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Formulation and Evaluation of Curcumin Loaded Nanoliposome on Brain Targeted

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ABSTRACT

The present work aimed to produce Curcumin based nanoliposomes that nanoliposome can target the site via the brain in a controlled manner. Nanoliposomes are important in controlling the carriage; Curcumin which is an active constituent of curcuminoids thus also provides an effective treatment for the central nervous system and plays a crucial role. The interaction of the drug was ruled out by FT-IR studies and no incompatibility and lipids and surfactant. To optimize the formulation, factors affecting the physical appearance of Nanoliposomes were investigated. Curcumin-loaded Nanoliposomes were formulated using cholesterol by physical dispersion method and characterized for particle size, drug content, stability, production yield. Prepared nanoliposomes gave the better physical, morphological concerning the concentration of the lipids, surfactant, and ratio of lipid and surfactant. Six different formulations of Nanoliposomes F1-F6 were formulated to obtain the optimized formulation. The TEM (Transmission Electron Microscopy) of Nanoliposomes showed that they were rounded in shape and porous in texture. *In-vitro* drug release of formulation indicates that formulation F6 was selected as an optimized formulation for incorporation into the nanoliposomes among all the six formulations and liposome showed drug release in a controlled manner at the end of 10 hours.

Keywords: Curcumin, Nanoliposomes, Physical Dispersion Method, Brain Targeting, Blood-Brain Barrier, Cholesterol, Bioavailability, In-vitro *Release*

1. INTRODUCTION

The main objective of the drug delivery system is to deliver a drug to a targeted site and to produce greater efficacy and reduces the toxic effects of the drug as compared to conventional drugs. In the pharmaceutical field, there are various novel dosage forms for novel drug delivery system to provide a sustained/targeted/prolonged drug delivery. To produce an optimal drug action, the active moiety could be transported by the suitable carrier system which delivers the drug to the targeted site of action and then trigger it to execute its task and for this, the carrier itself must be biodegradable, provide passive targeting, increase the efficiency and therapeutic index, provide stability via encapsulation, reduce the toxicity of the encapsulated agent and at the same time should improve the pharmacokinetic profile of the drug. [1]

1.1 Liposomes

The term liposome (meaning lipid body) was derived based on names of subcellular particles like lysosome and ribosome. It is defined as a spherule/vesicle of lipid bilayers enclosing an aqueous compartment.

The lipid most commonly used is phospholipids. Sphingolipids, glycolipids, and sterols have also been used to prepare liposomes. Liposomes may act as a solubilization matrix [2], as a local depot for controlled release of active compound, as permeability enhancer, [3-4] or as a rate-limiting membrane barrier for the modulation of systemic absorption of the drug**.** Their size ranges from 25 to 5000 nm. Depending upon their structure, liposomes are classified as:

1.1.1 MLV (multilamellar vesicles)

these liposomes are made of series of concentric bilayers of lipids enclosing a small internal volume.

1.1.2 OLV (Oligolamellar vesicles)

these are made of 2 to 10 bilayers of lipids surrounding a large internal volume.

1.1.3 ULV (unilamellar vesicles)

These are made of a single bilayer of lipids. They may be SUV (small unilamellar vesicles) of size 20 to 40 nm, MUV (medium unilamellar vesicles) of size 40 to 80 nm, LUV (large unilamellar vesicles) of size 100 to 1000 nm or GUV (giant unilamellar vesicles) of size greater than 1000 nm. [5,6]

Liposomes may be formulated with a range of characteristics including different sizes, charges, and drug retention, which can be tailored for a given drug and target site. There is a range of clinical products approved for the use which exploit liposomes to passively target drugs or vaccines to the appropriate site of action thereby improving specificity and reducing toxicity. Liposomes can also be actively targeted to specific cells or sub-cellular regions using targeting ligands attached to their surface, or by modification of the bilayer to give triggered release under appropriate conditions. [7, 8, 9]

Liposomes are synthetic vesicles consisting of one or more phospholipid bilayers, able to accommodate water lipids solvent. They are used as a delivery system for drugs, genes, and vaccines in therapeutics. [10]

1.2 Applications of Liposomes [11]

1. Liposomes use as drug or protein delivery vehicles
2. Liposomes use as an antimicrobial, antifungal, antiviral agent
3. Liposomes in tumor therapy
4. Liposomes in gene delivery
5. Liposomes in immunology
6. Liposomes as artificial blood surrogates
7. Liposomes as radiopharmaceuticals and radio diagnostic carriers.
8. Liposomes use in cosmetics and dermatology.

2. MATERIALS AND METHODS

Curcumin was received as a gift sample from (Lobie chemical, Mumbai), Phosphatidylcholine (Sigma- Aldrich chemical, USA), Cholesterol (Central drug house, Pvt. Ltd, New Delhi, India), Chloroform (Central drug house, Pvt. Ltd, New Delhi, India), Methanol (RFCL Limited, Okhla, New Delhi, India) and Acetone (Central drug house, Pvt. Ltd, New Delhi) were used in the study. All other chemicals and solvents were of analytical grade.

