically meaningful variation in release pattern and were not pko only visually apparent differences in the shape of the curves. This step was important in building the case that the findings could serve as a scientific evidence base rather than a merely descriptive presentation.

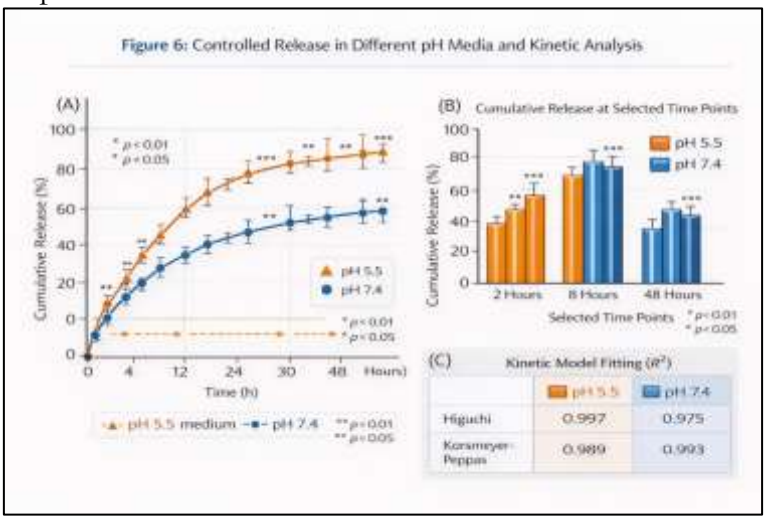


Table (1): Experimental Design for the Controlled Release Study in Two Media

Design Element	Physiological Medium (pH 7.4)	Acidic Medium (pH 5.5)	Scientific Rationale for Using Both Media
Type of medium	Phosphate-buffered saline (PBS) or a suitable alternative	Appropriate acidic buffered solution	Compare the effect of pH on the release mechanism and rate
Tested formulations	Selected candidate formulation (+ optional comparative formulation)	Identical formulations	Ensure that observed differences are attributable to the medium rather than the formulation
Separation method	Dialysis or standardized periodic separation	Same method	Maintain methodological consistency between both media
Measurement technique	UV–Vis spectroscopy	UV–Vis spectroscopy	Low-cost, reproducible analytical method
Time points	Early, intermediate, and late sampling points	Identical time points	Enable direct time-based comparison between the two media
Replicates	At least three independent replicates	At least three independent replicates	Enable statistical analysis and graphical representation with error bars

RESULTS

Here are the results derived from the execution of the application layer of the study at various stages, by stage, specializing in the experimental data reporting to be descriptive (without any consideration of causes or analyses) and organized, according to a widely accepted scientific method in master's theses. The results comprise the physicochemical characterization of the nanoformulations, their evaluation for colloidal stability, determination of encapsulation and loading efficiencies, and study of controlled release behavior in two different pH media. The results are shown in tables and figures, which facilitate the identification of macro-trends and differences between formulations.

Physicochemical Characterization and Colloidal Stability Results

The physicochemical properties results showed the three nanoformulations were significantly different, due to the single design variable considered in the DOE approach and with regard to size, polydispersity index and temporal stability, among other details. While the values determined right after manufacturing for all formulations were fairly close, those differences were amplified when monitoring variation as function of time, indicating the distinct behavior of each formulation upon exposure to storage conditions and to the surrounding medium. The initial characterization and temporal stability in detailed values are given in Table (1).

Table (1): Physicochemical Characterization and Temporal Stability Results of Nanoformulations

Formulation	Time	Hydrodynamic Size (nm) ± SD	Polydispersity Index (PDI) ± SD	General Appearance	Presence of Sedimentation
F1	Immediately	212 ± 15	0.29 ± 0.03	Slight turbidity	No

F1	24 hours	238 ± 18	0.34 ± 0.04	Moderate turbidity	No
F1	7 days	315 ± 27	0.46 ± 0.05	Pronounced turbidity	Yes
F2	Immediately	168 ± 12	0.20 ± 0.02	Semi-transparent	No
F2	24 hours	174 ± 13	0.21 ± 0.02	Semi-transparent	No
F2	7 days	188 ± 15	0.24 ± 0.03	Slight turbidity	No
F3	Immediately	142 ± 10	0.18 ± 0.02	Clear	No
F3	24 hours	147 ± 11	0.19 ± 0.02	Clear	No
F3	7 days	158 ± 12	0.20 ± 0.02	Clear	No

The findings showed that a few samples had quite constant diameter and polydispersity index during the observation period, while others showed a marked increase in the observed values between 0 and 72 h, illustrating a variance in colloidal stability for different samples. These results were then employed to choose the formulation that was best suited as a candidate for controlled release studies

Encapsulation Efficiency and Drug Loading Results

The EE and DL of the model compound in nanoformulations were determined by using UV-Vis spectrophotometric method. The findings showed quantitative variances between the various preparations. The means with standard deviation are given in table (2), the results are described without interpretation of what behind them.

