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Original Article

Optimization and Characterization of Lamivudine-Loaded TCS-PEG/MMT Polymeric Nanocomposites for Enhanced Antiretroviral Therapy

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ABSTRACT

Background: Acquired Immunodeficiency Syndrome (AIDS), caused by the Human Immunodeficiency Virus (HIV) was initially associated with rapid disease progression and high mortality rates. However, advances in drug delivery systems have aimed to optimize therapeutic outcomes, especially for medications facing challenges such as toxicity, uneven distribution, instability and formulation complexities. **Materials and Methods:** In this study, we introduce TCS-PEG/MMT composites, a novel formulation designed for antiretroviral activity. This formulation consists of a blend of Medical clay (MMT) and Thiolated Chitosan and Polyethylene Glycol (TCS-PEG), which are biodegradable materials. Using Design Expert software, we optimized the loading procedure of lamivudine onto chitosan (X1), MMT (X2) and PEG (X3) to achieve desired Entrapment Efficacy (EE) and particle size (PS). The desirability technique helped determine an optimal formulation with 3 g of chitosan, 3 g of MMT and 2.5 g of PEG. **Results:** The optimized formulation exhibited an EE of 79.58% and a PS of 592.32 nm. We conducted comprehensive analyses on the Optimized formulation (O-LMD-NC), including assessments of swelling characteristics, surface morphology, drug loading, entrapment efficiency and particle size. Water absorption of O-LMD-NC samples gradually increased over time, reaching a maximum of 13.5 g/g after 24 hr. *In vitro* drug release studies confirmed sustained release of lamivudine over a 24 hr period. **Conclusion:** Our findings suggest that LMD-loaded polymeric nanocomposites offer a promising approach to enhance the efficacy of AIDS treatment.

Keywords: Nanocomplex, Lamivudine, Montmorillonite, Human immunodeficiency virus, Optimization.

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) and Human Immunodeficiency Virus (HIV) infection constitute a cluster of diseases caused by the retrovirus HIV. Following initial infection, individuals may either be asymptomatic or experience a transient illness resembling influenza.[1] Subsequent to this initial phase, there typically ensues an extended period characterized by asymptomatic incubation. Progression of the disease leads to escalating impairment of the immune system, heightening vulnerability to opportunistic infections, neoplasms and conventional pathogens such as tuberculosis, occurrences uncommon among immunocompetent individuals. The term Acquired Immunodeficiency Syndrome (AIDS) denotes the manifestation of these late-stage infection indicators.[2]

Nucleoside reverse transcriptase inhibitors, exemplified by lamivudine, are commonly integrated with other antiretroviral agents in the management of HIV-1 infection. Its prolonged intracellular half-life renders it notably efficacious, permitting once or twice daily dosing. As a constituent of initial or subsequent combination regimens for HIV-positive individuals,

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it remains favored owing to its distinguished long-term safety and tolerability profiles among antiretroviral therapies. Lamivudine demonstrates preferential activation in both quiescent infected and uninfected Peripheral Blood Mononuclear Cells (PBMCs). In clinical assessments relative to other nucleoside analogues, it exhibits superior safety attributes and exhibits minimal susceptibility as a substrate for human DNA polymerase α, β and γ in nuclear and mitochondrial environments.[3]

Nanotechnology encompasses materials ranging in size from 1 to 100 nanometers, wherein the physical characteristics of various materials such as carbon, silicon and metals exhibit distinct properties at the nanoscale compared to the macroscale. It constitutes a domain of scientific inquiry delineated by the manipulation, application and conceptualization of processes and substances based on their size.[1]

Nanocomposites have garnered considerable attention in the modern era of nanotechnology due to their unique amalgamation of properties that surpass those of conventional composite materials.[2] Composite materials characterized by the presence of at least one nanometer-scale dispersion phase are categorized as nanocomposites. The nanoscale dispersion phase or nanofillers, owing to their substantial surface area-to-volume ratio, impart a distinctive array of exceptional properties, rendering them multifunctional.[3]

The properties of nanocomposites are predominantly governed by the extensive interfacial area between the polymer matrix and the nanoparticles. Surface characteristics play a pivotal role in dictating numerous chemical and physical interactions, thus a material with nanoscale dimensions exhibits markedly distinct attributes compared to one with larger dimensions but identical composition.[4] Thus, resulting nanocomposites provides improved multifunctional properties at very low loading of the nanofiller and at reduced cost.

The emergence of polymer nanocomposites as a multidisciplinary area of investigation is poised to significantly broaden the application scope of polymers across various sectors. The increasing demand for multifunctional, lightweight materials has spurred researchers to focus on polymer matrix-based nanocomposites.[5] Polymer clay nanocomposites represent a novel category of composites wherein the polymer matrix incorporates particles characterized by at least one dimension at the nanometer scale (10^{-9} m) within the dispersion phase.[6] This research addresses an important knowledge void by introducing a distinctive composite formulation termed TCS-PEG/MMT composites, which integrate a blend of Thiolated Chitosan-Polyethylene Glycol (TCS-PEG) with Montmorillonite (MMT), medical clay. The investigation is centered on the antiviral agent Lamivudine (LMD) with the aim of enhancing drug delivery efficiency while addressing concerns pertaining to drug toxicity, distribution, stability and formulation.

To attain the desired quality and product characteristics, optimization of formulation parameters is essential. Implementing "Quality by Design" (QbD) represents the most effective approach to ensure consistency among all formulation components. In contrast to the conventional "one factor at a time" methodology, QbD enhances and streamlines the optimization process, thereby enhancing its efficacy and cost-effectiveness.[7] The Quality by Design (QbD) process involves multiple stages aimed at elucidating interrelationships among various aspects. This approach facilitates a comprehensive understanding of the procedure, thereby streamlining the achievement of desired outcomes while minimizing expenditure, time and labor. The present study adheres to the Quality by Design (QbD) methodology as outlined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.[8] Statistical software was employed to uncover connections between formulation and process characteristics. The findings underscore the promising prospects of utilizing LMD-loaded polymeric nanocomposites as a promising approach to augment the efficacy of AIDS treatment. This innovative strategy holds promise for enhancing patient outcomes and addressing significant hurdles associated with antiviral therapy, concurrently improving drug delivery effectiveness.

In conclusion, the development and characterization of TCS-PEG/MMT composites represent a significant breakthrough in the battle against AIDS, offering a novel strategy to optimize the efficacy and dissemination of antiviral medications.

MATERIALS AND METHODS

All materials utilized in the research, such as Lamivudine from Aurobindo Pharma in Hyderabad, Chitosan, Polyethylene Glycol and Montmorillonite acquired from Yarrow Chemicals in Mumbai, were of analytical grade.

Preparation of LMD nanocomposites

Diffusion loading method

Synthesis of Chitosan- L-cysteine conjugates forming Chitosan (TCS)

A 1% acetic acid solution (50 mL) was employed to dissolve 500 mg of chitosan, followed by the addition of 100 mg of EDC to expedite the reaction with cysteine. Subsequently, 335 mg of Cysteine (CYS) was introduced upon complete dissolution of EDC and the pH was adjusted to 5 using 3 N NaOH. After stirring, the reaction mixture was allowed to incubate at room temperature for three hr. Dialysis against 5 mM HCl was performed five times over three days in the absence of light to eliminate unbound cysteine and isolate the polymer conjugates. Following this, dialysis against 5 mM HCl containing 1% NaCl was conducted twice to minimize ionic interactions between the

cationic polymer and the anionic sulfhydryl compound (Figure 1).

Preparation of TCS-PEG/MMT composites

Initially, separate solutions of Thiolated Chitosan (TCS) and Polyethylene Glycol (PEG) were prepared before being amalgamated to form the blend. Subsequently, various compositions of blend solutions were generated by combining the solutions on a Teflon dish. To ensure uniformity, Montmorillonite (MMT) of differing compositions (1 wt%, 3 wt% and 5 wt%) was incorporated into this blend solution, followed by continuous stirring for 8 hr at room temperature. After a period of three days of drying at room temperature, the resulting cast films were collected for characterization purposes (Figure 2).

