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Formulation and characterisation of curcumin loaded PLGA-Tf nanoparticle for increasing the availability of drug in the brain for the management of parkinson's disease

Deepika VERMA*[1], Kumud UPADHYAYA [1]

[1] Department of Pharmacy Kumaun University, 263136, Nainital Bhimtal, Uttarakhand, India.

* Corresponding Author. E-mail: deepikadlverma@gmail.com (D.V.); Tel. +91-9459700077

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ABSTRACT: Curcumin, an extract derived from Curcuma longa, boasts a myriad of medicinal applications. In our current research endeavour, we embarked on the formulation of curcumin nanoparticles via the meticulous micro emulsion precursor method, employing the Box-Behnken 32-level design approach. This involved the manipulation of three independent variables, namely, PLGA-Tf-curcumin concentration, stirring speed, and the concentration of the emulsifying agent (span 80). Our investigation revealed that all three independent variables wielded discernible influence over two crucial dependent variables: encapsulation efficiency (EE) and nanoparticle size. It was against this backdrop that we meticulously prepared a total of seventeen formulations. Among this array, formulation F3 emerged as the best to its remarkable EE (99.7±0.2) and a particle size of 214.7 nm. Delving further into our analysis, we scrutinized additional parameters, including drug content (99.7%) and cumulative percentage release (exceeding 99% within a span of 36 hours), both of which yielded highly favourable results. To elucidate the release kinetics, we harnessed the Zero Order, Higuchi, and Korsmeyer-Peppas kinetic models, each revealing an R-squared (R2) value remarkably close to unity. This signifies an exceptionally controlled and diffusion-driven drug release pattern, manifesting in a spherical manner. In this comprehensive assessment, we also scrutinized various other facets, including λmax (wavelength of maximum absorption), particle size distribution, X-ray diffraction, and FTIR analysis. Collectively, these analytical results reinforced the robust authenticity of our study.

KEYWORDS: Box-Behnken 32-level design; PLGA-Tf-curcumin concentration; encapsulation efficiency; Higuchi model; X-ray diffraction.

1. INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder that primarily affects movement control. It is characterized by symptoms such as tremors, rigidity, bradykinesia (slowness of movement), and postural instability. These arise due to the loss of dopamine-producing cells in the brain, particularly in the substantia nigra region [1-3]. Curcumin is a natural compound found in the turmeric, has been showing potential as a therapeutic agent in Parkinson's disease due to its anti-inflammatory and antioxidant properties [4,5]. The bioavailability challenge with curcumin restricts its effectiveness as it reaches the brain at significantly low concentrations, hampering its therapeutic impact [6, 7]. Nanotechnology offers a promising approach to enhancing the bioavailability of drugs like curcumin by encapsulating them in nanoparticles [8]. These nanoparticles can improve solubility, stability, and targeted delivery, ensuring better absorption and distribution in the body. This advancement holds potential for increasing the effectiveness of curcumin as a treatment for various conditions, including Parkinson's disease. The PLGA-Tf -(Transferrin) loaded curcumin nanoparticles can be used for targeting the brain for specific action. The main objective of this study is to formulate the targeted nanoparticle which target the specific region of the brain and also increase the bioavailability of poorly bio available drugs in the brain, especially in the substantial nigra region, which makes the drug more effective [9].

2. RESULT AND DISCUSSION

2.1. Organoleptic properties

The appearance of curcumin powder was bright yellow to orange-yellow colour. Taste was slightly bitter and earthy, and it has a mild, aromatic Odor that is often described as earthy, woody, and slightly spicy.

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2.2. Melting Point of extract

The average melting point of curcumin extract found to be 178.5±0.55°C, is within the reported range of the melting point of curcuminoids, which is 177-183°C. Therefore, the obtained result is consistent with the literature, and it validates the authenticity of the curcumin extract.

2.3. Partition coefficient of drug curcumin

The partition coefficients for curcumin were found range from approximately 1.5±0.2 in octanol/water systems at room temperature (around 25°C). The values suggest that curcumin tends to slightly favour the organic (octanol) phase but is not highly hydrophobic.

2.4. Solubility

Curcumin solubility in water (10µg/ml) was found less as compared to other organic solvent like methanol (87µg/ml) and ethanol (71µg/ml). However, in Phosphate buffer pH 6.4 and 6.8 the solubility was found 22 µg/ml and 28µg/ml respectively, which exhibits that the curcumin is hydrophobic nature and shows the more solubility in organic solvent as compared to hydrophilic solvent.

2.5. ʎmax of the *Curcumin*

The Figure.1 ʎmax value of curcumin was typically found in the range of 425 nm. This result indicates that curcumin contains significant amounts of curcuminoids. The value of the ʎmax was obtained as given in different literature, reflects the authenticity of powder as a curcumin. The equation y=0.0498x+0.0458 and R^2 value 0.9994 indicates the linearity of calibration curve.

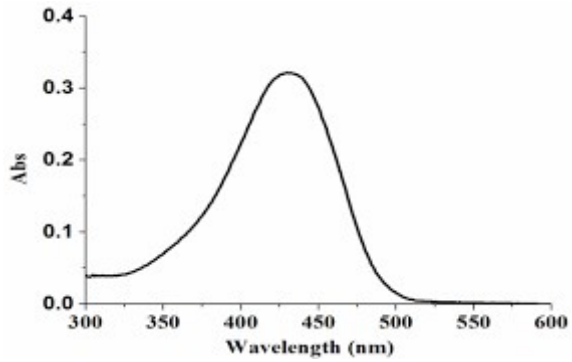

Figure 1. ʎmax of curcumin drug

2.6. Fourier Transformed Infrared Spectroscopy (FTIR)

From Figure 2 and Table 1, it was observed that all the characteristic peaks present in the curcumin FTIR spectra matched with reported values. It suggests that there is no strong evidence of chemical interaction or incompatibility between curcumin and PLGA-Tf in the nanoparticles.