2.1 Drug Description (Curcumin) [12]

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bisdemethoxycurcumin and also known as Turmeric. It has been clinically proven that it gives a protective effect on the liver on animals, provides anti-tumor action, and reduces inflammation. It also fights against infections and has several medicinal and healing properties **(**Fig 1).

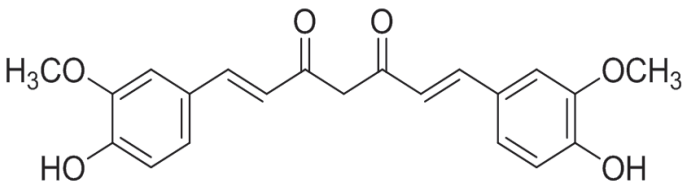


Fig.1: Structure of Curcumin

2.2 Preparation of Liposomes [13, 14, 15]

Liposomes were prepared by physical dispersion method using different ratios of lipids. In this method, the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over the flat bottom conical flask. The solution was then evaporated at room temperature without disturbing the solution. The hydration of lipid film form was carried out with aqueous medium phosphate buffer (pH 7.4). For this, the flask was inclined to one side and an aqueous medium containing a drug to be entrapped was introduced down the side of the flask, and the flask was slowly returned to upright orientation. The fluid was allowed to run gently over the lipid layer and the flask was allowed to stand for 2hr at 37°C for complete swelling.

After swelling, vesicles are harvested by swirling the contents of the flask to yield milky white suspension. Then formulations were subjected to centrifugation. Different batches of liposomes were prepared to select an optimum formula.

All batches of liposomes were prepared as per the general method described above and the composition of lipids for the preparation of liposomes. Composition of lipid for preparation of Nanoliposomes shown in **Table no-1** and Optimized formula for Nanoliposomes shown in **Table-2**

Each formulation contain 400mg of drug

S. No	Formulation Code	Phosphatidylcholine	Cholesterol
		PARTS	
1	**F1**	9	1
2	**F2**	8	2
3	**F3**	7	3
4	**F4**	6	4
5	**F5**	5	5
6	**F6**	4	6

Table 1- Composition of Lipids for Preparation of Nanoliposomes

S. No	Constituents	Quantity
1	Phosphatidylcholine	450mg
2	Cholesterol	50mg
3	Solvent	5ml
4	Drug (Curcumin)	400mg
5	Phosphate buffer pH 7.4	10ml

Table 2- Optimized Formula for Nanoliposomes Preparation

2.3 Evaluation Parameters

2.3.1 Preformulation Studies

2.3.1.1 Identification of drug and characterization

The physical appearance of the drug was determined by the drug's color and its nature (i.e. amorphous and crystalline).

2.3.1.2 Melting point determination

Capillary fusion method was used for determining the melting point of Curcumin. In this one-sided capillary tube was taken and a sufficient amount of drug was filled into the capillary [16]. The capillary was kept inside the melting point apparatus and then the temperature was increased gradually. When the drug started melting and melted properly, the temperature was noted down with the help of a Thermometer. Similarly, reading was taken six times and compared with reference value [17].

2.3.1.3 Determination of Solubility studies

Solubility was conducted using different solvents. Curcumin is freely soluble in acetone, chloroform, slightly soluble in alcohol, practically insoluble in water.

3. AUTHENTICATION OF THE DRUG

3.1 Calibration curve

About 1mg drug was accurately weighed in a digital balance and dissolved in phosphate buffer pH 7.4. The drug solution drug was scanned in the wavelength range from 200-400nm using UV- Visible spectrophotometer [SHIMADZU UV-1800] and phosphate buffer as a blank respectively [18, 19, and 20]. **Shown in figure-2**

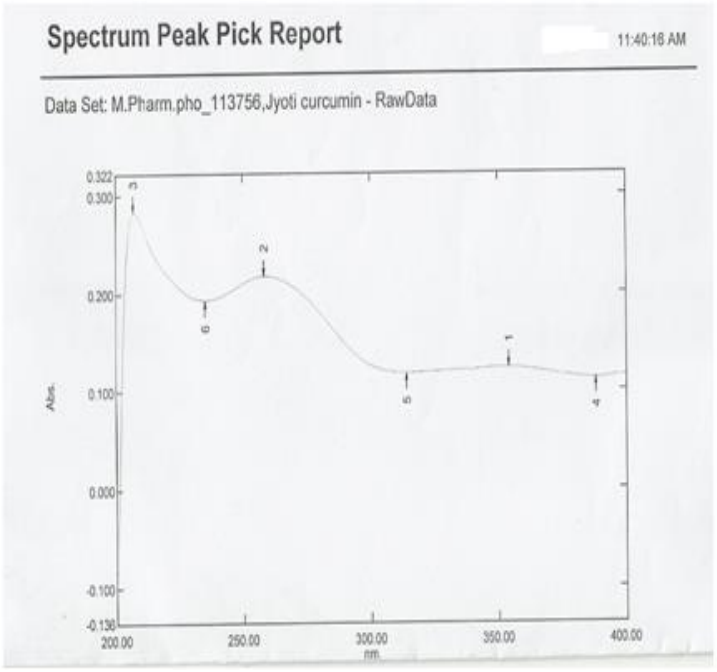


Figure-2: Calibration curve of Curcumin

3.2 Study of Pure drug sample by FT-IR spectroscopy

The weighed amount was added to KBr to form KBr pellet and subjected for scanning from 4000cm-1 to 400cm-1 using FT-IR spectroscopy (Perkin Elmer Spectrum Rx, Serial No.79225). Shown in **Figure 3 and Table 3**

