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QBD based and Box-Behnken Design based PLGA Nanoparticles for Sustained Release of Curcumin (Cur-PLGA-NS) In A549 Lung Cancer Cells

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KEYWORDS

PLGA Nanoparticle, QbD approach, Box-Behnken design, Curcumin, Lung Cancer

ABSTRACT:

Aim: Design of controlled release Polymeric nanoparticles (PNPs), using systematic optimization and control over critical formulation and process parameters is essential. The study aimed at using a QbD approach involving Box-Behnken design to develope PLGA based Polymeric Nanoparticle for controlled release of Curcumin in lung cancer.

Method: The curcumin loaded PLGA nanosphere (Cur-PLGA-NS) was prepared using the bottom-up method with the help of QbD approach using Box-Behnken design. The prepared NS were characterized by size, zeta potential, TEM, entrapment, and drug release. In vitro cytotoxicity and cell uptake study were also performed with A549 lung cancer cells.

Results: The optimized Cur-PLGA-NS with particle size of 143.1±12 nm and homogeneity in terms of PDI were obtained using the design with and no aggregation of particle, as confirmed by TEM analysis. High drug entrapment efficiency (81.26 %) and controlled release approximately 80.26% of the drug was shown within 24 hours. In vitro cytotoxicity assays against A549 lung cancer cells showed a significantly lower IC_{50} value for the nanoparticles (14.23 µg/mL) compared to curcumin suspension (20.09 µg/mL), indicating higher potency. Additionally, enhanced cellular uptake of Cur-PLGA-NS in A549 cells were observed.

Conclusion: From the study the QbD-optimized Cur-PLGA-NS demonstrated effective formulation characteristics highlighting its potential as a promising nanocarrier for curcumin delivery in lung cancer therapy.

1. Introduction

One of the leading causes of cancer-related mortality for both men and women is lung cancer. Globally, 3 million instances of lung cancer are expected to occur by 2035 [1, 2]. Depending on the stage and type of lung cancer at diagnosis, treatment options include radiation therapy, chemotherapy, and/or surgery. Due to a lack of early-stage diagnostics, the majority of lung cancers are discovered at advanced stages, with either distant metastases or local tumor invasion, making surgery impractical. As a result, chemotherapy and radiation therapy are now the only available treatments for lung

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cancer [3,4]. However, chemotherapy has a number of challenges, including systemic cytotoxicity, fast elimination, non-specific drug distribution, poor drug absorption, and the formation of drug resistance, which is a serious issue [1,5].

Curcumin, a naturally occurring polyphenol derived from the rhizome of the herb Curcuma longa (turmeric) can cause cancer cells to undergo apoptosis without harming healthy cells [6], and has the potential to be used as an anticancer medication [7,8]. The main reason for curcumin's anticancer effects is its capacity to block nuclear factor-kappa B (NF kappaB), which regulates apoptosis, cell division, and inflammation in healthy cells. Curcumin may also have an inhibitory effect on the overexpressed p-glycoprotein of tumor cells, according to current research [9, 10, 7]. Its weak water solubility (0.125 mg/L), poor bioavailability, severe metabolism, degradation, and rapid excretion, however, have limited its clinical application despite its promising qualities [9-11]. Many methods have been used to get over these limitations, such as Nano Drug distribution Systems (DDS), which include polymeric nanoparticles for safer systemic distribution and better local retention, liposomes, protein-based nanocarriers, and inorganic carriers [8].

Polymeric nanoparticles (PNPs) are sub-micronic carriers made up of an oily core inside the polymeric shell and a hydrophilic or lipophilic surfactant at the interface [12]. PNPs are physicochemically stable, resistant to enzymatic degradation, and have a large drug loading capacity because of their polymeric wall. PNPs may also lessen the toxicity of hydrophobic medications and enhance their pharmacokinetic profile [13-14]. Curcumin can be readily delivered using polymeric nanoparticles (NPs), which also provide increased permeability, resistance to metabolic processes, and prolonged circulation [9]. Because of its relatively low toxicity, biocompatibility, biodegradability, non-immunogenicity, and sustained release capabilities, the Food and Drug Administration (FDA) identified the poly (DL-lactide co-glycolide acid) (PLGA)-based nanoparticulate system as the most effective delivery method among the various polymers used in the formulation of cancer drug delivery systems [14-16].