Table 1. Functional group of curcumin

Functional group	Reported value	Observed peak value
O-H Stretching	3200-3600 cm^{-1}	3379 cm^{-1}
C-H Stretching (Alkanes)	2800-3000 cm^{-1}	3072 cm^{-1}
C=C Stretching (Alkenes)	1600-1680 cm^{-1}	1628 cm^{-1}
C=O Stretching (Carbonyl)	1600-1750 cm^{-1}	1603 cm^{-1}
C-O Stretching (Ethers and Esters)	1000-1300 cm^{-1}	1027 cm^{-1}
C-N Stretching (Amines and Amides)	9000-1350 cm^{-1}	976 cm^{-1}
O-H Bending (Alcohols and Phenols)	1350-1470 cm^{-1}	1327 cm^{-1}
C-Cl Stretching	700-850 cm^{-1}	809 cm^{-1}

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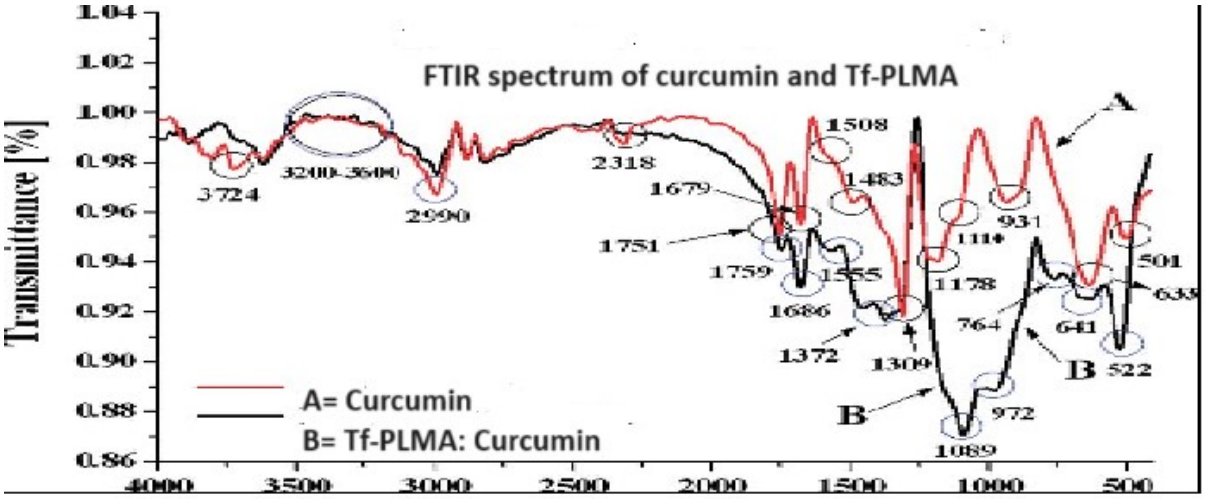

Figure. 2 FTIR spectra of PLGA- Tf- curcumin and pure curcumin drug

2.7. Optimization of the Formulation Process

The discussion on the characteristics of microemulsion using the 3^2 Box-Behnken Design was conducted during the formulation stage, where a total of 17 formulations were prepared. Each formulation was evaluated based on its dependent variables, which included Mean particle size (nm) and Entrapment efficiency (EE). These variables were studied at low and high levels of the three independent variables: PLGA-Tf-Curcumin, Stirring speed, and emulsifying concentration. To analyse the relationship between the independent variables and the observed responses, a response surface analysis was performed. Mathematical relationships were established, and a second-order polynomial equation (Eq. 1) was generated for each response variable. It was found that the equations for PLGA-Tf Conc.: Curcumin, stirring speed, and emulsifying concentration were quadratic in nature and included interaction terms. The coefficients of the polynomials fitted well to the experimental data, with the values of R^2 ranging between 0.9325 and 0.9968. This indicates a strong correlation between the independent variables and the observed responses. Additionally, the p-values were found to be less than 0.05 in all cases, indicating statistical significance.

(Eq.1) $Yi = b0 + b1\,X1 + b2\,X2 + b3\,X3 + b12\,X1\,X2 + b13\,X1\,X3 + b23\,X2\,X3 + b11\,X^2 + b22\,X2^2 + b33\,X3^2$

2.7.1. PLGA- Tf- Curcumin analysis

In the analysis of the response variable PLGA- Tf- Curcumin Figure 3 (a-b) presents the 3D-response surfaces and contour plots that provide insights into the relationship between the ratio of PLGA- Tf- Curcumin concentration of different components and the particle size. Figure 3 (a) illustrates a dome-shaped response surface and contour plot. This indicates that initially, there is an increase in the particle size with an increase in the ratio concentration of both PLGA Tf- Curcumin**.** However, after reaching a certain point, there is a gradual decrease particle size. This suggests that at intermediate levels of PLGA-Tf- Curcumin concentrations, the particle size was found to be maximum. In other words, there is an optimal range of PLGA -Tf- Curcumin that result in the most efficient particle size of nano particles.

Moving on to Figure 3 (b), it also displays a 3D response surface graph. Similarly, it shows an initial increase in the particle size with an increase in the concentrations of polymer- ligand and drug ratio (PLGA - Tf and curcumin). However, in this case, there is a sudden increase in the particle size after a certain point. Thus, at higher levels of polymer- ligand concentrations, the particle size was found to be maximum. This suggests the presence of an optimal range of polymer-ligand and drug concentration, which lead to the most efficient particle size. The polynomial Eq.2 also represents the statistical view over polymer- ligand concentrations, the particle size.