Antiretrovirals loading on TCS-PEG/MMT composites

Diffusion loading methodology was employed to integrate antiretrovirals into the composite materials. Specifically, 5 mL of insulin solution adjusted to pH 6 using a few drops of 0.01 N HCl was introduced to 1 g of the synthesized TCS-PEG/MMT composites. The mixture was allowed to incubate for 2 hr at 5°C. Subsequently, microparticles were subjected to a washing step with 15 mL of 0.01 N HCl followed by another washing step with 15 mL of distilled water. Following the preparation of hydrated particles with varying insulin loadings (5%, 10%, 15%, 20% and 25%), freeze-drying was conducted for 12 hr (Figure 3). The resulting material was sieved using a mesh size of number 20, thereafter sealed in an airtight screw-capped container and stored at 5°C until required.[9]

Experimental design and optimization

This study employs the Box-Behnken methodology to optimize the formulation of polymeric nanocomposites for antiretroviral drug delivery. It investigates three crucial factors: the concentration of chitosan (X1), the Montmorillonite content (X2) and the concentration of Polyethylene Glycol (PEG) (X3) and their influence on Particle Size (PS) and Entrapment Efficiency (EE).

Figure 1: Solution of Nanocomposite.

Chitosan (X1), known for its mucoadhesive properties and biocompatibility, impacts drug absorption and release. By varying the chitosan concentration within specified ranges (-1, 0, +1), the study assesses its effect on achieving maximum Entrapment Efficiency (EE). Incorporating Montmorillonite (MMT) (X2), naturally occurring clay with extensive surface area and swelling characteristics enhances drug loading and stability in the nanocomposite. Adjustment of MMT concentration (-1, 0, +1) aims to optimize Particle Size (PS) for effective medication administration. Additionally, Polyethylene Glycol (PEG) (X3), a hydrophilic polymer, is included to enhance dispersibility and solubility. Modulating PEG concentration within predetermined ranges evaluates its impact on EE and PS.

Through systematic experimentation using the Box-Behnken design, the study explores the relationships between independent factors (chitosan, MMT and PEG concentrations) and dependent variables (EE and PS). Formulation optimization is achieved through desirability functions and response surface analysis using Design Expert statistical software 12.0; ensuring specific requirements are met, such as maximizing EE while minimizing PS, to enhance overall performance of the polymeric nanocomposite system. ANOVA is employed for statistical inference regarding the influence of variables, guiding optimization by identifying significant factors and their interactions across 17 experimental trials. Various parameter fits for different models (Linear, Quadratic, Cubic and 2FI) are considered based on statistical parameters such as fit summary, R2, adjusted R2, predicted R2, fX model, ANOVA, coefficient of variance and prediction error. Quadratic models are recommended for particle size and entrapment efficiency in this investigation.[10]

Optimization concepts

Design Expert software offers a wide range of optimization techniques utilized in various industries and research fields. One fundamental method is factorial design, which enables researchers to systematically study the effects of multiple factors and their interactions on response variables. By carefully adjusting component levels, factorial designs allow for the identification of both individual and combined effects of factors on the studied system. Another powerful tool in Design Expert is Response Surface Methodology (RSM), a set of statistical and mathematical techniques used to describe the complex relationship between inputs and response variables. RSM utilizes predictive models to account for the curvature of the response surface, facilitating the determination of optimal conditions for either maximizing or minimizing the response.[11] Central Composite Design (CCD) is another essential tool offered by Design Expert. It combines axial and factorial points to create a response surface that considers possible curvature. By efficiently exploring the experimental space, CCD reduces the number of required experimental runs while enabling researchers to identify optimal process conditions. In scenarios such as formulation studies, where factors represent

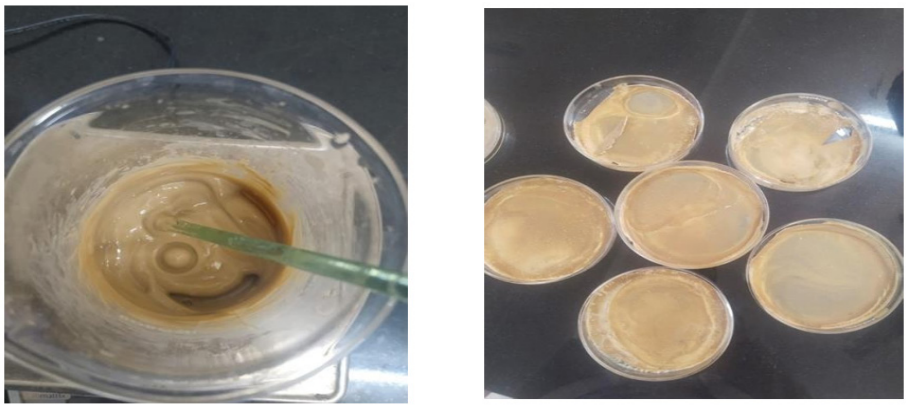

Figure 2: TCS-PEG/MMT composites.

the proportions of various components, mixture design becomes crucial.[12] By optimizing these mixing proportions, Design Expert assists researchers in achieving desired results in intricate systems. Robust Parameter Design is an innovative method facilitated by Design Expert, aiming to enhance process or product robustness against variable changes. By identifying parameter values that minimize the effect of variability on the response, output consistency and reliability are improved.[13] Moreover, Design Expert integrates the Desirability function, allowing researchers to combine various responses into a single desirability index, simplifying the optimization of multiple response variables simultaneously. To expedite the optimization process, Design Expert employs efficient algorithms to systematically search the experimental space for conditions that maximize or minimize the response.[14] Sequential Experimentation, facilitated by Design Expert, enables ongoing exploration and refinement of the experimental design space through iterative testing based on previous findings. In addition to these tools, Design Expert offers graphical tools such as response surface plots and contour plots to aid in data interpretation and visualization, providing researchers with deeper insights into the relationships between causes and responses. By combining robust statistical analytical techniques, visualization tools and optimization methodologies, Design Expert facilitates advancements across various scientific and industrial domains, empowering researchers and practitioners to conduct successful experimental design and optimization.[15]

Characterization of prepared nanocomposites

Particle size

The produced Nanocomposites (NC) underwent Photon Correlation Spectroscopy (PCS) using a Zetasizer device (Malvern Master Sizer 2000, UK) to ascertain particle size, particularly focusing on the Z-average mean. This analytical method assesses fluctuations in light intensity due to the Brownian motion of particles in a liquid. PCS analysis was conducted on each sample thrice to ensure reliability. By examining the autocorrelation function of these intensity fluctuations, the Zetasizer device determines the hydrodynamic diameter of the particles within the nanoscale range, providing precise and consistent particle size measurements. Repetition of measurements three times minimizes experimental variability, enhancing data integrity and ensuring the precision and repeatability of results. Such meticulous particle size characterization yields valuable insights into the characteristics and size distribution of the produced nanocomposites.[16]

Entrapment Efficiency

Drug loading capacity and entrapment efficiency of nanocomposites were assessed using UV-Vis spectroscopy at 271 nm. After dissolving 20 mg of nanocomposite in 2 mL phosphate buffer saline (pH 7.4) and centrifuging, supernatants were combined, treated with ninhydrin reagent and analyzed. Entrapment Efficiency (EE%) was calculated using the formula EE (%)=(T-F)/T×100, where F is the free LMD sulfate in supernatant, W is scaffold weight and T is total LMD concentration.[17]

$$EE\ (\%) = T\text{-}F/T \times 100$$

Preparation of checkpoint batch for validation of experimental design

An optimized batch of nanoparticles (designated as ONP-1) was synthesized and evaluated for various parameters including Entrapment Efficiency (EE), Particle Size (PS), Polydispersity Index (PDI), morphological characteristics and *in vitro* drug release profiles using optimized concentrations of Chitosan (CS), Tripolyphosphate (TPP) and Ascorbic Acid (AA). Relative error was calculated using the following equation to validate the experimental design.[18]