High-quality PNP production is challenging due to a number of issues in the manufacturing process, including the control and replication of important features of the target nanoparticles [17], even with the numerous advancements in laboratory-scale PNP synthesis. PNP preparation procedures are frequently massive, multi-step procedures with numerous affecting factors, making the process itself somewhat complicated. Use of quality by design (QbD) is crucial and recommended by ICH in order to guarantee the final product quality early in the method's development and to identify early on which parameters affect the process. A methodical approach to pharmaceutical development is known as quality by design [18]. It encompasses the assessment and comprehension of the manufacturing and formulation process. The objective is to create a regulated procedure that guarantees the product's quality [18–21]. Several methods and instruments for putting QbD into practice are described in ICH guideline Q8 (R2), including "multivariate experiments," "statistical process control methods," commonly referred to as design of experiment, and a "risk-based control strategy"[22].

Hence, in the present study a QbD approach was used to design PLGA based PNP using Box-Behnken design for controlled release of Curcumin and its efficacy in lung cancer was evaluated in terms of in vitro cytotoxicity and in vitro cell uptake.

2.0 Method:

2.1 Cur-PLGA-NS preparation:

The curcumin loaded PLGA nanoparticle (Cur-PLGA-NS) was prepared using the bottom-up method with saturation solubility, employing mixture of acetone and methanol as the nonpolar solvent and polyvinyl alcohol (PVA) and Tween 80 solution as the polar solvent. Briefly, 20 mg of PLGA and 10 mg of curcumin was dissolved completely in a mixture of acetone and methanol (3mL, 7:3 v/v) to form the organic phase. The resulting solution was added dropwise to deionized water containing Polyvinyl Alcohol (PVA) and tween 80 solution, with continuous stirring and sonicated at 4°C for 15 min to form the nanosuspension. The resulting nanosuspension was stirred to completely evaporate the organic solvent at room temperature. Then the CUR-PLGA-NSs were collected by centrifuging at 15,000 rpm for 50min at 4°C and freeze-dried [16].

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2.1.1 Particle size characterization:

Particle size and polydispersity index (PDI) were determined by dynamic light scattering using a particle size analyser (Zetasizer, Malvern Nano S 90). Determination was carried out at 25°C at a fixed angle of 90°. Before measurement, the Cur-PLGA-NS was adequately diluted at 1:9 ratios. This dilution was then vortexed and checked the size and PDI. Zeta potential of the prepared PLGA nanoparticle was evaluated by electrophoretic mobility technique using a Malvern Zetasizer Nano- ZS500. TEM studies for particle size characterization and morphology were performed using TEM JEOL JEM 2100 (200 KV). Prepared NP was added onto a 400-mesh carbon coated copper grid which was stained with 2% (w/v) phosphotungsten acid solution for 20 minutes. About 15 minutes after nanoparticle deposition, the grid was tapped with filter paper to remove excess water, and then it was air dried and the TEM images were obtained at 200,000× magnification [23].

2.2 Optimization by QbD approach

Using Design Expert® Software (Version 13.0.5.0, Stat-Ease Inc, Minneapolis, MN), the Box-Benhken design approach was applied using three factors at three levels in order to optimise the Cur-PLGA-NS that had been prepared. Based on the chosen independent parameters—the concentration of PLGA (A = X1), the concentration of PVA (B = X2), and the solvent: Non Solvent ratio(S: NS) (C = X3), the prepared Cur-PLGA-NS were optimised. Through preliminary research, the levels for each formulation variable were also determined. In addition, the architecture can be used to study quadratic response surfaces and construct models of second order polynomials. The study's size (Y1) and entrapement efficiency (EE) (Y2) were the chosen responses. Following the assignment of a range from low to high values to each independent variable (Table 1), seventeen experimental runs of the Cur-PLGA-NS formulations were produced and evaluated (Table 2).