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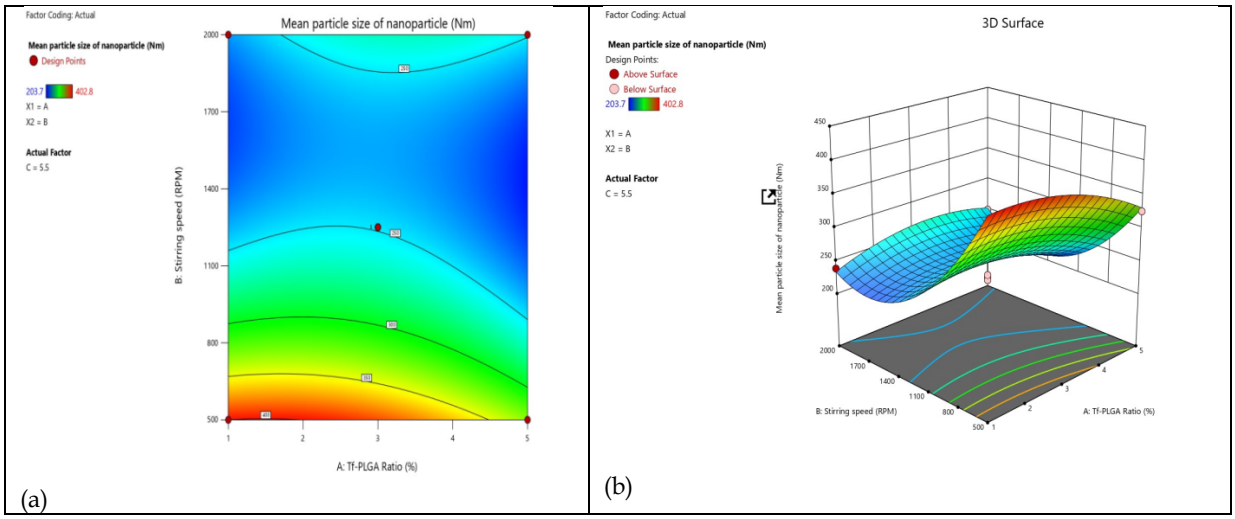

Figure 3. (a) Contour plots **(b):** 3D-response surfaces graph for particle size analysis

(Eq.2) Particle size (PS)=+70.60-1.35*A+7.99*B+3.75*C+7.42*A*B+10.76*A*C+8.31*B*C-18.51*A2-7.47*B2-18.44*C2

Equation in Terms of Actual Factors

Particle size (PS) =+70.60000, -1.35000*Amount of Tf-PLGA: curcumin, +7.99000*Amount of stirring speed, +3.75000 *Amount emulsifying agent (span 80), +7.42000*Tf-PLGA: curcumin*stirring speed, +10.76000 *Amount of stirring speed*emulsifying agent, +8.31000 *Amount ofTf-PLGA: curcumin *Amount emulsifying agent, -18.51500, *Tf-PLGA: curcumin ^{2}, -7.47500 *stirring speed^2 -18.44500 *amount of emulsifying agent^2.

Based on the polynomial equation derived from the analysis, it has been observed that the amount of PLGA-Tf- curcumin ratio and has a positive effect on particle size of nanoparticle i.e they have been contributing to increase the particle size. On the other hand, stirring speed has given the negative impact i.e. increasing the stirring speed reducing the particle size. This means that increasing the concentrations ratio of PLGA-Tf- curcumin leads to an increase in particle size.

2.7.2. Percent drug entrapment efficiency

Figure 4 (a-b) presents the 3D-response surfaces and contour plots and 3D response graph for the response variable "Percent drug entrapment A, the relationship between PLGA-Tf- curcumin ratio on drug entrapment efficiency is depicted. It was observed that at low concentrations of PLGA Tf-curcumin ratio, the percentage drug entrapment efficiency is lowest. This suggests that the combination of high PLGA-Tf--curcumin ratio concentration and a low stirring speed promotes a higher drug entrapment efficiency of drug. The improved entrapment of drug is often associated with the emulsifying ability of drug as well. The polynomial Equation 6 correlates all the factors which have involved in EE

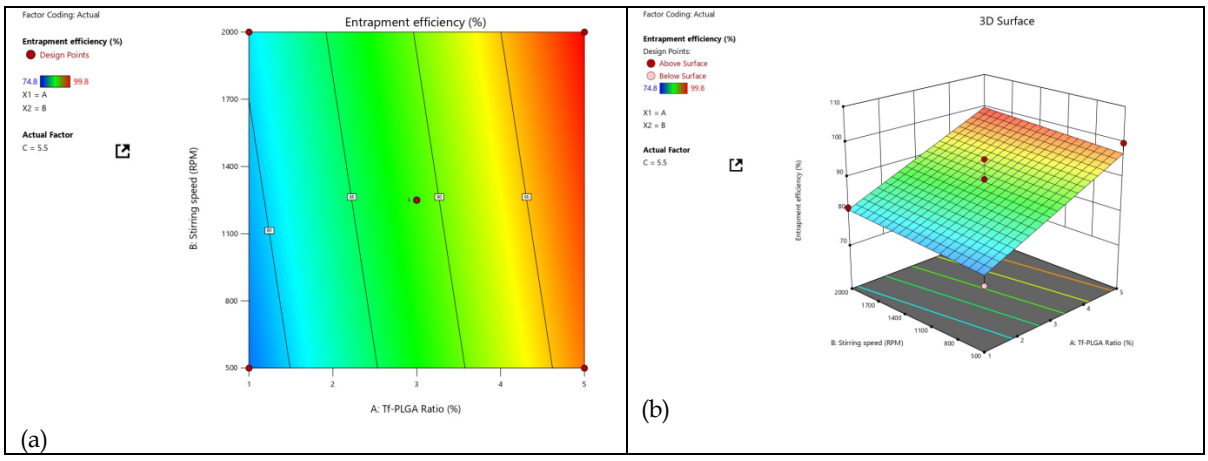

Figure 4. (a-b) (a) Contour plots **(b):** 3D-response surfaces graph for drug entrapment efficiency

Final equation (Eq.3) in Terms of Coded Factors:

Drug release after 30 min (Eq.3) = +96.43-2.40 * A+1.45 * B-2.52 * C-2.01* A * B -3.76 * A*C+4.28 * B* C-4.63 * A2+0.89*B2-3.34 * C2

Final Equation in Terms of Actual Factors: drug entrapment = +96.43000, -2.40500 * stirring speed, +1.45500 * Tf-PLGA: curcumin ratio, -2.52000 * stirring speed, -2.01000 * Tf-PLGA: curcumin ratio * stirring speed, -3.76000 * Tf-PLGA: curcumin ratio * emulsifying agent, +4.28000 * emulsifying agent * stirring speed, -4.63000 * Tf-PLGA: curcumin ratio^2, +0.89000 * emulsifying agent ^{2}, -3.34000 * stirring speed^2

The polynomial equation derived from the study indicates that the amount of stirring speed has negative effects on drug entrapment, while the PLGA-Tf-curcumin ratio has a positive effect. Based on this conclusion, optimum concentration of PLGA-Tf-curcumin ratio and stirring speed has been selected for further investigation. Span 80 is commonly used as a surfactant emulsifying agent in the formulation. Its lipophilic nature enables it to solubilize in the oil phase of the micro emulsion, forming a monolayer at the oil-water interface. This monolayer reduces the interfacial tension between oil and water, facilitating the formation and stability of nanoparticles.

2.7.3. Formula Optimization

The selection of an optimized formulation using a numerical optimization method called Design-Expert. The selected formulation is a nanoparticle drug delivery system (NPDDS). The desirability value for this formulation is 0.95, indicating a high level of desirability. The composition of the optimized nanoparticle formulation includes the following components and their respective quantities. PLGA-Tf- Concentration: Curcumin 5% Span 80:10% Stirring speed 1250 rpm Additionally, the values of the dependent variables obtained for this optimized formulation are as follows: Particle size: - 214.8 nm, drug entrapment efficiency: 99.7. These results indicate that the optimized nanoparticle formulation exhibits an optimised particle size and high percentage of drug entrapment efficiency which is favourable for drug delivery purposes.

2.8. Characterization of curcumin Nps

2.8.1. Particle Size Analysis by Dynamic Light Scattering (DLS)

The particle size of nanoparticle was determined by DLS method**.** Notably, the analysis revealed an optimum particle size of 214.5 nm. This specific measurement is particularly significant because it represents the most prevalent particle size in the sample.

2.8.2. SEM Studies

In the Figure 5 SEM images clearly illustrate the morphology of the nanoparticles, revealing that they exhibit a spherical shape. The SEM analysis accurately measured the size of the nanoparticles, demonstrating that they fall within the nanoscale range, specifically ranging from 100 to 400 nanometres (nm).

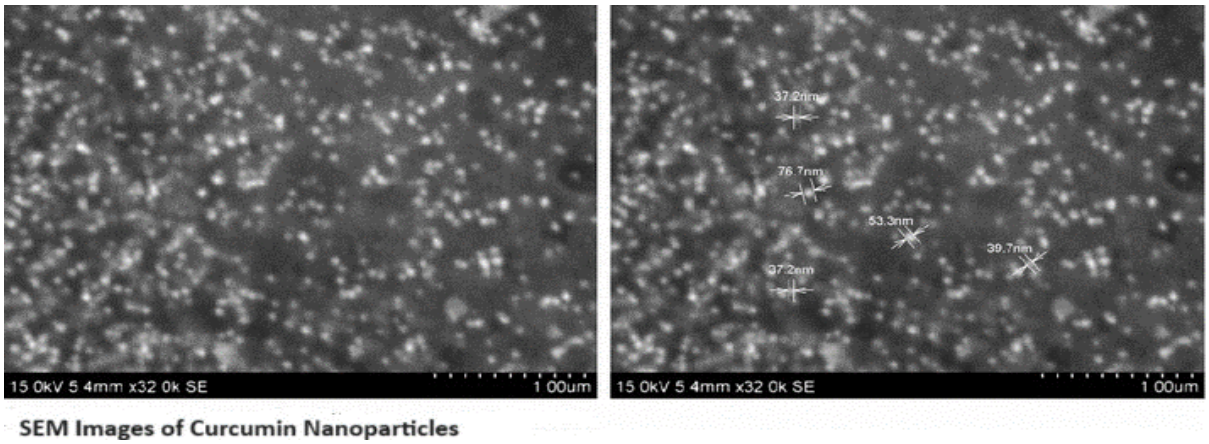

Figure 5. SEM images of PLGA- Tf- curcumin nanoparticles

2.8.3. Zeta Potential of nanoparticles

The Zeta potential of nanoparticles plays a crucial role in their stability. Typically, a Zeta potential with an absolute value (|Zeta potential|) greater than ±30 mV is considered favourable for ensuring electrostatic stabilization. The zeta potential of curcumin loaded nanoparticles has +32.5, as shown in Figure.6 which is indicating the good stability of nanoparticles.