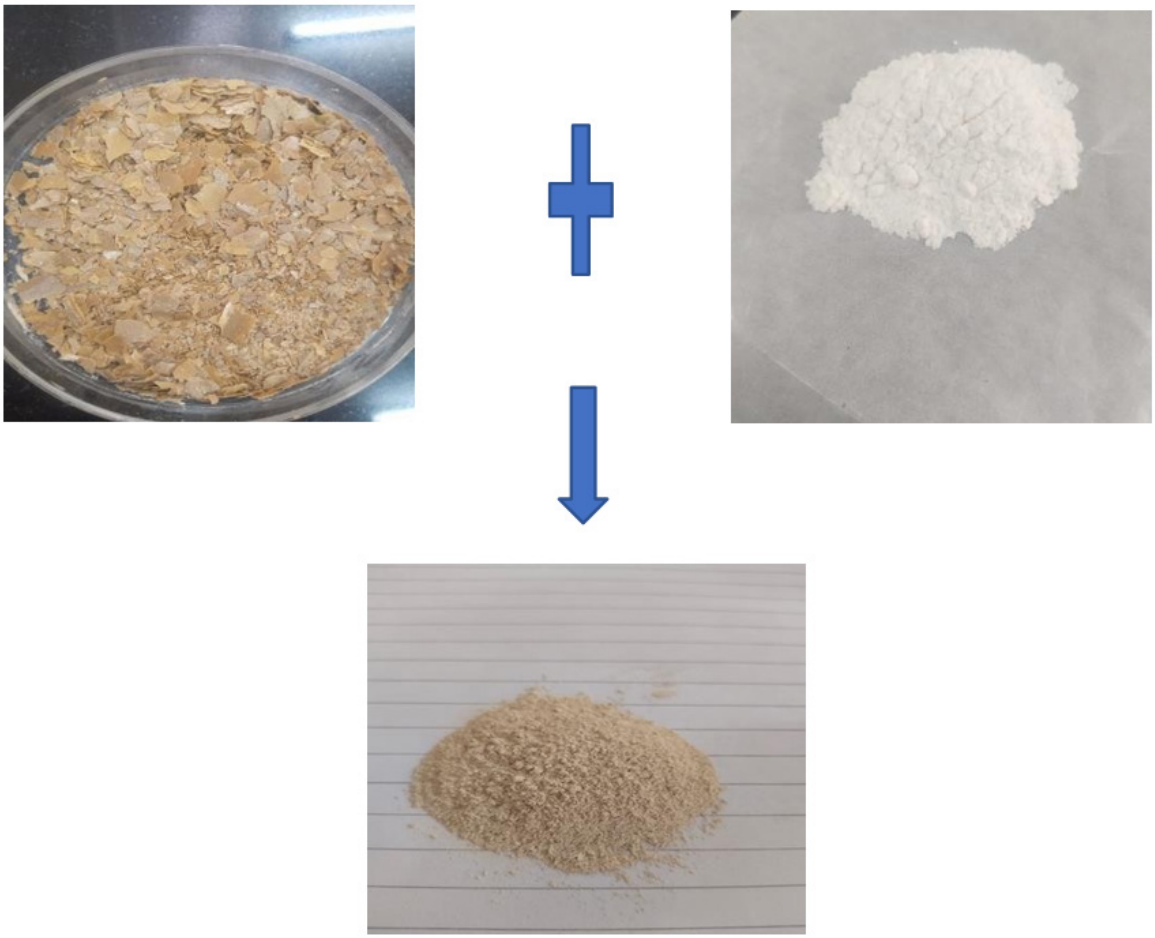

Figure 3: Drug loaded TCS-PEG/MMT composites.

Table 1: Experimental plan for Box-Behnken design in terms of actual and coded values.

Factors/ Independent Variables		Levels	Responses/Dependent Variables		Constraints
	-1	0	+ 1		
Chitosan - X1	1	2	3	EE	Maximum
MMT-X2	0.5	1.75	3	PS	Minimum
PEG (X3)	1	2.5	4		

$$\text{Relative error} = \frac{Predicted\ value - Practical\ value}{Predicted\ value} \text{x100.}$$

Characterization of O-LMD-NC

Microscopic Characteristics

SEM

The surface morphology and structural attributes of the optimized O-LMD-NC formulation were meticulously scrutinized using a JEOL JSM-6100 Scanning Electron Microscope (SEM) from Japan. Prior to imaging, samples underwent gold coating to enhance surface conductivity, ensuring accurate imaging results. SEM, renowned for its high-resolution capabilities, facilitated the observation of both micro- and nanoscale features. Mounted securely on a specialized sample holder, the SEM allowed for precise imaging, revealing intricate details of the nanocomposite structure. This comprehensive analysis yields invaluable insights into the material's surface characteristics and structural integrity, thereby advancing our understanding of its potential applications in biomedical engineering and drug delivery.

FT-IR

The Fourier Transform Infrared Radiation (FTIR) spectra were acquired using a Perkin Elmer Instruments IR spectrophotometer, a product of the United States, operating at room temperature. These measurements were conducted to discern potential interactions between the drug and the super disintegrants. The spectra of both components were meticulously collected and analyzed, aiming to qualitatively evaluate the peak patterns

observed. This qualitative examination facilitated comparison between the different components and sought to identify any molecular structural alterations or chemical interactions. Through analysis of spectral properties, researchers gained critical insights into the behavior and compatibility of the drug and super disintegrants within the formulation.[19]

Hausner ratio and Carr's Indices

The bulk and tapped densities of the O-LMD-NC formulation were assessed following the standardized protocol outlined in the United States Pharmacopeia (USP/NF, 2010), a widely recognized procedure in the pharmaceutical industry. Bulk density, indicative of a powder's mass per unit volume in a loose-packed state, contrasts with tapped density, representing the mass per unit volume when subjected to compaction. The Hausner ratio, derived from the ratio of bulk density to tapped density, serves as a pivotal indicator of powder flowability. A higher Hausner ratio suggests increased inter-particle friction or cohesion, signifying inferior flow characteristics. Additionally, Carr's index, computed as the percentage ratio of the difference between bulk and tapped densities to the tapped density, offers further insights into powder flow behavior. Elevated Carr's index values correlate with greater disparities between bulk and tapped densities, reflecting poorer flowability. This comprehensive analysis of bulk and tapped densities, coupled with Hausner ratio and Carr's index determination, provides valuable insights into the powder handling properties and flow behavior of the O-LMD-NC formulation.

Swelling degree

The Swelling Degree (SD) of O-LMD-NC was determined as follows: A 100 mL beaker containing a precisely weighed sample (0.2 g) was filled with 20 mL of deionized water. Subsequently, ultrasonic agitation was performed using an ultrasonic cleaner (KQ-600KDE NC, Kunshan, China) with a maximum power of 600 W and constant stirring for 30 min at 40°C. Following this, the suspension was centrifuged at 8000 rpm for 10 min. After discarding the supernatant, the centrifuge tube containing the sediment was weighed and then placed in a petri dish and dried overnight at 105°C in a hot air oven. This procedure was repeated to calculate the standard deviation. The swelling degree was determined as the ratio of the weight of the swollen sample to the initial dry weight.

$$SD=(w2-w1)/w0 \quad (1)$$

Where,

W0=weight of the dry sediment.

W1=Weight of the centrifuge tube.

W2= Weight of the centrifuge tube with swollen sediment.

Moisture absorption

Moisture absorption was determined using the angles and difference method. Initially, the dried samples were conditioned for 24 hr at 0% Relative Humidity (RH) using calcium sulfate as the desiccant. Subsequently, the samples were weighed, followed by conditioning in a saturated calcium nitrate solution at 20-25°C to ensure a relative humidity of 55%. The samples were weighed at predetermined intervals until equilibrium was achieved.