After the results, value for the dependent variables Y1 and Y2, were entered into the response columns of the experimental design programme, the model was run in triplicate for each of the seventeen formulations. The statistical analysis of the data was considered significant for any factor when the "p" values were less than 0.05. Box-Behnken design was was selected in order to examine the effects of independent variables (PLGA concentration, PVA concentration, and S: NS ratio) on dependent variables (Size and EE). Direct visualisation of the relationship between the response and independent variables is possible with 2-D contour plots. For each factor level combination, Y1 and Y2 represents the measured responses, where A, B, and C are the coded levels of independent variables. The primary effects (A, B, and C) show the average result of gradually increasing each factor's value from a low to a high one. Table 1 shows the parameters of the factorial design and the testing settings.

Table 1: Design parameter and experimental condition of Box-Behnken design

Independent Variable	Level used, actual (coded)	
	Low (-1)	High (+1)
PLGA concentration	1%	3%
PVA concentration	0.1%	0.5%
S:NS Ratio	1:15	1:5

2. 3 Quantitative estimation of curcumin by HPLC:

Concentrations of CUR for entrapment and release studies were measured using a Waters HPLC system (Milford, MA, Massachusetts, USA) equipped with a dual pump (Waters 515) and a UV detector (Waters 2489). The solvent system comprised acetonitrile:water (HPLC grade), and a Nova-Pak® HR C18 column (3.9×300 mm, 6µm particle size) maintained at 25.0±0.5°C was utilized. Empower chromatography software facilitated data acquisition, analysis, and reporting.

2.3.1. Analytical method development for HPLC analysis:

The developed analytical method was validated based on its linearity, repeatability, precision, intermediate precision, and system performance parameters, including theoretical plates, capacity factor, tailing factor, separation factor, and peak resolution. The development and validation of the method adhered to the guidelines set by the International Conference on Harmonization (ICH) [24, 25].

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The optimized mobile phase consisted of a mixture of acetonitrile and acidified water (adjusted to pH 3 with glacial acetic acid) in a ratio of 85:15 v/v. This mobile phase was filtered using a 0.2 nm Millipore membrane filter. Isocratic elution was performed with a flow rate of 1 ml/min, and samples and standards were prepared in HPLC-grade methanol. The column temperature was maintained at 35°C, the sample temperature at 25°C, and a wavelength of 425 nm was employed for detection.

For the preparation of standard solutions, 20 mg of standard curcumin was accurately weighed and transferred to a 100 ml volumetric flask. The volume was adjusted with methanol to achieve an accurate concentration of approximately 200 µg/ml. Lower concentrated solutions of standards were prepared by transferring aliquots from the stock solution, resulting in calibrants of 12.5, 25, 50, and 100 µg/ml. Fresh standard solutions were prepared daily through appropriate dilution of the stock solution with methanol for both intraday and interday analyses. Prior to injection into the chromatographic system, the solution was filtered through a 0.2 µm membrane syringe filter.

2.3.2 Validation of the method:

Validation of the analytical method was done according to the ICH guidelines. The method was validated for linearity, accuracy and precision.

Linearity:

The linearity of the measurement was assessed through the analysis of varying concentrations (12.5-200 µg/ml) of standard solutions. A calibration curve was constructed by integrating each chromatogram, illustrating the relationship between the average peak area and concentration through a regression equation. Computation of the correlation coefficient and slope of the peak further characterized the linearity. To ensure accuracy and reliability, all samples underwent triplicate analyses.

Accuracy:

Accuracy was determined by analyzing six separate reference drug solutions (100 µg/mL) with the appropriate mobile phase.

Precision:

The precision of the analytical method was examined concerning repeatability and intermediate precision. Repeatability, or intraday precision, was assessed by conducting six replicate injections of the curcumin standard at a concentration of 100 µg/ml on the same day at various time points. The percentage relative standard deviation (RSD) of the peak area was then calculated.

For intermediate precision, or interday precision, the relative peak area was determined at three different concentrations (25, 50, and 100 µg/ml) on separate days that spanned the entirety of the assay method. Precision was quantified as the % RSD of both the system and the samples, and all analyses were performed in triplicate to ensure robustness and reliability of the results.