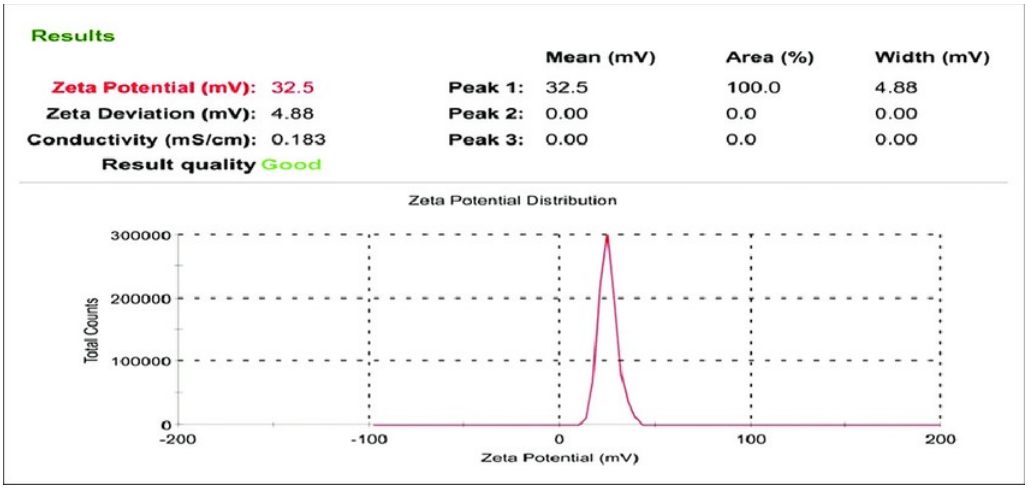

Figure 6. Zeta potential of curcumin loaded nanoparticles

2.8.4. X-ray Diffraction (XRD)

XRD study of curcumin loaded PLGA-Tf nanoparticles shown the less peaks as its pure drug curcumin. This indicating the internal phase of drug is transforming from crystalline to amorphous which depicts the enhancement of aqueous solubility of drug. Therefore, the information is valuable for understanding the physical properties of the nanoparticles and their suitability for various applications. The XRD is shown in Figure 7.

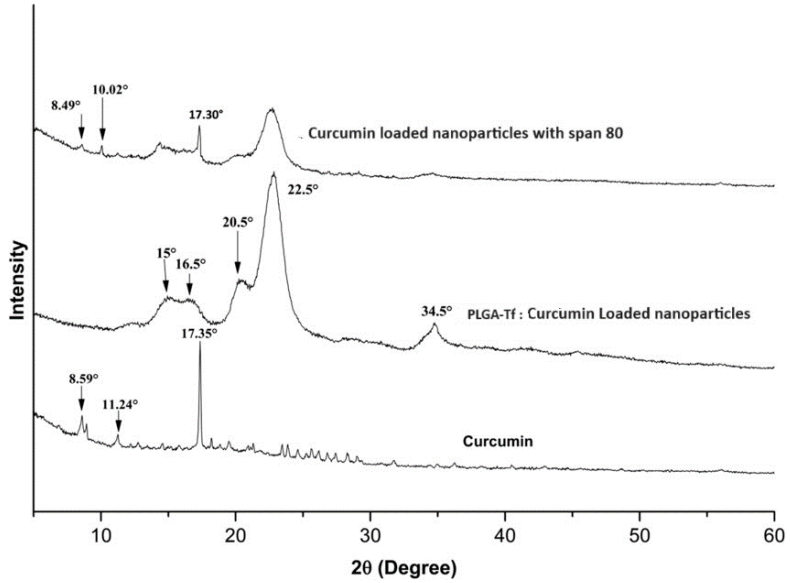

Figure 7. XRD of curcumin loaded nanoparticles

2.8.5. Drug Content

Amount of drug content was obtained as F3, F10 and F15 as these all formulation has shown he best results among all seventeen formulations. When drug content was analysed, it has obtained 99.7% in F3, 97.8% in F10, whereas 98.2 in F15. However, all these formations containing the best drugs content but still F3 was found to be the best.

2.8.6. Drug Loading and Encapsulation Efficiency (EE)

The EE was determined by all the formulations. It was noted that all formulations have a range of EE that is from74.8 percent (F4) to 99.7 percent (F3). Therefore, formulation F3 (run 3) showed the best EE as compared to other formulations. The reason was that all the formulation parameters, like PLMA-Tf-curcumin ration (5%), stirring speed (1250 rpm), and emulsifying concentration (10%) were taken as their optimized form, so given the best results.

2.8.7. In-vitro Release of drug

As per finding of all formulations it seems that most of the formulations failing in holding the adequate amount of drug content in nanoparticles and the entrapment efficiency were found also low. But three formulations viz., F3, F10 and F15 having the good drug content and entrapment efficiency which as 99.7, 98.7 and 99.2 respectively. Therefore, all these formulations have been considered for the release study of the drug. The data were obtained are shown in Table 2 and Figure 8. Table 2 depicts a study involving drug release from different formulations (F3, F10, and F15) and highlights significant differences in the release patterns of these formulations. All three formulations (F3, F10, and F15) exhibit drug release for more than 12 hours as shown in Figure 8. However, after six hours, F3 releases 33.9± 0.4 units of the drug, whereas F10 and F15 release 39.5 ± 0.3 and 43.6 ± 0.8 units, respectively. This suggests that F3 provides a more controlled and sustained release compared to F10 and F15. After 12 hours, F3 releases 51.8± 0.5 units, while F10 and F15 release 67.7± 0.5 and 81.7± 0.6units, respectively. Again, F3 exhibits a significantly slower release compared to the other two formulations. The same trend continues after 24 and 36 hours. F3 shows significant changes, releasing 81.9± 0.1 units at 24 hours and 99.2 ± 0.4 units at 36 hours. F10 and F15 do not exhibit significant differences in drug release after 24 and 36 hours. As shown that F3 consistently releases fewer drugs than F10 and F15, indicating a more controlled and sustained release pattern. At 36 hours, F3 releases 99.2 ± 0.4 units, while F10 and F15 release 93.6± 0.6± 0.6 and 95.5 ± 0.4 units, respectively. The passage attributes the differences in drug release to two main factors: the concentration of Span 80 and the stirring speed during the formulation process. F3 contains a higher concentration of Span 80 (10%) compared to F10 and F15 (5%). Span 80 is a non-ionic surfactant known to delay drug release, which explains the more controlled release in F3. Stirring speed also plays a crucial role. F3 is stirred at 1250 rpm, which is considered optimum for the process, whereas F10 and F15 are stirred at 2000 and 500 rpm, respectively. Stirring speed affects the coating process, influencing drug release patterns.

