PREPARATION AND CHARACTERIZATION OF LIPID-POLYMER-HYBRID NANO PARTICLES OF ANTI-ALZHEIMER'S DRUGS- A RESEARCH

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Abstract: Tramiprosate and luteolin are used as a means of treating Alzheimer's disease. Tramiprosate is a GABA-A receptor functional agonist due to this structure being similar to the GABA structure. Due to its tremendous antioxidant action, luteolin may be able to reduce the neurotoxicity of Aβ fragment 25-35 (Aβ 25-35) in the cortical neurons of mice. It has been widely established that neuroinflammation and glutamatergic excitotoxicity, which are linked to neuronal dysfunction and that they both contribute significantly to the onset and development of both locomotor and neurocognitive diseases. The goals of this work were to characterise and examine the physicochemical features of each solid form of an active pharmaceutical ingredient (API), each of which can display distinct physicochemical characteristics.

Keywords: Alzheimer's disease, Gama Amino Butyric Acid, Amyloid beta, Lipid-Polymer Hybrid Nanoparticles.

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INTRODUCTION

Alzheimer's disease (AD) is a long-term neurological condition that affects memory and cognition, is followed by behavioural changes such aggression, melancholy, hallucinations, delusions, anger, and agitation, and finally leads to dementia, physical impairment, and death[1]. Extra-neuronal protein aggregations (plaques), and intraneuronal protein aggregations (tangles) are all considered to be symptoms of Alzheimer's disease (AD) . Although it was known at the time that people with senile dementia might also display plaques and tangles in their brains, this was not considered to be a real disease condition in the elderly [2].

In this study, we used Anti-Alzheimer's drugs such as Homotaurine (Tramiprosate) and Luteolin. Tramiprosate **(IUPAC NAME: 3-Amino-1-Propanesulfonic acid)** is a tiny amino sulfonate substance that can be taken orally that binds to the amino acids Lys16, Lys28, and Asp23 of Aβ42. By stabilising Aβ -42 monomers, this interaction lowers the formation of oligomeric and fibrillar (plaque) amyloid aggregation. Neuroprotection against Aβ-induced later deposition is provided by inhibiting oligomer synthesis and elongation [3, 4-6]. The GABA-A receptor (GABA-AR) is conected to the mechanism of action. Tramiprosate functions as a functional agonist, and its chemical structure is similar to that of the neurotransmitter gamma-aminobutyric acid (GABA) [7, 8] and evaluated the effectiveness of peripherally delivered. Luteolin **(IUPAC NAME: 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one)** the ability to penetrate the blood-brain barrier (BBB). Reduced Aβ deposition, tau hyperphosphorylation, GSK activation, and microglial proinflammatory cytokines are evidence that peripherally given luteolin pre-treatment can inhibit TBI-induced AD pathology[9].

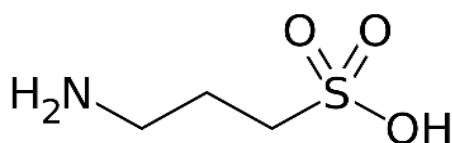The following structures of Tramiprosate (Homotaurine) and Luteolin

Figure 1: Structure of Tramiprosate

Figure 2: Structure of Luteolin

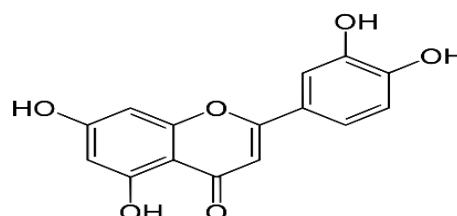Generally, the two different methods can be used to develop lipid-polymer hybrid nanoparticles (LPHNP). In one

technique, the polymer core and lipid shell are prepared separately and then combined. In the alternative method, the

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hybrid nanoparticles are generated using a one-pot nanoprecipitation and self-assembly method. In this study, LPHNP is prepared by a single nanoprecipitation method. In this method, different polymers, lipids, and solvents such as Poly Ethylene Glycol (PEG-6000), PLGA-50:50, Soya lecithin, Ethanol, Acetone, and Phosphate buffer solution (PBS) P^H-7.4 are used. The pre-formulation study explains FTIR and Solubility studies of the drug molecules.

MATERIALS AND METHODS

Materials:

Tramiprosate-5gm and Luteoline-5gm were purchased from Carbanio, supplied by Prince scientific, Hyderabad, Telangana. PLGA 50:50 was purchased from Gradient Science, Jeedimetla, Hyderabad, Telangana. PEG-6000 and Soya Lecithin were purchased from Vijaya Enterprises, Santhosh Nagar, Hyderabad, and Telangana. All other chemicals and solvents used were of analytical grade.