In vitro drug release study

In vitro drug release assessment of LMD from O-LMD-NC was conducted. A standard curve for LMD was established to characterize its release properties. Absorbance of the solution at 291 nm was measured using a UV-vis spectrophotometer. The release study utilized Phosphate Buffer Solution (PBS), where 50 mL of PBS containing 100 mg of LMD equivalent weight was prepared and maintained at 37°C. To maintain a constant volume, 1 mL of PBS solution was withdrawn at regular intervals and replaced with an equal volume of fresh PBS. The extracted solution underwent derivatization before absorbance measurement using a UV-vis spectrophotometer. The amount of LMD released was determined using the standard curve and plotted against time for duration of up to 24 hr. Each measurement was performed in triplicate, with three repetitions for each measurement.

Release kinetics

In vitro release experiments are essential for elucidating the kinetics of drug release from formulations. To discern the release data and elucidate the underlying release mechanisms, various mathematical models are employed.[20]

Stability study

O-LMD-NC samples were subjected to a stability analysis chamber for duration of three months under elevated temperature and relative humidity conditions (25±2°C/60%±5% RH, 40±2°C/75%±5% RH). Additionally, freshly prepared nanoparticles were maintained at a controlled temperature of 5±3°C as a reference. After a 90-day period, the samples were retrieved for stability assessment, comparing the Entrapment Efficiency (EE) of the nanoparticles with that of the control formulations.

FTIR

The combination of key components within the formulation, along with the discernible bands observed in the pure Lamivudine (LMD) Fourier Transform Infrared (FTIR) spectra, provides essential insights into the intricate molecular interactions occurring within the system. Notably, prominent peaks were evident in the FTIR spectra of pure lamivudine at wavenumbers of 3448 cm^{-1}, 2999 cm^{-1}, 1767 cm^{-1} and 1458 cm^{-1}, indicative of the formation of N-H, O-H, C=O and C=N bonds, respectively (Figure 4). These spectral attributes elucidate the molecular

RESULTS AND DISCUSSION

Compatibility study by FTIR

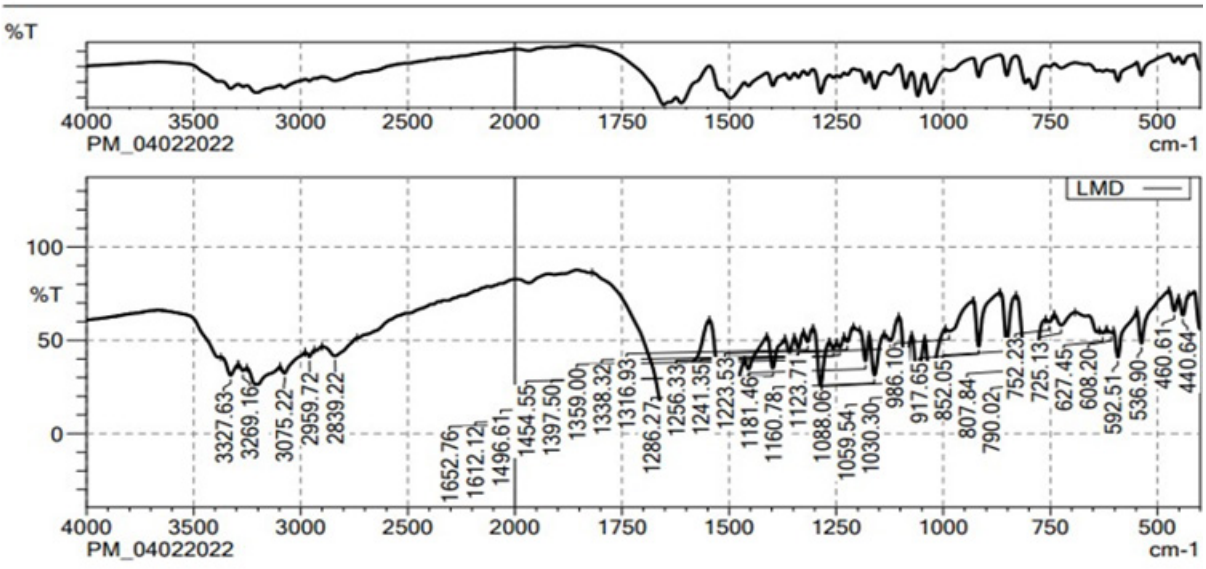

Figure 4: FTIR spectrum of LMD.

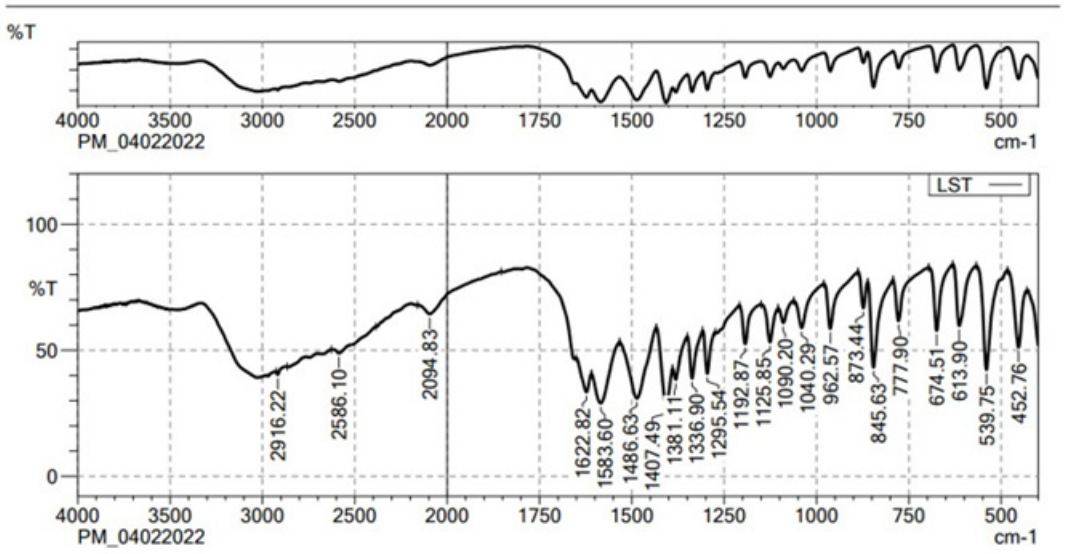

Figure 5: FTIR spectrum of physical mixture of optimized formulation of LMD.

Table 2: Experimental runs and observed responses.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A:chitosan	B:MMT	C:PEG	EE	PS
		g	g	g	%	nm
1	1	1	0.5	2.5	69	1105
11	2	2	0.5	4	56	1058.5
10	3	2	3	1	71	1235
15	4	2	1.75	2.5	84	826.9
9	5	2	0.5	1	64	650.5

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A:chitosan	B:MMT	C:PEG	EE	PS
5	6	1	1.75	1	65	1125.5
8	7	3	1.75	4	55	829.5
4	8	3	3	2.5	78	958
7	9	1	1.75	4	41	793.2
3	10	1	3	2.5	75	796
2	11	3	0.5	2.5	72	704.7
13	12	2	1.75	2.5	82	824.9
14	13	2	1.75	2.5	83	826.8
16	14	2	1.75	2.5	81	839.5
12	15	2	3	4	68	415
17	16	2	1.75	2.5	79	826.1
6	17	3	1.75	1	51	790.7