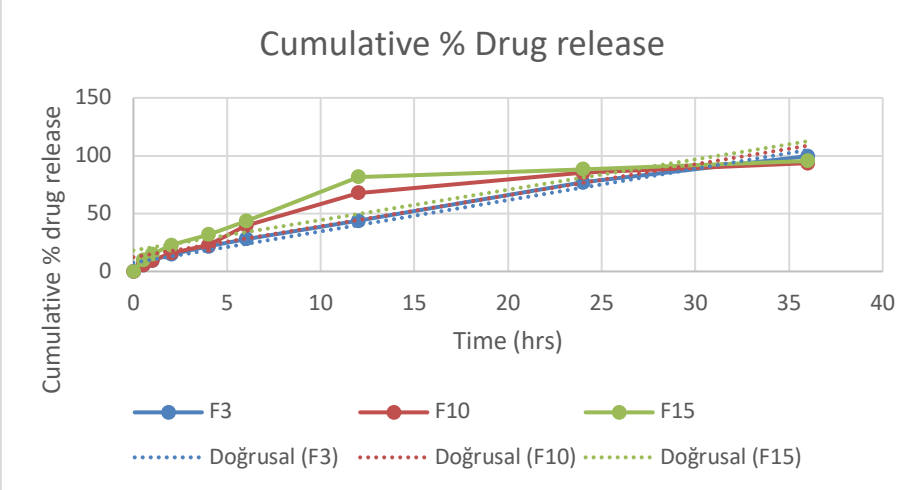

Figure 8. Cumulative % drug release

Table 2. Cumulative percent drug release

Time in (hrs)	F3	F10	F15
0	0	0	0
0.5	5.22± 0.5	5.35± 0.4	9.6± 0.2
1	9.52± 0.4	9.54± 0.6	14.7± 0.4
2	15.7± 0.2	15.8± 0.3	22.8± 0.6
4	23.5± 0.6	22.8± 0.4	31.8± 0.8
6	33.9± 0.4	39.5 ± 0.3	43.6± 0.8
12	51.8± 0.5	67.7± 0.5	81.7± 0.6
24	81.9± 0.1	85.4± 0.9	88.2± 0.4
36	99.2± 0.4	93.6± 0.6	95.5± 0.4

First order release kinetics exhibits % log drug remaining versus time in hours. It depicts formulation F3, F10 and F15 releases the drug in nonlinear manner. Higuchi model express the % release of drug with respect to root time. The model expresses it significant by linearity of graph and R^2 value. The graph plotted between cumulative % drug release and square root of time. The R^2 has given which represents the linearity of graph. The release of drug represents by plotting a graph between log % cumulative drug release log time. It suggests the release of drug in controlled and sustained manner.

2.8.8. R^2 Value of All Kinetics Model

In the context of drug release kinetics modelling, R^2 (R-squared) is a statistical measure used to assess how well the observed data points fit a particular mathematical model. The value of R^2 ranges from 0 to 1, with higher values indicating a better fit between the model and the observed data. The value of R^2 is shown in Table 3. In zero-order kinetics; R^2 measures how well the experimental data fit a zero-order release model, where drug release is constant over time. A high R^2 value 0.9927(close to 1) indicates that the zero-order model provides a good fit to the data, suggesting that drug release is independent of time. In the Higuchi model, R^2 evaluates how well the square root of time fits the data. It assesses the drug release is consistent with diffusion through a matrix. A high R^2 0.9906 values suggest a good fit to the Higuchi model, indicating that drug release follows a diffusion-based mechanism. Therefore, on the basis of above findings the F3 has shown the best release of drug (99.2± 0.4) in 36 hours in controlled and sustained manner. The R^2 also suggested that formulation F3 is best formulation and follow the zero order and Higuchi model for release of drug which is best suited for a controlled and sustained release dosage form.

Table 3. R^2 value of different kinetic model

Formulation	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmayer -Peppas (R^2)
F3	0.9927	0.9057	0.9906	0.9266
F10	0.8807	0.8643	0.9692	0.9814
F15	0.817	0.9647	0.9464	0.9765

2.8.9. Stability Studies

Stability study was performed as per ICH guidelines. All the parameters were evaluated like organoleptic properties, drug content, entrapment efficiency and kinetics release. It was observed that there was no significant difference was noted in the above-mentioned parameters. So, it can say that formulation F3 was found to be stable.

3. CONCLUSION

Curcumin, an extract derived from Curcuma longa, possesses a wide array of medicinal properties, including anti-inflammatory, antioxidant, and antiseptic effects. It has also exhibited potential as a therapeutic agent for Parkinson's disease. However, its limited bioavailability in the brain hampers its effectiveness. To overcome this challenge, we formulated Tf-PLGA-curcumin nanoparticles designed for brain targeting and enhanced drug bioavailability. The formulation development utilized a Box Behnken 3^2-level design, and nanoparticles were prepared using the microemulsion precursor technique. Comprehensive evaluations encompassed drug characterization, particle size, particle size distribution, drug content, encapsulation efficiency (EE), and drug release kinetics. The results yielded satisfactory outcomes. Particularly noteworthy is the drug release profile, a critical facet of our research. Notably, formulation F3 demonstrated an impressive nearly 99 percent drug release within a span of 36 hours, indicative of controlled and prolonged action. These release kinetics findings align well with our in vivo study. Furthermore, there exists promising potential for conducting animal studies to substantiate and solidify our research outcomes.

4. MATERIALS AND METHOD

Pure curcumin was procured from the open market, other chemicals like PLGA,transferrin, and Span 80 were gifted from Varav biogenesis Ltd. Kala Amb, H.P., India. The rest other chemicals and reagents were laboratory grade.

4.1. Preformulation studies of drug

4.1.1. Organoleptic properties

Curcumin is a natural compound found in turmeric, and it possesses various organoleptic properties, which are characteristics that can be perceived by our senses.