1. Solubility studies[10,11]

It is possible to define qualitative solubility as when the two phases are combined to form a homogeneous mixture. According to the introduction of the combinatorial chemistry, then the properties of the newly developed active compound will get shift towards higher molecular weight and the lipophilicity of the compounds will get increase and resulting in a decrease in the aqueous solubility of the drug.

Fourier transform infrared spectroscopy (ATR-FTIR)[12]

Homotaurine's FTIR spectra were examined at ambient temperature (25 ± 1 °C) using a traditional detector with a minimum resolution of 2 cm-1 region (Bruker EQUINOX 55 FTIR analyzer fitted with liquid nitrogen-cooled mercury cadmium telluride (MCT)). A diamond was utilized as an inside replication component, which was set at a 45° frequency position and scanned 32 times to produce one replication with 21 resolutions. All spectra were given an FTIR correction, and the area from 4000 to 400 cm-1 was chosen towards the path of the bands, with a peak suitable finished with the Opus software system.

EXPERIMENTAL

Preparation of LPHNP by Nanoprecipitation Method

In this preparation method, Lipid-Polymer Hybrid Nano Particles (LPHNP) were synthesized by using a single-step Nanoprecipitation Method. Preparation of LPHNP by using Homotaurine and Luteolin with the help of different polymers, lipids, and solvents such as Poly Ethylene Glycol (PEG-6000), PLGA 50:50, Soya lecithin, Ethanol, Acetone, and Phosphate buffer solution (PBS) P^H-7.4 are used.

For the nanoprecipitation approach, it is necessary to dissolve a) the drug and the polymer (PLGA 50:50) in acetone, an organic solvent that is water soluble, and to dissolve b) the drug and Soya lecithin-PEG-6000 in ethanol. To obtain a uniformly dispersed liquid crystalline phase, the Soya lecithin-PEG-6000 solution must be heated above the gel-to-liquid transition point. After that, the PLGA gradually added drop by drop to the aqueous lipid dispersion while being continuously stirred for 30 minutes at a speed of 1000 rpm. Due to hydrophobic interactions, the lipids around the polymer begin to self-assemble, with the hydrophilic head groups of the lipids facing out toward the external aqueous solution and the hydrophobic tails of the lipids pointed toward the inner NP. This causes the polymer to coil into NPs. The lipid-PEG sterically stabilises the hybrid by fusing the hydrophobic lipid tails into the inner lipid shell and extending the PEG chains into the aqueous environment. The organic medium has been removed, and the LPHNPs that result are then centrifuged at 10,000 rpm for 60 minutes. When two phases are quickly combined by hydrodynamic movement, uniform NPs with a relatively narrow particle size distribution are produced.

  a) Drug = only using homotaurine and not using luteolin in the preparation method for Homotaurine.

  b) Drug = only using luteolin and not using homotaurine in the preparation method for Luteolin.

  a) Drug = using homotaurine and b) Drug = using luteolin for combination preparation method for Homotaurine and Luteolin.
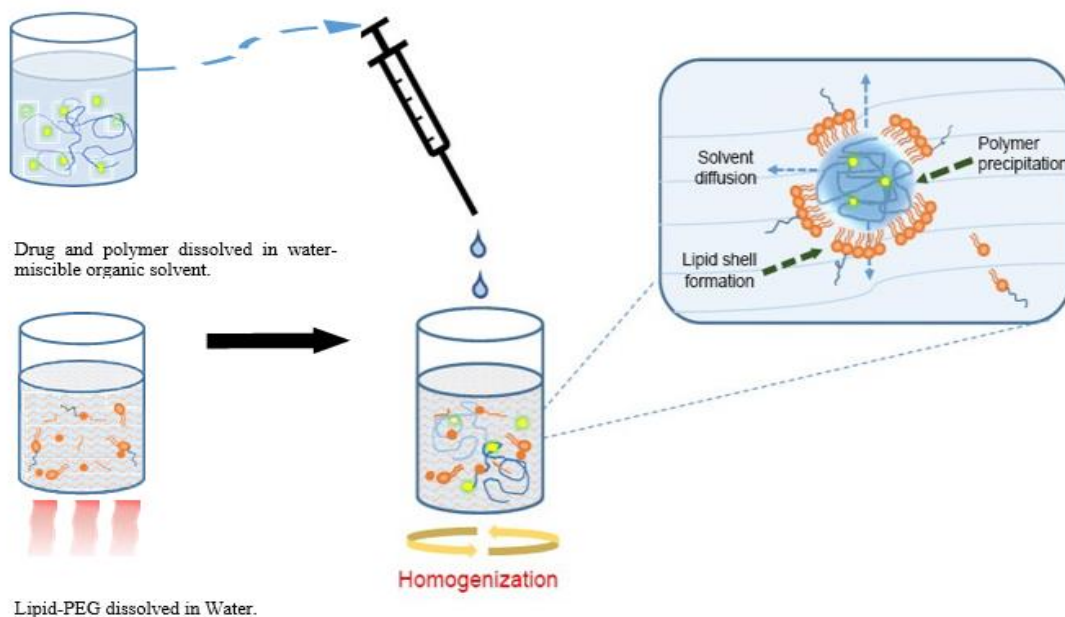