2.4 Drug Entrapment Efficiency (DEE)

The drug entrapment efficiency (% EE) of the prepared Cur-PLGA-NS was determined by cold centrifuging 2 mL of prepared Cur-PLGA-NS for 30 minutes at 12,000 rpm to separate the free CUR from the Cur-PLGA-NS. The clear supernatant layer was collected and the concentration of unentrapped CUR was calculated by developed HPLC method (Waters). The following formula was used to calculate the % DEE:

EE (%) = [(Concentration of total drug – concentration of free drug) / Concentration of total drug) x100] .....Eq1 [26]

2.5 In-vitro drug release study

An in vitro drug release study was conducted on Cur-PLGA-NS formulation and CUR-suspension to assess the rate and pattern of release of CUR encapsulated inside NPs by using a cellulose dialysis membrane (molecular weight cut off between 12,000 K-14,000 K). Briefly, 1 ml of the prepared formulation was taken into dialysis bag and dialyzed against 100 ml phosphate buffer (pH 7.4) as release medium with 1% Tween 80 as solubilizing agent. The solution was magnetically stirred (IKA HS7 magnetic stirrer) continuously at 100 rpm at a temperature of 37±1°C for 24 hr. At 1, 2, 3, 4, 5, 6, 7, 8 and 24 hr, the dialysate aliquots were taken out and replaced with the same amount (2 mL) of fresh media. The samples were further diluted with dissolving media and the concentration of CUR was determined using the developed HPLC (Waters) method using the calibration

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curve (R2 = 0.98) and the graph of drug release (%) v/s time (hr) was plotted [27,28].

2.6 In-vitro Cytotoxicity studies

To assess the cytotoxicity of Cur-PLGA-NS and CUR suspension against the human lung cancer cell lines A594, an MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed. Prior to the assay, A594 cells were incubated in F12K media and supplemented with 10% fetal bovine serum (FBS), 100 U/ mL penicillin and 100 mg/mL streptomycin and incubated at 37 °C in a 5% CO_2 incubator under sterile conditions. The cells were seeded on a 96-well plate at a density of 1.8 × 106 cells/well and incubated overnight. Following this, different concentrations of CUR-PLGA-NS and CUR suspension were added to the wells in triplicate and incubated for 24 hr. After the incubation period, 20 µL of MTT solution was added to each well, and the plate was incubated for an additional 4 hr at 37° C. This allowed mitochondrial dehydrogenases to react with MTT, forming formazan crystals. The medium was then removed, and 200 µL of DMSO was added to each well to dissolve the formazan crystals. The plate was shaken for 15 minutes at room temperature. Finally, the optical density of each well was measured at a wavelength of 570 nm using a microplate reader (Synergy H1). The IC_{50} values of Cur-PLGA-NS and CUR suspension were calculated and presented as the mean ± standard deviation (SD) [29, 30]. The % cell viability was calculated using the equation: [(Abs570 of treated cells / Abs570 of untreated cells) x 100].....Eq2 [13]. Percentage cell viability and the IC50 value was calculated.

2.7 In-vitro cell uptake study

In-vitro cellular uptake of CUR-PLGA-NSs and CUR suspension in A549 human lung cancer cells was evaluated using a fluorescence microscope (XD-RFL Fluorescence Microscope, SDPTOP, China). Briefly, A549 cells were cultured in a 12-well plate at a density of 2 × 10^5 cells/mL and incubated at 37 °C in a CO_2 incubator for 24 hours. After incubation, the spent medium was carefully aspirated, and the cells were washed with 1 mL of 1X PBS. Next, the cells were treated with the required concentration of CUR-PLGA-NSs or CUR suspension in 1 mL of culture medium and incubated for 2 hr. Then the cells were washed twice with 1X PBS to remove any residual formulation and 500 µL of mounting medium was added to each well [31]. The cells were then observed under 20X objective of fluorescence microscope using filter cube with Excitation 470/40 and Emission 525/50 and the images were analyzed using ImageJ Software v1.48.

3.0 Results & Discussion:

3.1 Formulation of Cur-PLGA-NS

Homogenous PLGA based Curcumin loaded nanosuspension (Cur-PLGA-NS) were obtained using the bottom-up method with saturation solubility, employing mixture of acetone and methanol as the nonpolar solvent and polyvinyl alcohol (PVA) and Tween 80 solution as the polar solvent.