4.1.2. Melting Point determination

The temperature at which a solid and a liquid are in equilibrium at one atmosphere of total pressure is known as the normal melting point of a solid. By placing a tiny quantity of curcumin into a tiny capillary tube, affixing it to the stem of a thermometer positioned in the centre of a heating bath, progressively heating the water, and monitoring the temperatures at which melting starts and ends, the melting point of curcumin was discovered [10].

4.1.3. Solubility studies

The shake flask method was used to gauge curcumin solubility in a variety of aqueous and non-aqueous solvents. 2 mL of each container were filled with extra curcumin, and the mixes were then put inside glass vials and sealed. To promote initial mixing, each sample was treated to 5 minutes of vortex mixing using a vortexer (GeNei, Bangalore, India). Each vial was spun using a centrifuge at 10,000 rpm for 10 min after achieving equilibrium for an additional 72 h at 25 °C (Remi Laboratory Instruments, Mumbai, India). Each sample supernatant was filtered through a membrane filter (0.45 mm) to get rid of any undissolved drugs that could have been present. After their dilution with methanol solvent, all samples drug content was calculated using UV Visible spectrophotometer at 425 nm. The experiment was performed three times, and the means of each were recorded [11].

4.1.4. Partition coefficient of drug

Weighed 10 mg of curcumin powder accurately and dissolved in 50 ml water. Placed the curcumin sample in a glass vial and added a 50 ml volume of n-octanol to the vial. Capped the vial and shaken the mixture vigorously for 30 minutes to allow the mixture for equilibration set up between curcumin and octanol solvent. After the equilibration period, allow the mixture to stand until phase separation occurs. n-octanol and water separated into distinct layers due to their immiscibility. Once both the phases shown the distinct layer separated from each other and analysed the concentration of curcumin in the sample phase by UV-Visible spectrophotometer at 425 nm wavelength using Eq.4

$$(\text{Eq. 4})\text{Partition coefficient (P)} = \frac{\text{Concentration of curcumin in organic phase}}{\text{Concentration of curcumin in aqueous phase}}$$

The partition coefficient (P) represents how readily the drug partitions between the octanol and water phases. A higher P value indicates a greater affinity for the organic phase (octanol), while a lower P value indicates a greater affinity for the aqueous phase (water) [12].

4.1.5. λmax of the Curcumin

The λmax measured using a UV-Vis spectrophotometer by preparing a 5µg/ml dilute solution of the extract in a methanol solvent and scanned the solution over a range of 400-800 nm wavelengths. The λmax was determined from the resulting spectrum by identifying the wavelength at which the absorbance is highest.

4.1.6. Calibration Curve of curcumin

First of all, series of dilutions of the curcumin drug was prepared the in methanol solvent. The concentration range was kept form 2-20 µg/ml by using serial dilutions. Than measured the absorbance of each dilution at the 425 nm λmax. A graph was plotted between the absorbance values against the corresponding concentrations of the dilutions. Than fitted a straight line to the data points using linear regression. The equation of the line was formulated in the form y = MX + C, where y is the absorbance, x is the concentration, M is the slope, and C is the y-intercept. The correlation coefficient (R^2) of the calibration curve was calculated to assess the linearity of the data [13].

4.1.7. Fourier Transformed Infrared Spectroscopy (FTIR)

The curcumin was scanned in the range from 4000 to 400 cm–1. The Fourier Transformed Infrared Spectroscopy (FTIR) (IR Affinity-1, Shimadzu, Japan) technique was used to analyse the functional groups and chemical bonds present in a *curcumin* [14].

4.2. Method of preparation of Curcumin loaded poly lactic-co-glycolic acid (PLGA)- transferrin (Tf) Nanoparticles

*4.2.1. Loading of Curcumin onto PLGA- transferrin (Tf) Nanoparticles***.**

The main components of the solvent phases include 100% ethanol as the organic solvent, ethyl oleate as the continuous phase, Tween 80 as the emulsifying agent, and PEG 400 as the stabiliser. After dissolving the curcumin in 15 mL of an ethanol solution containing surfactant and a 5% co-surfactant concentration, 5 mL of oil was added. The mixture was then agitated continuously for 20 min at room temperature at 15000 rpm. Once the mixture had been agitated for 20 minutes, 1 mL of PLGA-transferrin (Tf) solution (1% w/v) was added drop by drop. Centrifugation was used to separate the curcumin-loaded nanoparticles, and samples were then cleaned multiple times with ethanol to remove any extra curcumin that had stuck to the surface of the nanoparticles [15].

4.2.2. Formulation and Optimization of Microemulsion system (MES)

Using Design of Experiments (DoE) version 13.0.15, particularly the Box-Behnken design with three factors and two levels, is a sound approach for optimizing the formulation process of curcumin-loaded Tf-conjugated PLGA nanoparticles. Three square complete factorial designs were chosen for optimisation based on the results of the pre-optimisation investigations. Particle size and encapsulation effectiveness were shown to be significantly influenced by the phase ratio and sonication period. Based on the information from the pre-optimization, two levels were selected for the design matrix, which was fixed. Particle size and encapsulation effectiveness are crucial for the formulation's performance; hence they were picked as the replies. In Table 4, the three-square design matrix and the factors with their levels are displayed. Particle size and entrapment effectiveness responses were statistically assessed and are displayed in Table 5 [16].