Figure 3: Nanoprecipitation method of LPHNP13

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LPHNP Formulation Optimization and Characterization

RSM based on CCD experimental studies was carried out using the Design-Expert 8.0.4.1 software (Stat Ease Inc., Minneapolis, MN, USA) to enhance the LPHNP formulations. The selected elements were the PLGA amount (X1) and concentration of Lecithin (X2), and responses included particle size (Y1), zeta potential (Y2), encapsulation efficiency (Y3), and the size distribution represented by the polydispersity index (Y4), as illustrated in Table 11. Particle size minimization (Y1), zeta potential (Y2) of < -20 mV, encapsulation efficiency (Y3) maximisation, and polydispersity index(Y4) minimization were the objectives for investigating the improved LPHNP formulation.

EVALUATION PARAMETERS

Particle size and Poly dispersity index:

LPHNP was diluted 100 times in deionized water [14], and Dynamic Laser Scattering (DLS) was used to determine the size, distribution, and zeta potential of the particles (ELSZ-2000 particle size analyser, Otsuka Electronics, Otsuka, Japan). By using the polydispersity index (PDI), the particle distribution would be depicted.

Drug entrapment efficiency

The entrapment efficiency (EE) (percent) is the percentage of active substance encapsulated in nanoparticles as a percentage of the initial pharmacological dose. A UV-Vis spectrophotometer was used to calculate the EE of nanoparticles. Centrifugation (at 22000 rpm for 45 minutes) and filtration were used to extract the unencapsulated medication from the nanoparticles. The acquired samples were then diluted in P^H 7.4 Phospate buffer solution (1:10) and tested in order. The sample absorbance was determined using quartz cells with a thickness of 1 cm that operated at particular drug wavelengths (Homotaurine-202 nm and Luteolin-211 nm). The following is how the EE percent was calculated:

% EE = [(Drug added- Free "unentrapped drug")/Drug added]*100

Zeta Potential

The zeta potential of a particle represents the particle's total charge as well as the formulation's stability. The Zeta sizer Nano-ZS90, Malvern Instrument Ltd., UK, was used to assess the zeta potential using the differential light scattering (DLS) approach. Milli-Q water was used to scatter nanoparticle samples. All measurements were carried out in triplicates at 25 °C.

***In vitro* release studies and release kinetics**

In a 10 ml Franz diffusion cell containing 10 ml of phosphate buffer, the release investigations were conducted. A 10 ml Franz diffusion cell was filled with 10 ml of phosphate buffer pH 7.4. A magnetic stirrer was used to fabricate the Franz diffusion cell, and the medium was equilibrated at 37 ± 5^0 C. One end of a dialysis membrane was sealed after it was removed. The dialysis membrane and other end were sealed when the separation of unentrapped nanoparticles was completed. The sample was suspended in the medium with the dialysis membrane. At predetermined intervals, 1ml of aliquots were retrieved, screened after collection, and the device was immediately refilled with the same amount of fresh buffer medium.

RESULTS AND DISCUSSION

Table 1: Solubility of Homotaurine

Properties	Results
Solvents	**Concentration (mg/ml at 25°C)**
Water	30
pH 7.2	12
Methanol	8
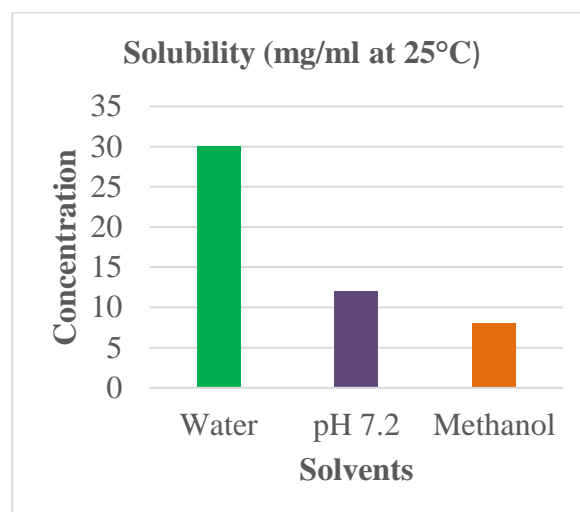
Figure 3: Solubility of Homotaurine in various solvents