Table (2): Encapsulation Efficiency and Drug Loading Results of Nanoformulations

Formulation	Encapsulation Efficiency (EE%) ± SD	Drug Loading (DL%) ± SD
F1	54.8 ± 3.6	4.1 ± 0.4
F2	65.3 ± 2.9	4.9 ± 0.3
F3	71.2 ± 3.1	5.2 ± 0.4

The results show that the different formulations exhibit a diverse capacity to encapsulate the model compound in the nanocarrier, which is well distinguished by the measured values of the three formulations.

Controlled Release in Two Different Media

The controlled release of a model compound from the selected formulation was investigated in two pH media, a physiological medium (pH 7.4) and an acid medium (pH 5.5). The percentage cumulative release was calculated at different time intervals, and the results were tabulated and plotted as time-dependent release curves. The cumulative release in both media is summarized in Table (3).

Table (3): Accumulated Release (%) of the Model Drug in Two Different Media

Time (h)	Cumulative Release (%) at pH 7.4 ± SD	Cumulative Release (%) at pH 5.5 ± SD
1	18.4 ± 2.1	26.7 ± 2.4
3	29.6 ± 2.5	41.3 ± 3.1
6	42.1 ± 3.0	58.9 ± 3.6
12	55.8 ± 3.4	72.5 ± 4.0
24	68.9 ± 3.8	85.7 ± 4.2

The time release curves showed a distinct pattern and speed of release between the two media, and an enhanced cumulative release was seen in the acidic medium when compared to the physiological medium at all the time points under investigation. The results also

suggested an initial burst release stage during the first hours, then a more prolonged release stage with progressing time in both media.

Release Kinetics Modeling Results

The cumulative release data were fitted to several kinetic models to identify the kinetics of the release process. The findings indicated that the fit coefficients of determination (R^2) were different for each model and release media. The data showed better agreement with certain models than others, suggesting that the type of release mechanism is different in different environments. The coefficients of determination of the kinetic models are shown in Table (4).

Table (4): R^2 Values for Various Models of Drug Release Kinetics

Kinetic Model	R^2 at pH 7.4	R^2 at pH 5.5
Zero-order	0.89	0.91
First-order	0.93	0.95
Higuchi	0.96	0.97
Korsmeyer–Peppas	0.95	0.96

DISCUSSION

The discussion is focused on the quality of the results obtained from the implemented framework for the research objectives, specifically the dependency of physicochemical properties of nanoformulations on the functional performance in terms of colloidal stability, encapsulation efficiency, and organ-specific controlled release patterns in two media with different pH values. The results of physicochemical characterization in table (1) showed a well-defined distinction between the there formulations in terms of hydrodynamic size, PDI and temporal stability, which was the result of a single design variable used in this study. For instance, F1 showed significant particle size and PDI enhancements with time and caused sedimentation on day 7, whereas F2 and F3 displayed somewhat more constant values, and the PDI for F2 and F3 was low enough to imply good colloidal homogeneity. Such a behavior substantiates the recommendation that the stability of the system should not be evaluated on the basis of the first measurements only but rather on the potential of the formulation to preserve its characteristics with time, which in turn is a decisive parameter in picking up a formulation for functional application. The link between the stability results and the drug loading and encapsulation efficiency results in table (2) highlighted that stable formulations showed also higher encapsulation efficiency in comparison with the unstable formulation. Formulation F3 showed the maximum encapsulation and loading capacity, followed by F2, while F1 demonstrated the minimum values. This trend would indicate an association with improved colloidal stability and less loss of model compound in the course of processing or storage, which ultimately controls the quantity of drug in the nanocarrier. However, a high encapsulation efficiency on its own is not sufficient to consider a formulation as best in terms of time-dependent stability and release behaviour. This accounts for choosing the candidate formulation on the basis of a combination of indicators rather than just a single-parameter one. The differentiated media are the most informative controlled release results in the two Ph results among the findings of this study. There is a plain dissimilarity in both release rates and profiles between the physiological medium (pH 7.4) and the acidic medium (pH 5.5) as can be seen from table (3). The total release percentage was always higher in the acidic medium at all the time intervals studied and the gap between these two bridging orchids widened as time elapsed, demonstrating the nanosystem response to environmental changes. Moreover, the release profiles showed an initial burst release in first few hours followed by a sustained release, which is general phenomena in nanos delivery systems and

is attributed to the drug fractions located near the surface and embedded in the matrix of the carrier. This phenomenon exemplifies that pixel-oriented physicochemical properties of the carrier can be engineered to produce significant functional differences without employing sophisticated targeting schemes.