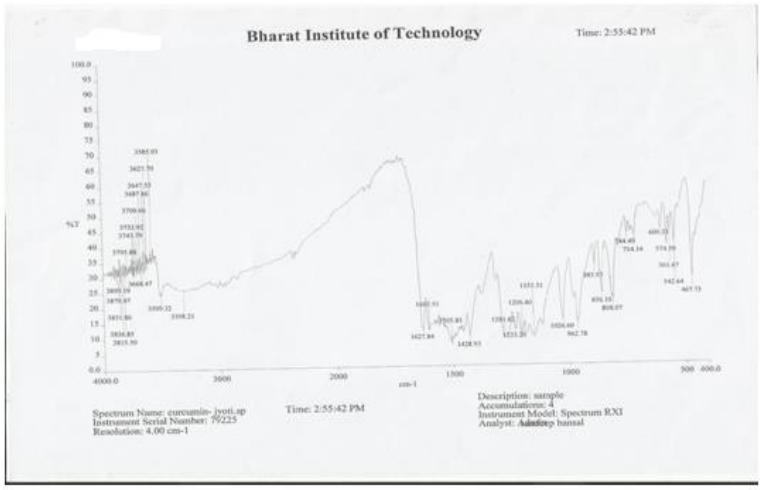


Figure -3 FT-IR value of pure drug sample

Reference Value	Observed Value	Functional Group Determination
3510.2cm^{-1}	3501cm^{-1}	Phenolic OH (Stretching)
1627.8cm^{-1}	1629cm^{-1}	Ketone C=O(Stretching)
1596cm^{-1}	1603cm^{-1}	Aromatic C=C
1276cm^{-1}	1276cm^{-1}	C-O

Table No 3. FT-IR value of pure drug sample observed value of Curcumin in FT-IR

4. RESULTS AND ANALYSIS

4.1 Identification of drug [21]

Physical appearance: Shown in Table no-4

1.	**Color**	Yellow
2.	**Odor**	Pungent
3	**Texture**	Fibrous

Table No 4. (Identification of Drug)

4.2 Melting Point range

The melting point of Curcumin was observed by Capillary Fusion Method. The observed melting point of the drug was found to be**181ºC±1.28** in the range reference value, with the decomposition as reported in Merck index, thus indicating purity of samples. Shown in **Table no-5**

Apparatus	Observed value	Reference value
Digital melting point	181°C±1.28	179-183°C

Table No 5. Melting Point of Curcumin

4.3 Thin layer chromatography

For Thin Layer Chromatography of Curcumin, precoated Silica gel aluminum plates (2×4cm) were used. The solvent system was Chloroform: Methanol (9:1). The Rf value was found to be in the range of **0.92** which was nearly the same as that of the reference value. [22] This test confirms that there is no interaction between the drug and the carrier. **Shown in Table no-6**

Thin layer chromatography	Rf value (observed)	Rf value (Reference)
Curcumin	0.92±0.015	0.90

Table No 6. Rf value of Curcumin

4.4 Study of Drug-Excipients compatibility by Fourier Transform Infrared (FTIR) Spectroscopy [23, 24, and 25

Drug-Excipients compatibility studied were carried out by using an FT-I R spectrophotometer. The FT-IR spectra of pure drug sample alone, lipids sample, and physical mixture with excipients after 15 days for study any interaction between them. The FT-IR spectra drug, cholesterol, Phosphatidylcholine, and both combination mixture were shown in the figure respectively. FTIR of cholesterol **shown in Figure -4 &**FTIR of Drug+Lipid Mixture **shown in Figure-5**