Table 4. Ranges of the Factors Investigated Using Box–Behnken Experimental Design for Micro emulsion of drug *curcumin*

Independent Factors	**Range**	
Independent variables (factors)	Low (-1)	High (+1)
X1 = Tf -PLGA conc: curcumin (100 mg) (%)	5	20
X2 = Stirring speed (rpm)	1	10
X3 = Emulsifying conc. (%) (%)	5	10

Table 5. Formulation and Optimization of Microemulsion system (MES)

Run	Tf-PLGA Conc.: Curcumin (100mg) (%)	Stirring speed (rpm)	Emulsifying conc. (%)	Mean particle size (nm)	Entrapment efficiency
1	3	500	1	402.5	85.4
2	5	1250	1	238.5	97.5
3	5	1250	10	214.8	99.7
4	1	500	5.5	402.8	74.8
5	3	1250	5.5	298.7	89.5
6	3	500	10	395.4	84.7
7	3	1250	5.5	269.8	95.2
8	1	1250	10	203.7	79.5
9	3	1250	5.5	228.7	88.7
10	5	2000	5.5	248.9	98.7
11	3	1250	5.5	220.8	85.8
12	3	1250	5.5	225.3	89.5
13	3	2000	1	318.9	84.9
14	1	2000	5.5	238.8	81.2
15	5	500	5.5	325.7	99.2
16	1	1250	1	285.2	82.5
17	3	2000	10	228.7	91.7

4.3. Characterization of curcumin Nps

4.3.1 Particle Size Analysis by Dynamic Light Scattering (DLS)

The DLS technique is used to provide the information about the average particle size and size distribution of the sample. The colloidal suspension of curcumin nanoparticles was prepared in hexane solvent and placed it in a suitable cuvette. The instrument emitted a laser beam into the sample, and as nanoparticles in the suspension moved due to Brownian motion, they scattered light. The scattered light was collected at the specified scattering angle, and the auto correlation function of intensity fluctuations was calculated [17].

4.3.2. Scanning Electron Microscopy (SEM)

SEM provides high-resolution images of nanoparticles, allowing visualization of their morphology and size. The SEM images of nano particles was determined by preparing a sample of curcumin nanoparticles and placed for analysis [18].

4.3.3. Zeta Potential

Zeta potential measures the surface charge of nanoparticles. It is crucial for assessing their stability, as nanoparticles with high absolute zeta potentials are less likely to aggregate. The zeta potential determined as first, prepared a colloidal suspension of curcumin nanoparticles in a suitable solvent like n-hexane and ensured that the sample was well-dispersed and free of aggregates [19].

4.3.4. X-ray Diffraction (XRD)

X-ray Diffraction is used to determine the crystalline structure of materials, including nanoparticles. First of all, prepared a small amount of curcumin nanoparticle sample and chosen the appropriate X-ray wavelength and configuration. Placed the sample onto a sample holder, ensuring it formed a thin, even layer. Inserted the sample holder into the XRD instrument and initiated the measurement. X-rays were directed onto the sample, and the scattered X-rays were detected and recorded as a function of the scattering angel. Identification the presence of crystalline peaks and determine the crystalline phases present in the sample [20].

4.3.5. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy allows identifying chemical bonds and functional groups in curcumin nanoparticles, helping confirm the presence of curcumin and study any chemical modifications that may occur during nanoparticle formulation. First of all, configured the FTIR and made it ready for analysis by

setting of appropriate parameters. Now, placed a small amount of curcumin nanoparticle sample on an attenuated total reflection (ATR) crystal and recorded a baseline spectrum of the solvent to subtract it from the sample spectrum. Placed the sample holder into the FTIR spectrometer and initiated the measurement. The instrument collected an infrared spectrum by measuring the transmittance of infrared light at various wave numbers [21].

4.3.6. Drug content

The analyze of the curcumin loaded NPs was done as prepared a solution in a methanol (Model No. 1700, Shimadzu Corporation, Tokyo, Japan). Set the wavelength range from 400 -800 nm and scanned the speed. Further, transferred a small volume of curcumin nanoparticle solution into a UV-Visible cuvette and record the absorbance spectrum. The drug content was determined as per given Eq. 5

$$(Eq.5)\text{Curcumin content}\left(\frac{\text{mg}}{\text{g}}\right)=\frac{(\text{C x V x D})}{\text{W}}$$

C = Concentration of curcumin obtained from the calibration curve, V = Volume of sample taken, D = Dilution factor (if any), W = Weight of curcumin nanoparticles powder taken [22].

4.3.7. Drug Loading and Encapsulation Efficiency

Drug entrapment efficiency refers to the percentage of the drug that is successfully encapsulated or entrapped in nanoparticles. The drug entrapment efficiency was calculated by comparing the amount of drug that is entrapped within the delivery system to the total amount of drug that was initially added to the system. The formula as Eq.6 is used for calculating drug entrapment efficiency [22].

$$(Eq.6)\text{Drug Entrapment Efficiency}=\frac{\text{Amount of Drug Entrapped}}{\text{Total Amount of Drug Added}}X100$$

4.3.8. In-vitro Release of drug

The in-vitro release of curcumin from nanoparticles was evaluated by the dialysis method, which involves placing the curcumin-loaded nanoparticles in a dialysis bag with a specific pore size 0.1µm, and then submerged the bag in a release medium (phosphate-buffered saline (PBS)). *In vitro* dissolution profile was carried out in 900 mL of phosphate buffer pH 6.8 at 37°C ± 1°C and 75 rpm using Franz diffusion equipment. The samples were taken out at Specific time intervals (up to 24 hrs), measured at 425nm using analytical techniques double beam UV-Vis spectrophotometry. Release kinetics was characterized using various mathematical models that describe the release behaviour from nanoparticles. Fit release data to mathematical models that describe various release kinetics as follows: [23].
Zero-order release: $C(t) = k0t$, First-order release: $\ln(C(t)/C0) = -kt$, Higuchi release: $C(t) = kH\sqrt{t}$, Korsmeyer-Peppas release (for non-Fickian diffusion): $M(t)/M\infty = kKPtn$

4.3.9. Stability studies Accelerated

Stability studies were performed for evaluating the long-term stability of curcumin-loaded nanoparticles. The sample were kept at 40 ±2°C and 75 ±5 % relative humidity (RH) into vials, ensuring that each container was tightly sealed to prevent moisture ingress for next three month and every 15 days' time interval and all the parameters were checked and results were compared to the initial results [24].

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