Additional analysis of the release kinetics by means of application of the release data in different mathematical models (Table 4) inferred that the Higuchi model attained the maximum goodness-of-fit value in both media, while the Korsmeyer–Peppas model had also a relatively high value. This consensus pattern of release indicates that the release is primarily a diffusion controlled process which may be influenced by other processes due to interactions between the carrier and medium, specially in acidic environment. The higher values of the goodness-of-fit for the acidic medium when compared to those for physiological medium support the suggestion that dC/dt may be a function of pH and that this dependency could be brought to light through a change in the release rate influencing the release mechanism. This observation is in line with the overall objective of the work to relate physicochemical characterisation with application in performance. The significance of these results should be considered in light of the limitations of the study, as the experiments were performed with a model compound and using simplistic in vitro release systems, thereby limiting the extent to which they can be directly translated to clinical scenarios. However, the consistency of results at different stages and the evident relationships between stability, encapsulation efficiency, and release pattern ensure the suitability of the chosen experimental design to realize the aim of the study based on resource constraints. The findings also indicate that translational research in a resource-poor research setting has the potential to produce highly interpretable data given tight control of variables and systematic design and analysis of experiments.

Overall, this discussion emphasizes that the physicochemical characteristics of nanoformulations are not simple descriptive parameters but governing the functional behavior of the system. The rational control of these properties allows the modulation of the drug release profiles and environment--sensitivity in a quantifiable and analyzable way. The results also demonstrate that combining physicochemical characterization, with functional measurements and kinetic analysis, yields a consistent and applied framework, which could be considered a starting point for more advanced future work

CONCLUSIONS AND RECOMMENDATIONS

First: Conclusions

A series of novel scientific insight is obtained from this study that validates the physicochemical characterization as the pivotal aspect while designing or evaluating the nanoformulations for anticancer drug delivery. The findings showed that the ability to manipulate basic physicochemical characteristics—including hydrodynamic size, polydispersity index, and colloidal stability—is a key determinant for whether a nanoformulation can be brought forward to functional evaluation. A clear trend was observed where formulations that were stable with time gave more predictable encapsulation and loading efficiency, showing a positive correlation between colloidal stability and the ability to retain the loaded compound. Moreover, the results of encapsulation efficiency and drug loading indicated that an optimal formulation design, which balances the amount of stabilizer and phytantriol, not only results in enhanced stability, but also reduces the loss of model compound during preparation and storage, leading to improvement of system's functional efficiency. These results confirm that it is not possible to evaluate the quality of a nanoformulation by an isolated single parameter but only by integrated physicochemical and functional parameters.

Release test results for the function-directed system demonstrated a dependent release with a faster release rate and a higher cumulative release percentage in acidic medium than those in the physiological solution. This implies that nanoformulation physicochemical parameters might provide a certain level of environmental responsivity to the release profile without employing complicated active targeting systems. Kinetic modelling of release predicted diffusion control as the dominating release mechanism, but also identified differences in goodness-of-fit parameters for the two media which may be attributed to environmental influence on system behaviour. In conclusion, the results from this study indicate that physicochemical characterization is not simply a descriptive underpinning step, but the key enabler through which functionality of nanoformulations is realized. Additionally, the work highlights that acking, and the rest, sd0/constant all apply to all the factors to working with an obvious reading as three the by one variable experimental design can also be used to provide a straightforward and unambiguous understanding of the process, even for resource-limited studies purposes. In that line of reasoning, this work establishes the basics of an integrated applied model that could be relevant for academic investigations aiming at directly and scientifically linking nanodesign with functional performance.

Second: Recommendations

Based on the obtained results, the present work suggests to consider the physicochemical characterization as a priority in the design of nanoformulations and to not confine the assessment only to formulations in their initial post-preparation state of measurement, but also to consider the temporal stability of the formulation as a potentially decisive parameter in determining which formulations to take forward to functional testing. The study also suggests that the development of a nanoformulation should include a compromise between colloidal stability and encapsulation efficiency, as focusing too much on either parameter may lead to suboptimal systems.

The authors encourage also future investigations that systematically expand on other design parameters (e.g., carrier type, surface decoration strategies) and their bearing on release behavior and environmental responsiveness, within the limitations imposed by the one-variable-at-a-time concept, to ensure interpretative clarity and keep a tight control over experimental complexity. It is further recommended to apply the applied framework,which was introduced in this work, to real pharmaceutical agents instead of model compounds, to examine if the presented conclusions can be generalized to genuine therapeutic systems.

In addition, if resources allow, we recommend inclusion of additional assays (e.g., protein interaction assays or simplified cellular models) to further elucidate the biology of nanoformulation behavior without sacrificing the stepwise process that begin with physicochemical characterization and proceed functional evaluation. Finally, such integrated applied frameworks are recommended for adoption in graduate research as well, as they strike a practical-scientific balance for application-based research in low resource contexts.

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