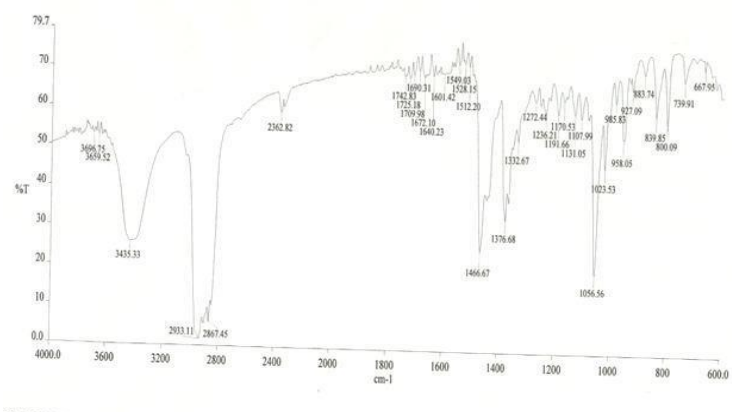


Figure-4 FT-IR of Cholesterol

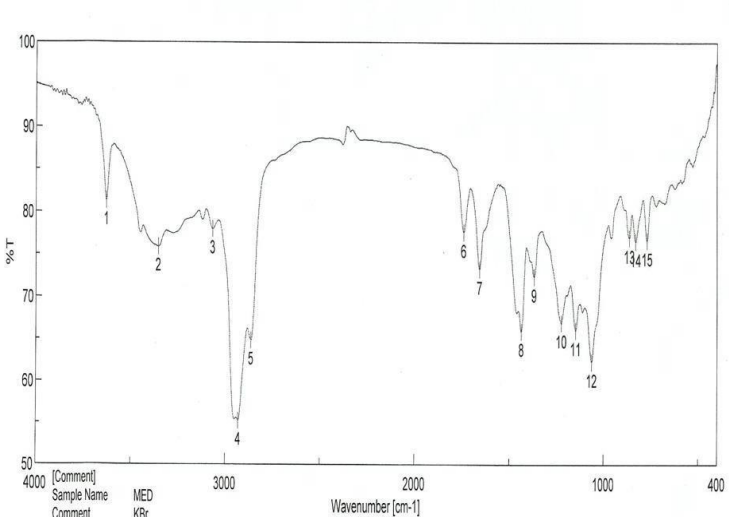


Figure 5: FT-IR Value of Drug + Lipid Mixture

4.5 Development of the Calibration Curve of Curcumin by UV- Visible spectrophotometer (SHIMADZU UV-1800)

4.5.1 Scanning of Drug, Curcumin for the Determination of Absorption Maxima

The UV spectrum of Curcumin showed a maximum peak at **432nm**. Hence all the further UV estimation was done at a maximum wavelength of 432nm. UV scan profile **Shown in Figure-6**

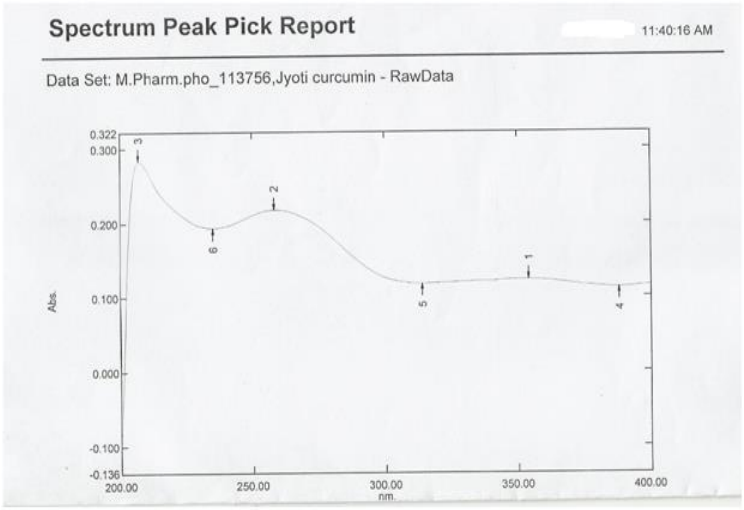


Figure 6: UV Scan Profile of Curcumin

4.6 Quantitative Estimation of Curcumin

4.6.1 Calibration Curve of Curcumin in Phosphate Buffer (pH 7.4)

The absorbance of the Curcumin solution was measured at 432 nm against the phosphate buffer (pH 7.4) as a blank and obtained data as shown in **Table 7**. By using obtained data a plot was drawn in **Figure no: 8.**

Concentration (µg/ml)	Absorbance (nm)
0	0
5	0.229±0.02
10	0.448±0.15
15	0.648±0.23
20	0.868±0.01
25	1.068±0.22
30	1.272±0.02

Table no: 7 Calibration Data of Curcumin in Phosphate Buffer (pH 7.4)

4.6.2 Calibration Curve of Curcumin in 0.1N HCl

The absorbance of the Curcumin solution was measured at 432nm against the 0.1 N HCl as a blank and obtained data as shown in **Table 8**. By using obtained data a plot was drawn and shown in **Figure 9**

Concentration (µg/ml)	Absorbance (nm)
0	0
5	0.226±0.01
10	0.481±0.12
15	0.693±0.04
20	0.891±0.14
25	1.101±0.02
30	1.353±0.15