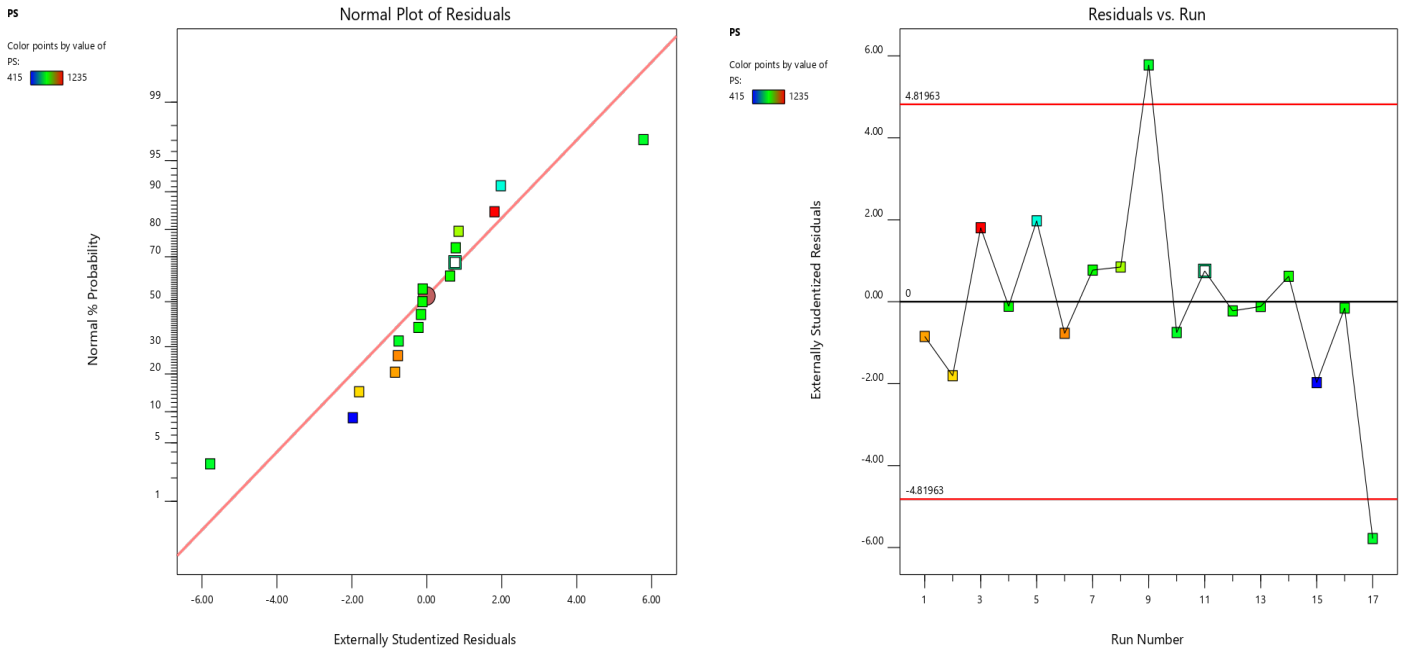

Figure 6: The normal plot of residuals and residuals vs Run for Response-PS..

architecture of the lamivudine molecule by revealing the presence of significant functional groups. Furthermore, the physical combination exhibited a notable spectral peak spanning from 1500 cm^{-1} to 3000 cm^{-1}, suggesting potential interactions between the drug molecules and the polymer matrix (Figure 5). This observation implies successful incorporation of the medication into the polymeric microsphere matrix, affirming the structural integrity of the formulation and its capability to facilitate precise and controlled drug delivery.

Preparation and optimization of Nanocomposites

Utilizing Design Expert Software 12.0, a Box-Behnken Design (BBD) was employed to conduct 17 experimental runs by manipulating three factors: the concentration of chitosan (X1), the montmorillonite content (X2) and the concentration of Polyethylene Glycol (PEG) (X3) at three discrete levels coded as (-1, 0 and +1). Particle size (PS) and Entrapment Efficiency (EE) were selected as response factors in this study. The detailed configuration of the BBD is presented in Table 1. The PS of the experimental formulations ranged between 415 and 1125.5 nm, while EE varied from 41 to 84% across the 17 batches, as illustrated in Table 2. An Analysis of Variance (ANOVA) was employed to ascertain the quantitative impacts of the components. Furthermore, multiple regression analysis was conducted on the data to derive polynomial equations, employing a quadratic model for melting point and a linear model for yield. Model terms were considered significant if their p-values were below 0.05. A

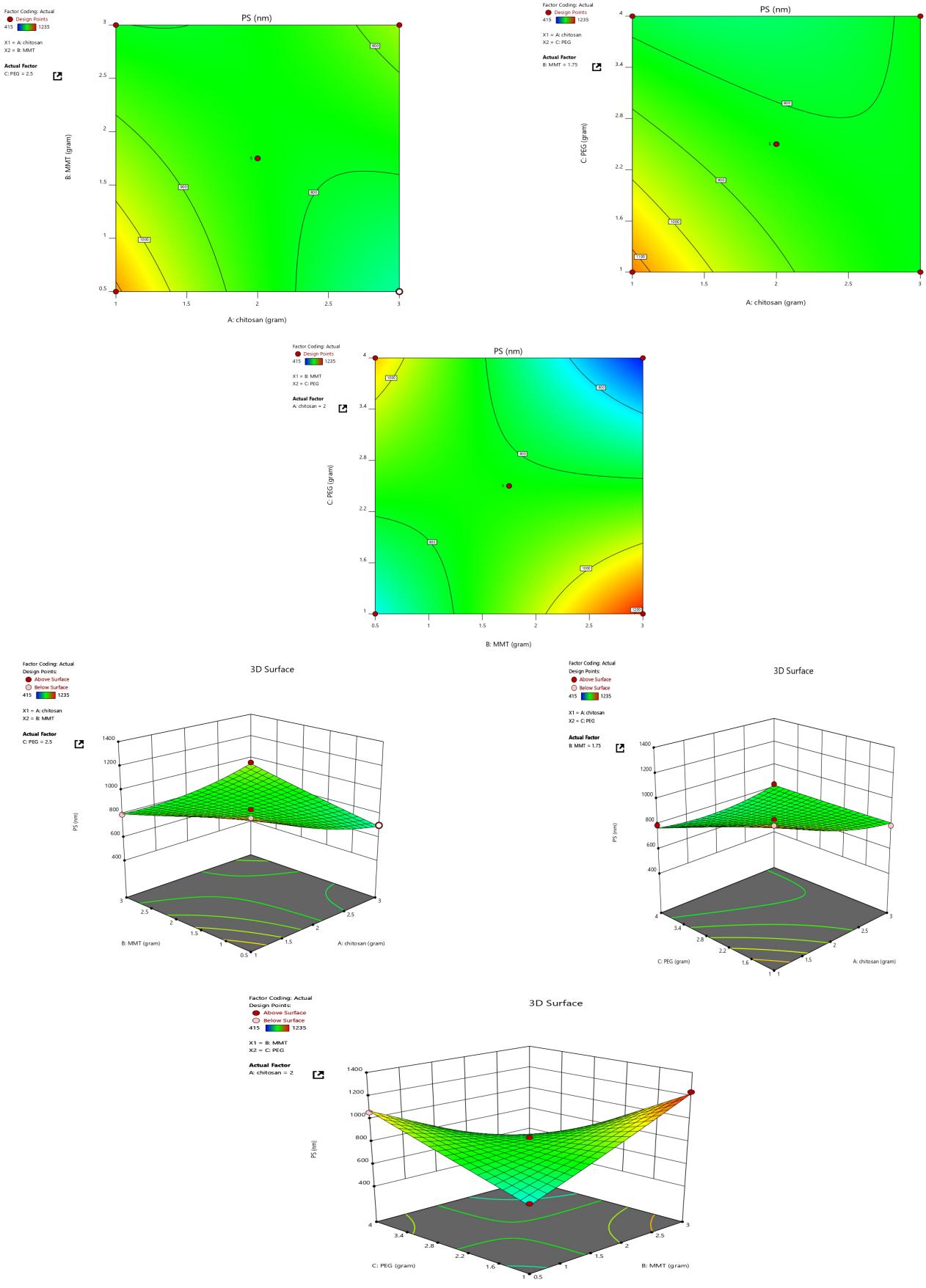

Figure 8: Contour plots (a) and 3-Dimensional Response surface graphs (b) for the response particle size.