3.1.1 Particle size characterization:

Particle size and polydispersity index (PDI) of CUR-PLGA NS were measured using dynamic light scattering (DLS) with a Malvern Zetasizer. The mean particle size was found to be 143.1±12 nm, with a PDI of 0.167±0.17 (Fig. 1A). Zeta potential measurements indicated good stability of the nanoparticles, with value of -13.3 mV (Fig. 1B). Further analysis of particle size and morphology was carried out using transmission electron microscopy (TEM). TEM images (Fig. 1C) revealed that the nanoparticles were spherical, homogeneously sized, uniformly dispersed, and free from aggregation. The observed particle size was consistent with the DLS results.

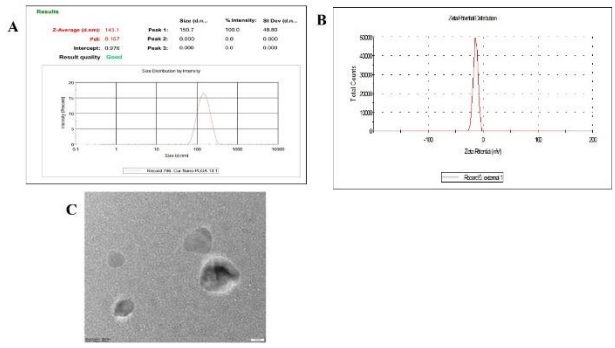

Fig. 1: Particle size characterization with size distribution (A), zeta potential (B) and TEM image (C) of CUR-PLGA NS.

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3.2 Optimization of Cur-PLGA-NS

Box-Behnken design was chosen for optimization of the NC formulation based on 3 independent variable {PLGA Conc. (A = X1), PVA conc. (B = X2), and the S:NS ratio (C = X3)} and total 17 run was observed (Table 2). After the observed values for the dependant variable size (Y1) and EE (Y2) was put on the design results were analysed using polynomial coded analysis with linear process order along with ANOVA as a model for significance. Different combinations of independent variables, such as AB, AC, and BC, can meet specific objectives (e.g., increasing or decreasing Y1—particle size, Y2— EE, or both) while accounting for various factors influencing NC formulations. The equations representing these relationships in terms of coded factors are provided in Equations (3) and (4).

Size =156 -11X1 + 13.87X2 -23.38X3 -10X1X2 -17X1X3 -6.25X2X3 +5.88X12 +9.13X22+ 16.13X32 .....Eq. (3)

EE = 80 -0.775X1 + 0.1125X2 + 0.1375X3+0.0250X1X2-0.0250X2X3+0.250X1X3-8.85X12+0.125X22+0.125X32 .....Eq. (4)

After analysis of the response 1 (Y1= Size) with polynomial analysis and linear process order model was found to be significant (p-value=0.0055 and F-value=8.24). Contour plot and polynomial equations showing that increase in concentration of PLGA and S: NS ratios resulted in decreased size whereas increase in PVA concentration results in increased values of Y1 (Size) as shown in Fig 2A. Analysis of response 2 (Y2 = EE) was also analysed using polynomial analysis and linear process order and the model was found to be significant after ANOVA (p-value<0.0001 and F-value=1035.38). The Contour plot and polynomial equations showed that increase in concentration of PLGA resulted in decreased size whereas increase in PVA concentration and S:NS ratio results in increased values of Y1 (Size) as shown in Fig 2B.

For optimization process, independent variable A, B and C was set in range value and dependent variable Y1 was set at minimum possible value and Y2 was set at maximum possible value and total 2 solutions were obtained. From all given solutions, solution 1 was selected as optimized formulation with 2% of PLGA Conc., 0.25% of PVA Conc. and 1:15 of S:NS ration as it was giving the desired values of Y1 (size) (143.75 nm) and Y2 (EE) 79.95 % with a desirability score of 0.895 (Fig. 2 (C)).