Table 8: Calibration Data of Curcumin in 0.1N HCl

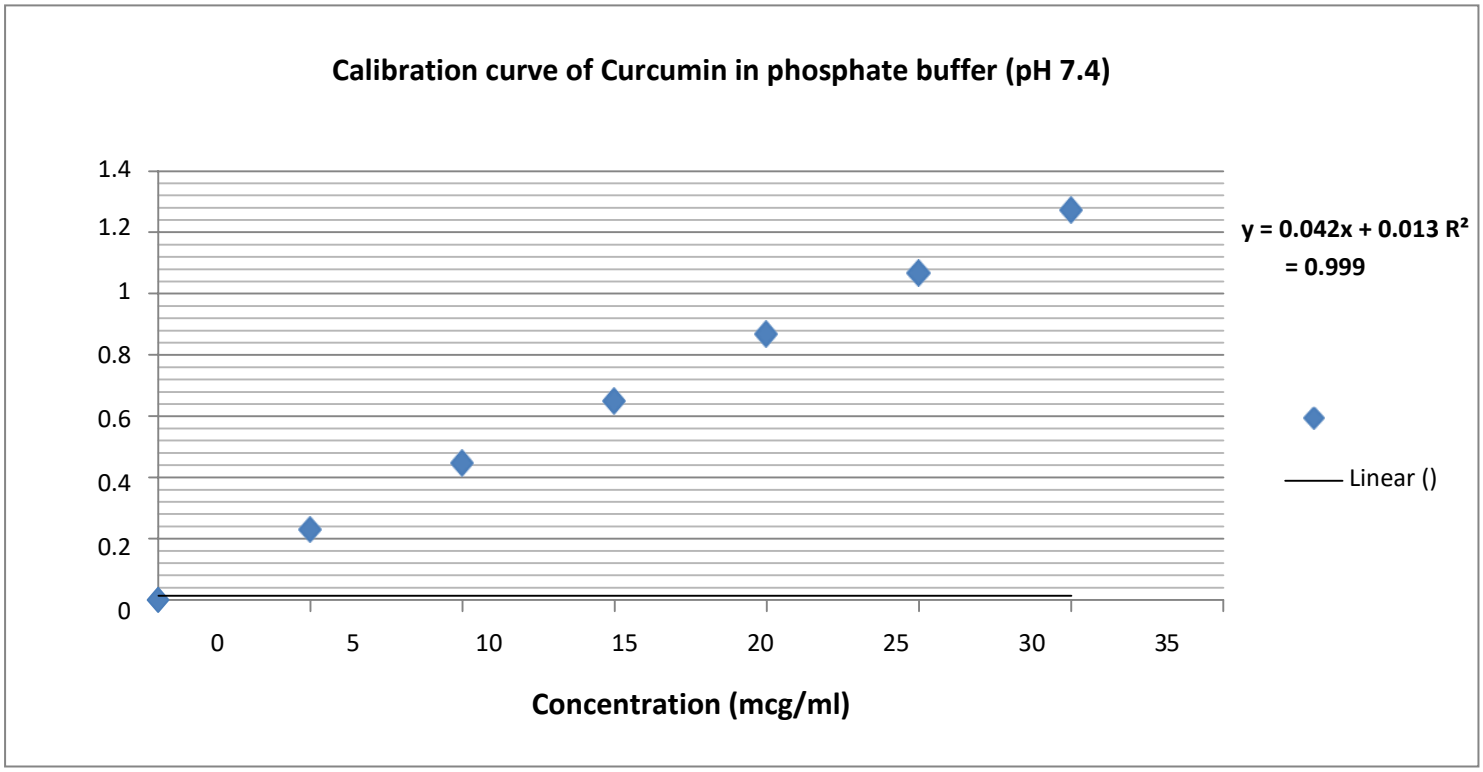


Figure 8: Calibration curve of Curcumin in phosphate buffer (pH 7.4)