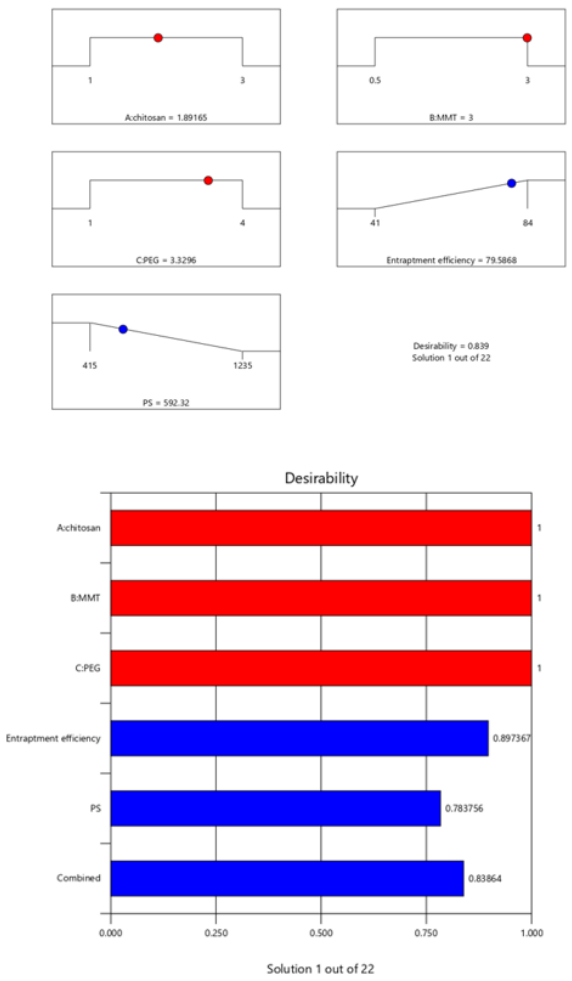

Figure 8: Desirability plot and Bar graph for optimized formulation.

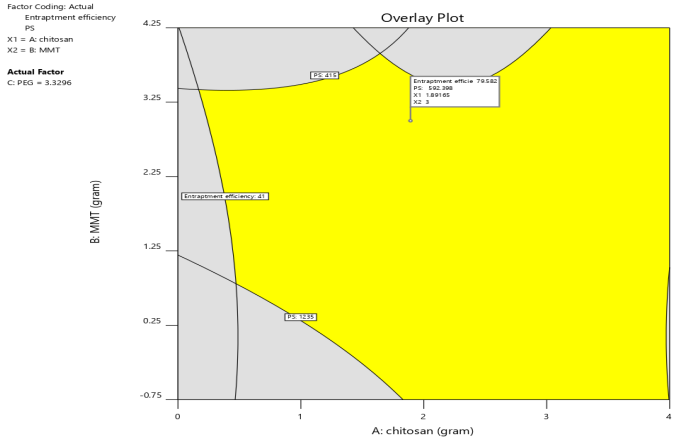

Figure 9: Overlay plot of desirability.

negative sign indicated an antagonistic or inverse influence of the component on the selected response, while a positive value signified a synergistic effect conducive to optimization[21]

The quadratic model was determined to be the optimal choice for predicting both Particle size and Entrapment Efficiency,

.Surface Morphology (SEM)

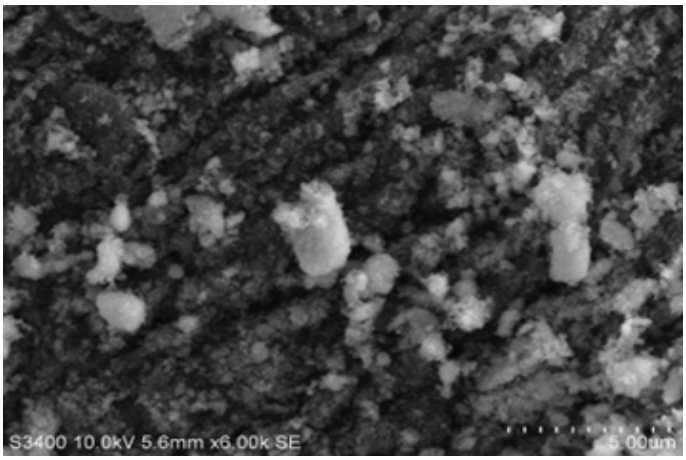

Figure 10: Surface morphology O-LMD-NC.

Swelling degree

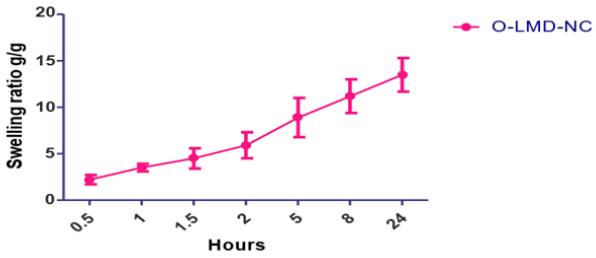

Figure 11: Water uptake study of O-LMD-NC.

In vitro Drug release

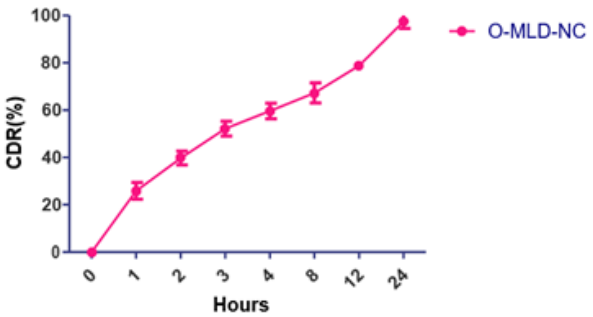

Figure 12: *In vitro* drug release profile of O-LMD-NC.

resulting in R2 values of 0.9961 and 0.9857, respectively. All models demonstrated significance with the experimental data, as evidenced by Adjusted R2 and Predicted R2 values. Specifically, for Particle size, these values were 0.9911 and 0.9412, respectively. Similarly, for Entrapment Efficiency, they were determined to be 0.9672 and 0.8567, respectively.

Drug release kinetics

Table 3: Drug release kinetic study for O-LMD-NC.

Formulation code	Zero order	First order	Higuchi	Peppas		Best fit model
	r2	r2	r2	r2	n	
O-LMD-NC	0.7394	0.9753	0.944	0. 9476	0.3949 (Fickian release)	Peppas

Table 4: Stability studies for O-LMD-NC.

Test	Initial	25°C±2°C+60%±5% RH		40°C±2 C+75%±5% RH	
		3 M	6 M	3 M	6 M
Description	Complies	Complies	Complies	Complies	Complies
Particle size (nm)	592.32	591.48	588.82	591.78	590.25
LMD- Drug release (%) (at the end of 24 h)	97.57	96.78	96.02	96.29	95.81

Furthermore, 'Adequate Precision' was employed to assess the signal-to-noise ratio and the values exceeded the desired cutoff of 4. The achieved Particle Size and Entrapment Efficiency values, which matched the specifications of the experimental design, were 56.192 and 23.388, respectively. The results for both investigated reactions were graphically represented, demonstrating a strong correlation between the experimental and anticipated data. The adequacy of the suggested models is assessed through the F value, which ideally should be non-significant. A model is considered fit when its F value is greater than 0.05. In suggested model, non-significant F values are desirable and, in this case, we obtained values of 53.46 and 199.25 for Particle Size and Entrapment Efficiency respectively. The low probability (0.01%) indicates that the model F-values are unlikely to arise due to noise, further confirming the appropriateness of the models[10].