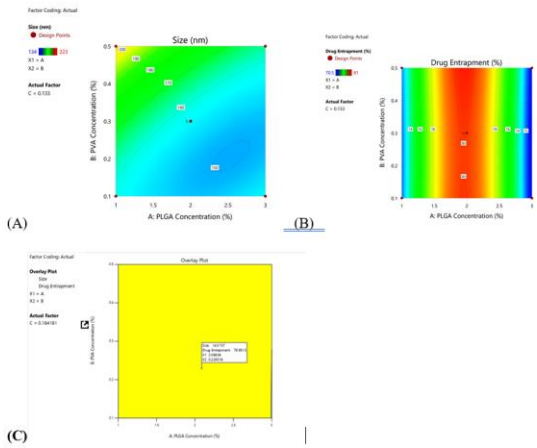

Fig. 2 (A) Effect independent variable on dependent variable Size (Y1), (B) Effect independent variable on dependent variable EE (Y2), (C) Optimization of response Y1 (Size) and Y2 (EE) along with desirability.

3.3 Analytical method development for HPLC analysis:

Various solvent systems in varying ratios as the mobile phase, flow rates, and chromatographic settings were used in the development and optimization of the HPLC process. Based on the research, the composition of the mobile phase was optimized as a mixture of acetonitrile and water that had been acidified to pH 3.0 using acetic acid in a ratio of 85:15 (v/v) while being maintained at a flow rate of 1 milliliter per minute. A quantitative study of curcumin was used to further validate the approach.

3.3.1 System suitability:

Retention time and peak area percentage RSD values for six replicate injections of the system suitability solution were within 2%, suggesting minimal variance in the measured values (Figure 3 and Table 3). Curcumin's tailing factors (T) were 1.192, indicating that all of the peaks were symmetric (T < 2). According to the number of theoretical plates, the column's effectiveness exceeded 1500. These findings support the applicability of the circumstances and HPLC equipment, which were subsequently used to sample analysis and additional

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validation. The accuracy, precision, linearity, LOD, LOQ, and system applicability of the optimized designed method were all confirmed.

Table 2: Formulation Table as suggested by Design Expert

Formulation	PLGA Concentration %	PVA Concentration %	S:NS ratio	Size nm	Entrapment Efficiency %
FF1	2	0.3	1:7.5	156.4	80.1
FF2	3	0.5	1:7.5	152.4	70.5
FF3	3	0.3	1:15	221.2	70.5
FF4	1	0.1	1:7.5	170.4	72.1
FF5	1	0.3	1:5	169.5	72.1
FF6	2	0.1	1:15	180.1	80.5
FF7	2	0.3	1:7.5	156.4	80.1
FF8	3	0.3	1:5	134.8	70.5
FF9	2	0.3	1:7.5	156.4	80.1
FF10	2	0.5	1:5	170.4	81
FF11	1	0.3	1:15	188.1	72
FF12	2	0.3	1:7.5	156.4	80.1
FF13	2	0.1	1:5	152.5	80
FF14	3	0.1	1:7.5	147.9	70.5
FF15	2	0.3	1:7.5	156.4	80.1
FF16	2	0.5	1:15	223.3	80
FF17	1	0.5	1:7.5	215.6	72

Table 3 System suitability data of the analytical HPLC method

Curcumin (100 µg/mL)	Retention time (min)	Peak area	Tailing factor	Theoretical plate
Mean	3.198	1606980.5	1.192	54,682
%RSD	0.11	1.007	1.01	1.002

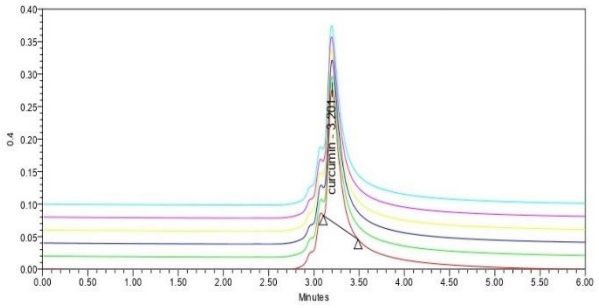

Fig 3: HPLC chromatogram of six replicate injections of standard curcumin

3.3.2 Linearity:

As shown in Figure 4, the calibration curves were linear across the 12.5-200 µg/mL concentration ranges that were examined. For the conventional curcumin, the regression equations were found to be Y = 1.80e+04 X - 9.82e+04. In the experimental concentration range, the normalized Intercept/Slope and correlation coefficient (r2) were determined to be -0.051263 and 0.998538, respectively, using this equation. High levels of correlation and good linearity of the approach were indicated by the correlation coefficient, which was greater than 0.999.