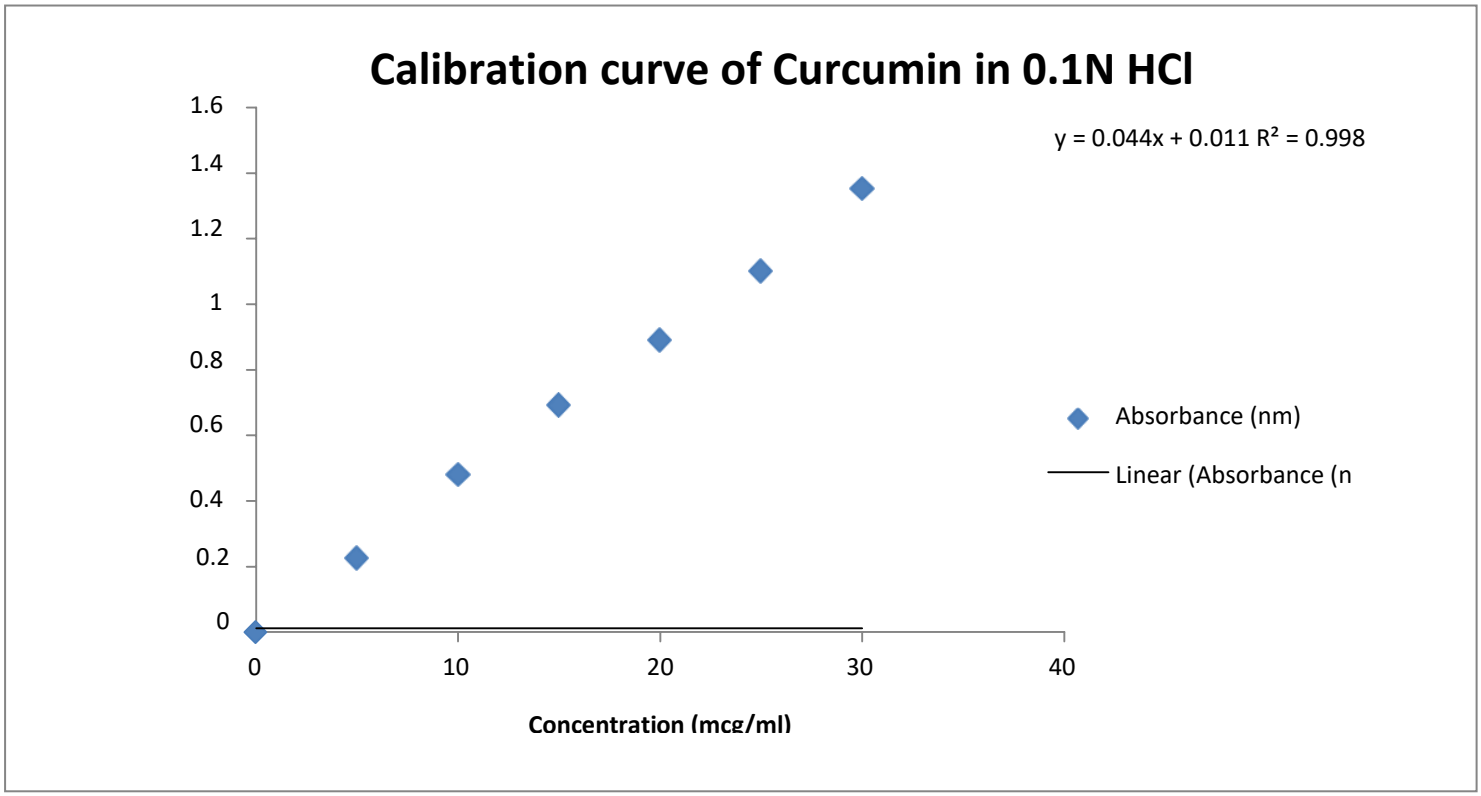


Figure 9: Standard plot of Curcumin in 0.1N HCl by UV spectrophotometer at 432nm

4.6.3 Solubility

Solubility study of Curcumin was performed using Magnetic Stirrer with Hot Plate in 0.1N HCl and phosphate buffer by equilibrium solubility method. **Table 9** shows the solubility profile of Curcumin in different media.

Medium	Concentration (µg/ml)
Phosphate buffer (pH 7.4)	2.19±0.01
0.1 N HCl	1.02±0.15

Table 9: Solubility Studies of Curcumin in Different Media

4.7 Formulation Development

4.7.1 Selection of lipids

The selections of polymers like cholesterol and Phosphatidylcholine for the formulation of brain targeting nanoliposomes were depending upon the particle size and entrapment efficiency found in the result. So, based on results of entrapment efficiency, Cholesterol and Phosphatidylcholine showed higher drug entrapment. **Shown in Table 10**

4.7.2 Method of Preparation

The technique used for the preparation of nanoliposomes.

Physical Desperation Method: shown in figure 10

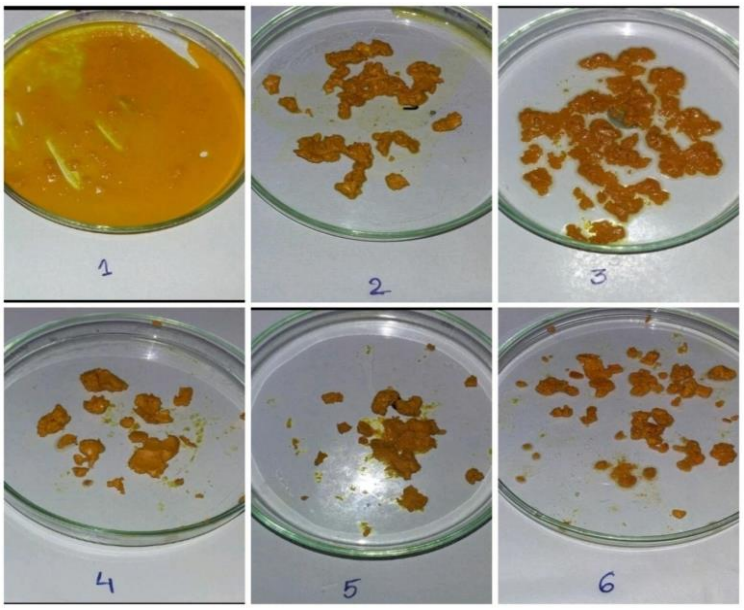


Figure 10: Formulation of nanoliposomes

S.NO	Formulation code	Phosphatidyl choline	cholesterol	Drug (mg)	Chloroform (ml)	Phosphate buffer pH 7.4 (ml)
		Ratios (mg)				
1.	**F1**	9(450)	1(50)	400	5	10
2.	**F2**	8(400)	2(100)	400	5	10
3.	**F3**	7(350)	3(150)	400	5	10
4.	**F4**	6(300)	4(200)	400	5	10
5.	**F5**	5(250)	5(250)	400	5	10
6.	**F6**	4(200)	6(300)	400	5	10

Table No 10: Selection of Lipids

4.8 Physicochemical Characterization and Evaluation of the Formulations

4.8.1 Percentage Yield

The % yield of all formulation code from F1-F6 was found to be **20.72 to 73.01%**. **Table 11**

Formulation Code	% Yield
F1	20.72%
F2	24.34%
F3	30.49%
F4	40.32%
F5	50.71%
F6	**73.01%**