In the ANOVA results table, both the F and *p* values were utilized to assess the significance of the model coefficients. The *p* values for Y1 and Y2 were determined to be <0.0001 and <0.0001, respectively. It is crucial for p values to be below the significance threshold of <0.05. In this context, both responses are considered desirable for the model. The coded equations demonstrate synergistic and antagonistic effects on each model. For Entrapment Efficiency (Y1), synergistic effects are observed with variables B, C, AC, A^2 and C^2, while antagonistic effects are associated with variable A, AB, BC and B^2. Conversely, for Particle size (Y2), synergistic effects involve combinations such as A, C, AB, AC, BC and A^2, while antagonistic effects are linked to variable B, B^2 and C^2 (Figure 6). Response Surface Methodology (RSM) was utilized to delve deeper and investigate the influence of independent variables on responses.[10,4] Response Surface Methodology (RSM) employs three-dimensional Response Surface Graphs (RSG) to facilitate the examination of primary effects and interaction effects. Contour plots illustrate measured responses graphically. Regarding particle size, in particular, the RSG and parallel contour plots indicate that the reaction decreases as concentrations of X1X2 and X2X1 rise. Particle size increases as concentrations of these variables rise, as depicted by the contour and 3D response surface graphs (Figure 7).

Setting objectives for each response and generating an overlay graph were essential steps in optimizing the independent variables (Figure 9). The experimental design encompassed defining minimum and maximum values for each independent factor. Through the program, diverse concentration trials were conducted and the responses obtained during these trials aided in formulating an optimized recipe that accounted for all variables and responses. A design with a significance level of 100% is indicated by a value of 1, representing the highest desirability on the desirability value scale, which ranges from 0 to 1. Desirability plots exhibit varying colors; red signifies high Desirability (D=1) while dark blue denotes the least desirable values. Other colors on the desirability graph correspond to values ranging from 0 to 1. At the optimal concentrations of the independent variables, we achieved a desirability value of 0.839, suggesting an 83.9% alignment with our design objectives (Figure 9).[22]

Optimization of multiple series of models derived from experimental investigations can be achieved through the application of the Desirability function [D]. Each response was calibrated to two distinct limits, namely the minimum particle size and maximum Entrapment Efficiency (EE), to generate the overlay graph. All selected variables were encompassed within the design space. The contour plot for critical responses (Figure 8) was superimposed with the combined desirability plot for all responses, revealing a maximum D value of 0.839

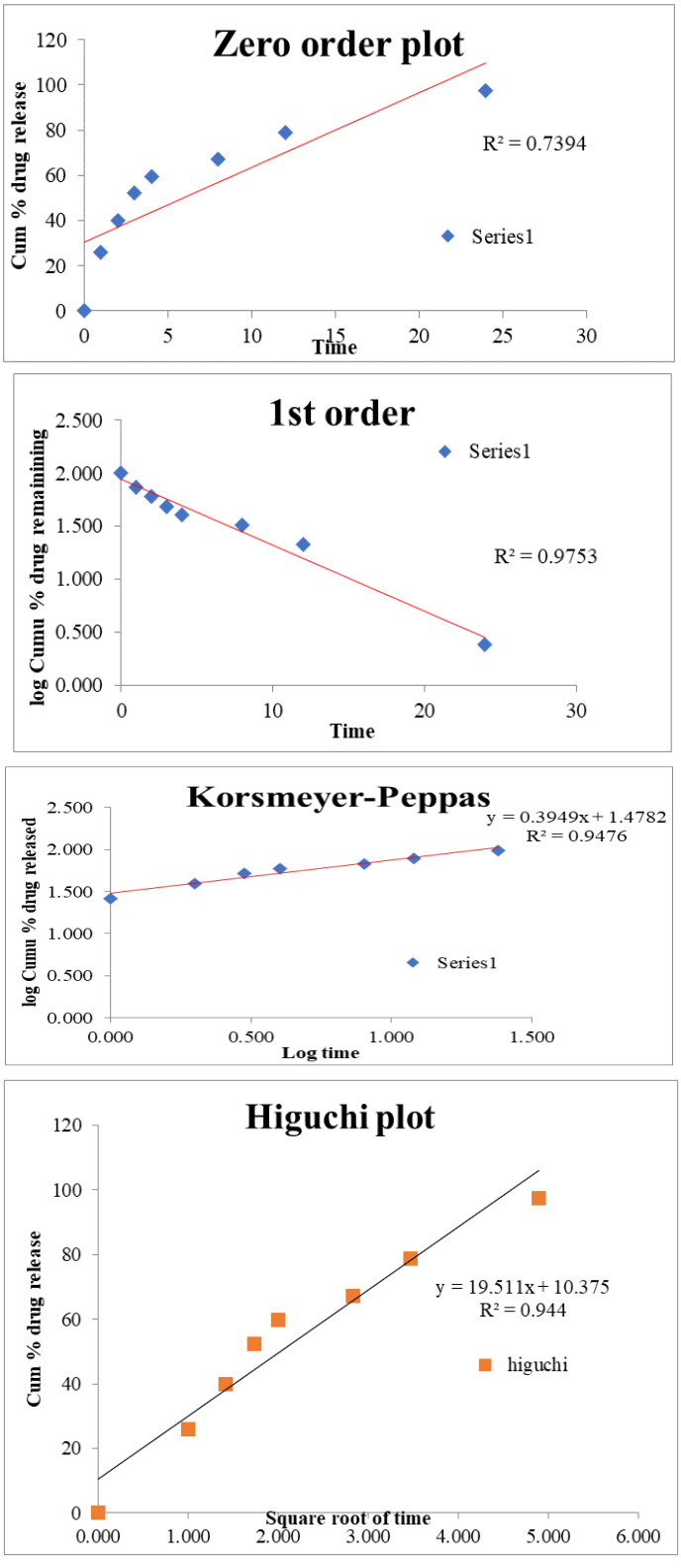

Figure 13: a) Zero order release kinetics of O-LMD-NC, b) First order release kinetics of O-LMD-NC, c) Korsmeyer-Peppas kinetics of O-LMD-NC d) Higuchi of O-LMD-NC.

at optimal concentrations of independent variables. Utilizing this approach, a formulation comprising 1.89 g of chitosan, 3 g of Montmorillonite (MMT) and 3.3 g of Polyethylene Glycol (PEG) may potentially fulfill the criteria of the ideal formulation. Consequently, employing these optimal concentrations could yield a particle size of 592.32 nm and an entrapment efficiency of 79.58%. These projected optimal concentrations were utilized to devise and assess an improved version of O-LMD-NC. A comparison between theoretical values and actual data was conducted to validate the experimental design. The accuracy of the design was affirmed by observing a relative error of less than 3%.

Surface Morphology (SEM)

The size and morphology of O-LMD-NC were elucidated through Scanning Electron Microscopy (SEM) surface morphology characterization. The analysis revealed a size distribution ranging from 500 to 1000 nm, with select nanocomposites exhibiting a rod-like architecture (Figure 10). Notably, these nanocomposites maintained their rod-like morphology even upon extension, indicating a propensity for elongated structures (Figure 10). This detailed examination provides valuable insights into the nanocomposite's dimensional attributes and structural stability, which are pivotal considerations for its application in various biomedical and drug delivery contexts.

Hausner ratio and Carr's Indices

Following a meticulous assessment of the flow attributes of the optimized formulation, it was observed that the Nanocomposite (NC) possesses favorable flow properties. A comprehensive analysis, coupled with precise measurements of diverse flow parameters, substantiated the exceptional flow characteristics exhibited by the NC. These findings underscore the potential utility of the formulation in applications such as pharmaceutical manufacturing and biomedical engineering, where precise and uniform material distribution is imperative. Moreover, the evaluation of flow properties provides valuable insights into the handling characteristics and rheological behavior of the NC, crucial considerations for its practical application across various industrial and research domains. In summary, the detailed examination of flow characteristics enhances our understanding of the NC's behavior under different processing conditions, facilitating informed decision-making regarding its application and further refinement.