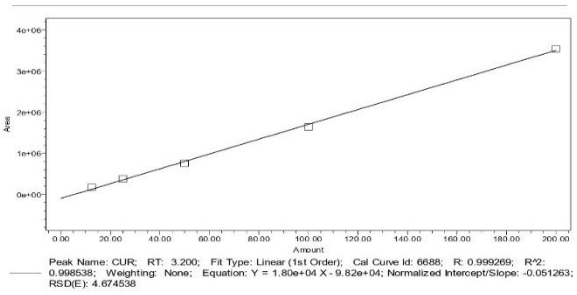

Fig 4: Linear regression graph of curcumin for the experimental concentrations

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3.3.3 Precision:

The percentage relative standard deviation (RSD) of the peak area of six replicate injections (n=6) of the curcumin standard at a concentration of 100 µg/mL on the same day at different time periods was calculated to determine repeatability, or intraday precision, and the result was 1.007 (Table 4). Table 5 shows the intermediate precision, also known as the inter-day precision, calculated using the percentage RSD of the experimental value at three newly generated concentrations (25, 50, and 100 µg/mL) on different days. Within the experimental concentrations, all three RSDs were determined to be far below the approved threshold of 2%. To make sure the results were reliable and robust, precision was measured three times. As a result, the method's precision was deemed satisfactory. The findings demonstrated that, within the concentration range, there is a strong correlation between the peak area and drug concentration.

Table 4 Repeatability/intraday precision of the HPLC method

Conc (µg/mL)	Area	Mean	STDEV	% RSD
100	1586224			
100	1597557			
100	1611426	1606980.5	16176.21	1.007
100	1634653			
100	1606820			
100	1605203			

Table 5: Inter-day precision

Theoretical concentration (µg/mL)	Inter-day precision			
	Experimental concentration (µg/mL)		%RSD	Average Recovery %
	1st day	2nd day		
25	25.2 ±0.021	25.3 ±0.019	0.3	99.8 ±1.41
50	50.7 ±0.049	50.4 ±0.038	0.4	101.2 ±0.98
100	100.4 ±0.151	100.3 ±0.098	0.1	100.8 ±0.73

3.3.6 Accuracy:

The average percentage recoveries for inter-day accuracy were found to be 99.8, 101.2 and 100.8 for the concentrations of 25, 50 and 100 µg/mL respectively as presented in Table 5. All percentage recoveries were within 98–102%, indicating the good accuracy of the method.

3.3.7 Limits of detection and quantitation:

The LOD and LOQ for curcumin were determined to be 0.154µg/mL and 0.475µg/mL, respectively. The high sensitivity of the proposed method is reflected by the signal to noise ratio of 3.19 and 10.67, for LOD and LOQ, respectively.

3.4 Drug Entrapment

The entrapment efficiency of prepared CUR-PLGA NS was found to be 81.26±4.1% indicating high and successful entrapment of CUR in the PLGA core. The high drug entrapment is likely due to its strong solubility in the lipophilic core. Encapsulation in nanocarriers improves the solubility and stability of poorly water-soluble drugs like CUR [32].

3.5 Drug Release:

The in vitro drug release for CUR-PLGA NS and CUR suspension were performed in phosphate buffer (pH 7.4) containing tween 80 (1% v/v) using a cellulose dialysis bag technique. The cumulative % drug release of CUR-PLGA NS was observed approximately 80.26±2.2% after 24 hr, whereas only 52.69±3.7% was observed for CUR suspension (Fig. 5). The high and sustained drug release of CUR-PLGA NS may be attributed to the stable incorporation of the drug within the PLGA core, while the polymeric shell likely restricted rapid release, indicating that the drug was firmly embedded and protected from quick diffusion into the dialysate [5, 33].