Table 11: Evaluation Parameter of Percentage Yield

4.8.2 Particle size

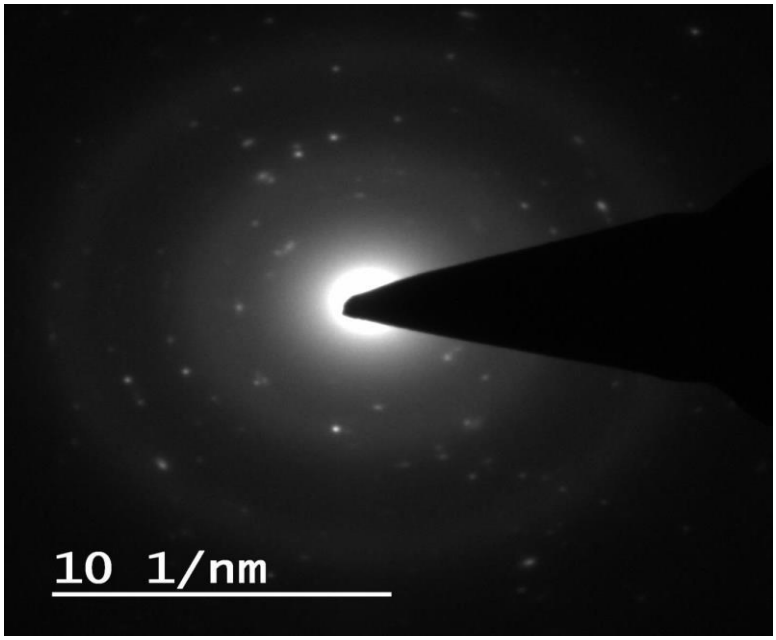
The particle size of Curcumin loaded nanoliposomes was determined using an optical microscope with an ocular micrometer which was calibrated using a stage micrometer. The particle diameter was found in the range of **905.11±0.032 to 921.12±0.57 µm.** It was due to the significant effect of the concentration of the lipid. Particle size increased with different ratios in lipids. **Table no 12**

Formulation Code	Particle Size(µm)
F1	905.11±0.032
F2	745.45± 0.04
F3	914.23±0.08
F4	929.11±0.33
F5	933.09±0.13
F6	**921.12±0.57**

Table No 12: Evaluation Parameter of Particle Size

4.8.3 Surface Morphology of Nanoliposomes

The TEM photomicrographs of the drug-loaded nanoliposomes and their surface morphology are shown in the figure and. Morphology of the drug-loaded Curcumin nanoliposomes was found discrete and spherical with a rough outer surface shown respectively **Figure 11& Figure 12**



Fie 11: TEM of Curcumingur

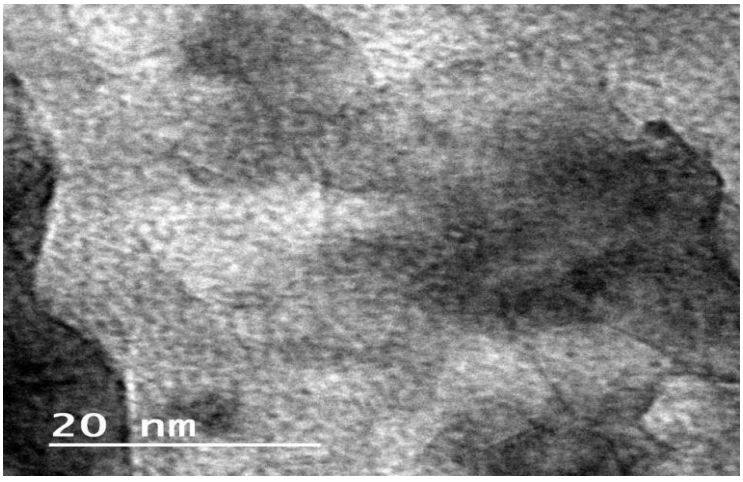


Figure 12: TEM of Curcumin

4.8.4 Drug Entrapment Efficiency

The drug entrapment efficiency of all the formulations was in the range of **81.34±0.33 to 91.87±0.011%**. The drug entrapment efficiency of nanoliposomes values of the different formulations was observed. Based on the result shows that varying the concentration of lipids automatically increases the drug entrapment efficiency. **Table 13**

Formulation Code	Drug Loading (%)
F1	10.09±1.03
F2	10.75±0.71
F3	12.00±0.77
F4	10.09±0.087
F5	10.91±0.91
F6	**12.34±1.03**

Table No 13: Evaluation Parameter of Drug Entrapment Efficiency

3.8.5 Drug Loading

The drug loading of all formulations (F1-F6) was found to be in the range of **10.09±1.03 to 12.34±1.03%.** **Table 14**

Formulation Code	Drug Loading (%)
F1	10.09±1.03
F2	10.75±0.71
F3	12.00±0.77
F4	10.09±0.087
F5	10.91±0.91
F6	**12.34±1.03**

Table No 14: Evaluation parameter of Drug loading (%)

4.8.6 Drug Release

The drug Release of all formulations (F1-F6) was found to be in the range of **77.89 to 89.60±0.33%. Table 15 and figure 13**

Formulation code	*In vitro* drug release (%)
F1	77.89±0.19
F2	80.47±1.08
F3	83.87±1.10
F4	88.13±1.09
F5	86.27±0.90
F6	**89.60±1.06**

Table No 15: *In Vitro* Drug Release (%)