Swelling degree

In the quest for effective nutrient delivery and cellular signal transduction, the utilization of Nanocomposites (NC) possessing an appropriate water-holding capacity stands paramount. Thus, a comprehensive assessment of the NC's water-holding capacity was undertaken as a critical parameter. Over a 24 hr timeframe, the nanofibrous scaffolds exhibited substantial expansion with increasing immersion duration. Notably, the optimized O-LMD-NC samples demonstrated a consistent augmentation in water absorption content, culminating at 13.5 g/g following the 24 hr immersion period (Figure 11). This observation is particularly

noteworthy. The heightened water absorption properties observed in the composite formulation can be attributed to the relatively higher concentration of Montmorillonite (MMT) within its composition. Consequently, the optimized formulation showcases exceptional water retention characteristics, thereby underscoring its potential for applications requiring robust hydration properties.

Moisture absorption

Due to its exceptional expansibility, Montmorillonite (MMT) serves as a physical barrier that effectively restricts moisture escape, thereby significantly enhancing composite swelling. This particular water retention behavior promotes cell adhesion. Moreover, adjusting the degree of mixing and clay integration can alter the hydrophilicity of the composite. In real-world biological scenarios, the pH conditions at the delivery site profoundly influence the carrier matrix's swelling capability and structural integrity maintenance. The presence of immobilized phases, such as Montmorillonite (MMT), results in fluid absorption and desorption occurring at a notably slower rate in nanohybrids where biopolymers are intercalated between silicate layers. This observation underscores the highly desirable attributes of nanohybrid systems: the ability to regulate fluid dynamics and release kinetics, crucial for precise and sustained drug delivery in medical applications. Hence, acquiring a comprehensive understanding of the interactions between biopolymers and silicate layers within nanohybrids is imperative to optimize their performance and tailor them for specific biomedical applications.

In vitro Drug release

In vitro drug release experiments were conducted for O-LMD-NC, employing linear standard calibration curves for LMD established beforehand to facilitate the computation of drug release kinetics. These experiments were conducted over duration of 24 hr and the drug release profiles were assessed in Phosphate-Buffered Saline (PBS) at pH 7.4. Notably, sustained drug release from O-LMD-NC was observed throughout the testing period, with an initial rapid release occurring within the first 2 hr, possibly attributed to the higher chitosan and Montmorillonite (MMT) content in the polymer composite. Subsequently, the release rate gradually declined over the subsequent 3 hr, reaching equilibrium after 24 hr. Specifically, 59.97% of the LMD was released within the initial 3 hr period, with sustained release continuing thereafter, reaching 97.57% by the end of the 24 hr period. Minimal LMD release was observed from the nanocomposite at equilibrium (Figure 12). The observed release behavior correlated with the water swelling profiles, indicating that the water swelling ratio of MMT served as the primary determinant of LMD component release via diffusion. The higher water permeability of the polymer matrix contributed to the larger swelling ratio of the nanocomposite, thereby enhancing the solubility of various constituents.

Drug release kinetics

A comprehensive examination of the release kinetics of the Lamivudine-loaded Nanocomposite (O-LMD-NC) formulation has yielded intriguing insights into its drug release behavior. The kinetics of drug release were meticulously analyzed using various mathematical models, including the zero order, first order, Higuchi and Peppas models (Figure 13, a-d). The correlation coefficients (r^2) obtained for these models were 0.7394, 0.9753, 0.944 and 0.9476, respectively, indicating a robust fit. Particularly noteworthy is the highest coefficient of determination (r^2=0.9753) observed for the first order model, suggesting a strong adherence of the experimental data to this kinetic model. Furthermore, the Peppas model exhibited a respectable correlation coefficient (r^2=0.9476), implying that the drug release mechanism may follow a non-Fickian (anomalous) transport behavior, characteristic of polymeric systems. Taken together, these findings provide valuable insights into the release kinetics of the O-LMD-NC formulation, laying the groundwork for further refinement and development of controlled drug delivery systems with enhanced therapeutic efficacy (Table 3).

Stability Studies

Throughout the duration of the trial period under both conditions, no noticeable physical alterations were observed in the formulation. The particle size remained unchanged, with minimal variation in medication release observed after 18 hr. Encouraging results were obtained from the performance evaluation of the test formulation in controlled environments. The formulation consistently met the specified criteria for relative humidity (RH) and temperature, namely 25°C±2°C and 60%±5% and 40°C±2°C and 75%±5%, respectively. Notably, the particle size analysis revealed stability over a 3 to 6-month period, with readings ranging from 588.82 nm to 592.32 nm and consistent values across multiple time points. Furthermore, Lamivudine (LMD) exhibited a consistently high drug release profile at the 24 hr mark, with values ranging from 95.81% to 97.57%, underscoring the formulation's efficacy in facilitating drug release within the specified timeframe (Table 4). Taken together, these findings affirm the durability and reliability of the test formulation across various environmental conditions, signifying its potential for further advancement and utilization in pharmaceutical formulations.

CONCLUSION

In this study, we present the development of a novel composite formulation tailored for the oral administration of lamivudine, a crucial antiviral agent. Specifically, we synthesized TCS-PEG/MMT composites by incorporating Montmorillonite (MMT), medicinal clay, into a biodegradable blend of Chitosan and Polyethylene Glycol (TCS-PEG). Utilizing the desirability technique, we optimized the formulation process to achieve an

Entrapment Efficiency (EE) of 79.58% and a targeted Particle Size (PS) of 592.32 nm. Subsequently, upon attaining these desired concentrations, we generated an optimized blend termed O-LMD-NC. Furthermore, post-release assessments confirmed that the carrier system, comprising clay and polymeric components, exhibited no adverse effects on biological systems. This finding underscores the material's potential for controlled release of Lamivudine (LMD), ensuring both safety and efficacy in drug delivery applications. The successful development and evaluation of this composite formulation signify a significant advancement in oral drug delivery technology, holding promising prospects for enhancing therapeutic outcomes across various medical conditions, particularly in HIV/AIDS management.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

ABBREVIATIONS

AIDS: Acquired immunodeficiency syndrome; **HIV:** Human immunodeficiency virus; **TCS:** Thiolated Chitosan; **PEG:** Polyethylene Glycol; **PBMCS:** Peripheral blood mononuclear cells; **MMT:** Montmorillonite; **LMD:** Lamivudine; **NC:** Nanocomposites; **Qbd:** Quality by Design; **ANOVA:** Analysis of Variance.

SUMMARY

In this study, we introduce a novel composite formulation tailored specifically for the oral delivery of lamivudine, a crucial antiviral medication essential for AIDS treatment. The composition, TCS-PEG/MMT composites, combines a biodegradable blend of Chitosan and Polyethylene Glycol (TCS-PEG) with the medicinal clay montmorillonite (MMT). Through careful optimization using the desirability technique, we created an optimized blend named O-LMD-NC, achieving an exceptional Entrapment Efficiency (EE) of 79.58% and a targeted Particle Size (PS) of 592.32 nm. Subsequent tests showed that biological systems were not adversely affected by the carrier system, comprised of clay and polymeric components. This underscores the material's potential for controlled release of Lamivudine (LMD), ensuring safety and effectiveness in drug delivery applications. Importantly, post-release evaluations confirmed the formulation's reliability and efficacy by validating continuous release of lamivudine over a 24 hr period. The development and evaluation of this composite formulation represent a significant advancement in oral medication delivery technology. By leveraging Montmorillonite's unique characteristics and the biocompatibility of chitosan and polyethylene glycol, we have created a versatile platform with promising potential for enhancing treatment outcomes across various medical conditions. This innovation holds particular significance for managing HIV/AIDS, where improved medication delivery methods can greatly enhance patient outcomes and treatment efficacy. In conclusion, our research underscores the potential of TCS-PEG/MMT composites as a promising approach to enhance the effectiveness of AIDS treatment and advance oral medication delivery technology. Through meticulous optimization and comprehensive assessment, we have laid the groundwork for future research and application of this hybrid formulation to meet the evolving demands of drug delivery in diverse medical settings.

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