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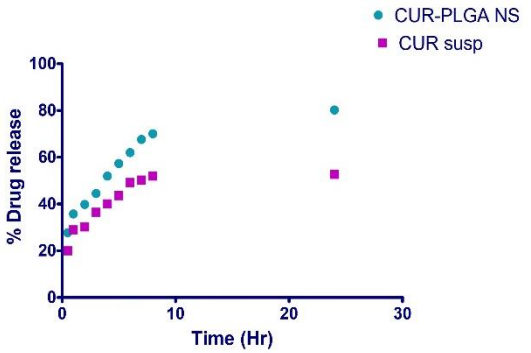

Fig. 5: In vitro drug release profile of CUR-PLGA NS and CUR suspension in PBS (pH 7.4)

3.6 In-vitro Cytotoxicity study inA549 cell lines:

The MTT assay was used to assess the effect of the produced CUR-PLGA-NS delivery systems on the viability of A549 human lung cancer cells. Using the MTT colorimetric assay, the cell viability of the CUR-PLGA-NSs and free CUR suspension was assessed in vitro against A549 lung cancer cell lines at 24 hr after treatment (Fig. 6). Compared to the free CUR solution, the CUR-PLGA NP treated groups showed a more pronounced cell inhibitory effect. The IC50 value for treatments using CUR-PLGA NP formulations was found to be lower (14.23±2.11 µg/ml) than that of CUR suspension (20.09±2.81 µg/ml). The difference in cell survival shown between the CUR suspension and CUR-PLGA-NS may be attributed to the enhanced cellular accumulation of the nanoparticles, as demonstrated by the in vitro cellular uptake results.

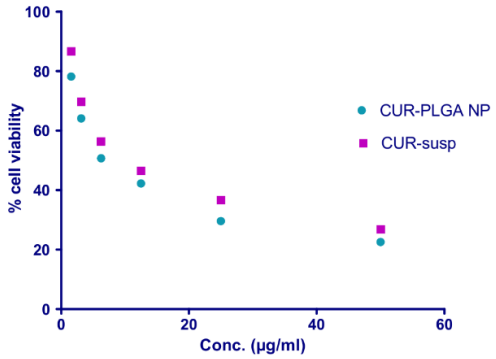

Fig. 6: Cell viability study of CUR-PLGA NP and CUR suspension on A549 cells after 24 hr of treatment

3.7 In vitro cell uptake studies

With the use of a fluorescent microscope (XD-RFL Fluorescence Microscope, SDPTOP, China), the in vitro cellular absorption of CUR-PLGA-NSs and CUR suspension against A549 human lung cancer cells was assessed. According to the data, cellular absorption increased throughout time (2, 4, and 6 hours), peaking at 6 hours for the two treatments (Fig. 7). In contrast to the curcumin suspension exhibiting the less absorption, the cellular uptake in CUR-PLGA-NS was somewhat higher. The improved permeability and retention in the tumour cells may be the cause of this higher cellular absorption. This could have led to a higher drug concentration and thus more cytotoxicity.

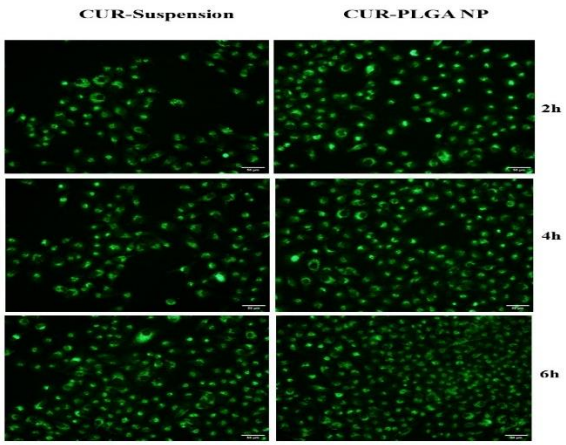

Fig. 7: Fluorescence microscope images showing uptake of CUR-PLGA NP and CUR suspension at 2, 4, and 6 hours of treatment in A549 cells

Conclusion:

Polymeric nanoparticles (PNPs), particularly those based on PLGA, offer significant advantages for the delivery of hydrophobic drugs like curcumin due to their stability, biocompatibility, biodegradability, and ability to enhance drug permeability and circulation time. In this study, curcumin loaded PLGA-nanoparticles were successfully developed by the application of Quality by Design (QbD) approach to enable systematic optimization and control over critical formulation and process parameters using the Box-Behnken design. The formulation demonstrated efficient drug entrapment, particle size optimization with high and sustained release of drug. The in vitro cytotoxicity and cellular uptake

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study showed promising results indicating its potential efficacy in lung cancer treatment.

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