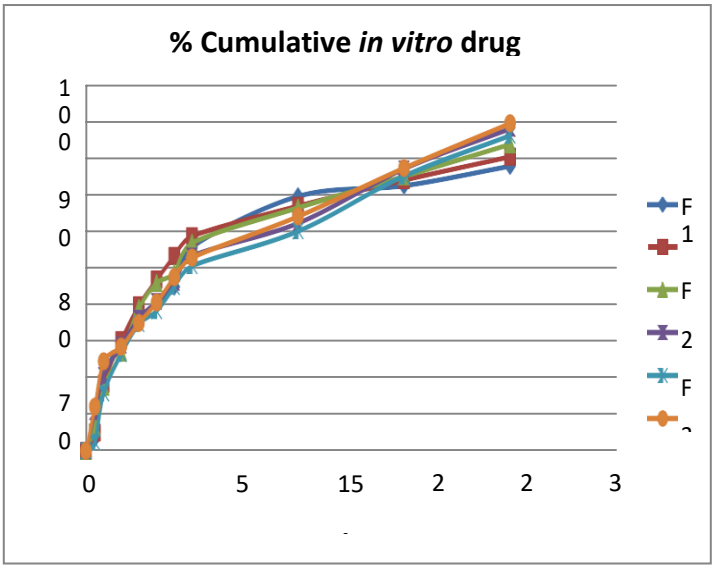


Figure: 13 shows % cumulative drug release of Curcumin

4.8.7 In Vitro Drug Release Study

The *Invitro* release of all formulations was found to be **77.05±0.19 to 89.60±1.06 %** respectively. **Table no: 16**

Time (h)	% Cumulative Drug Release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	3.38±0.19	5.04±0.20	6.51±0.08	10.74±0.13	2.38±0.20	12.1±0.05
1	20.29±0.21	18.38±0.19	17.51±0.21	20.6±0.17	15.76±0.49	24.51±0.25
2	29.67±0.22	30.3±0.18	26.88±034	28.9±0.23	26.56±0.30	28.47±0.2
3	36.14±0.36	39.84±0.21	39.08±0.23	36.29±0.34	34.74±0.60	35.01±0.55
4	40.6±0.35	46.84±0.28	45.92±032	40.88±0.45	38.29±0.11	40.6±0.45
5	44.89±0.21	53.29±0.34	48.61±0.21	46.04±0.45	44.91±0.23	47.54±0.30
6	55.76±0.21	58.74±0.06	56.89±0.10	53.29±0.23	50.6±0.10	52.84±1.01
12	69.65±0.95	67.01±1.01	66.51±0.83	62.29±0.75	60.1±0.85	64.14±0.90
18	72.54±0.03	74.07±0.098	75.09±0.76	77.15±0.08	75.21±0.054	77.39±0.54
24	77.89±0.98	80.47±0.23	83.87±0.44	88.13±0.66	86.27±0.12	89.60±0.33

Table No 16: Cumulative *in vitro* drug release of Curcumin loaded nanoliposomes of all formulation code (F1-F6)

4.8.8 Stability Study

All the data found satisfactory there is no change in organoleptic characteristic. **Table 17 and Table 18**

S.NO.	Number Of Days	Percentage Drug Remaining					
		F1	F2	F3	F4	F5	F6
1	0	98.90	101.2	98.34	98.25	101.4	101.2
2	7	98.18	101.2	98.32	98.21	101.01	101.4
3	14	97.95	100.34	98.28	98.17	100.94	100.95
4	21	97.78	100.15	98.24	98.13	100.67	100.18
5	28	97.68	99.99	98.16	98.07	100.43	99.99

Table No 17: Stability Study of all Formulation at Room Temperature

S.NO.	Number of Days	Percentage Drug Remaining					
		F1	F2	F3	F4	F5	F6
1	0	98.20	101.2	98.34	98.25	101.4	101.6
2	7	98.13	101.1	98.29	98.19	101.1	101.2
3	14	97.87	99.94	98.21	98.15	100.74	100.84
4	21	97.73	99.89	98.17	98.09	100.57	100.59
5	28	97.64	99.84	98.13	98.03	100.33	100.34

Table No 18: Stability Study of all Formulation at 40^0C

5. CONCLUSION

Nanoliposomes of Curcumin were prepared by the Physical Dispersion Method by using various concentrations (ratios) of lipids. From the Preformulation study, it was observed that the melting point of Curcumin was to be 180-182°C which indicates the purity of the sample. In the chromatographic studies (TLC) of Curcumin, the Rf value was to be 0.69-0.92 which indicates that there is no interaction between the drug and the carrier. Based on compatibility studies found there is no interaction between drugs and lipids. Prepared nanoliposome by physical dispersion method shows good entrapment efficacy and drug loading property for all formulation. The *in vitro* release data showed controlled release behavior from all formulations. The release of Cholesterol coated Curcumin nanoliposomes was found to be 89.60% at 37°C in the pH 7.4 phosphate buffer. In the Surface Morphology of the drug-loaded curcumin, nanoliposomes were found discrete and spherical with a rough outer surface. According to the results, all formulation F6 was found to be the best formulation concerning all other formulations. The formulation F6 shown the enhancement of bioavailability, better absorption, slow metabolism, and improve systemic elimination because it retains a long time in the systemic circulation.

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