Table 2: Solubility of Luteolin

Properties	Results
Solvents	**Concentration (mg/ml at 25°C)**
DMSO	57
Ethanol	55
DMF	6
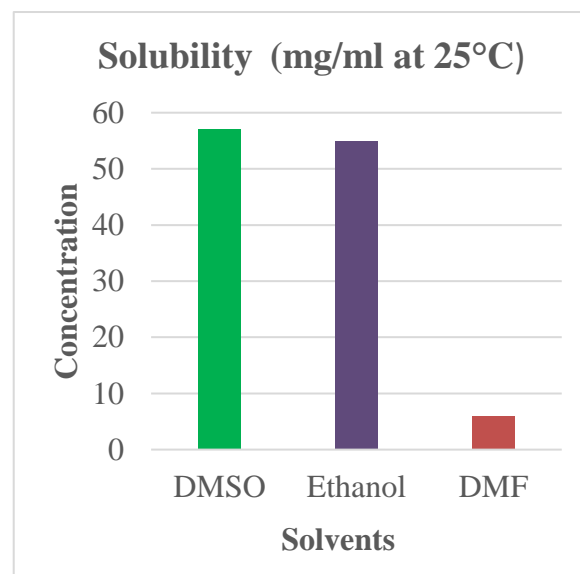
Figure 4: Solubility of Luteolin in various solvents

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**Fourier Transformation Infrared Spectroscopy (FTIR)**
i.Homotaurine:
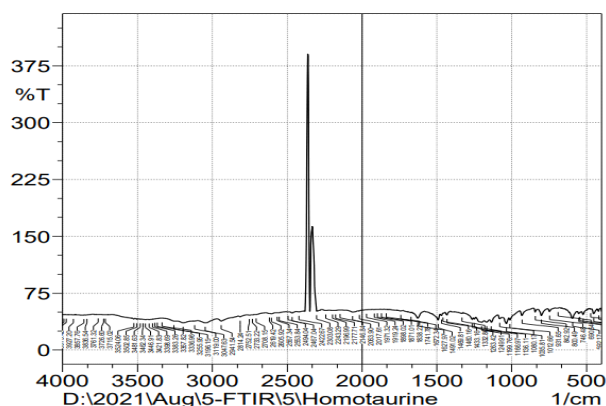
Figure 5: FTIR Spectra of Homotaurine

Table 3: Interpretation of FTIR spectra of Homotaurine

S. No	Functional Group	Reported Frequency (cm^{-1})	Observed Frequency (cm^{-1})
1	S=O Stretching	**1400-1000**	1332.86
2	Aliphatic -CH2 stretch	**3000-2500**	2494.04
3	Sulphonic acid O-H stretch	**3300-2500**	2303.06
4	N-H Stretching	**3000-2800**	3047.68
5	S=O bending	**1400-1000**	1060.12

ii. Luteolin:
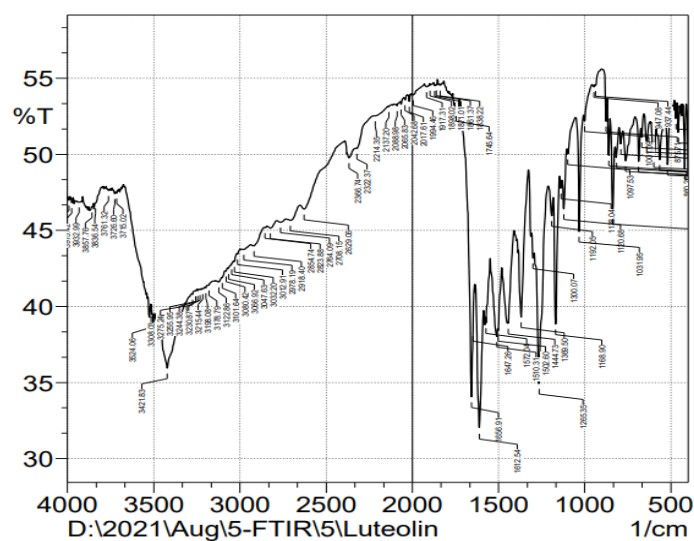
Figure 6: FTIR Spectra of Luteolin

Table 4: Interpretation of FTIR spectra of Luteolin

S. No	Functional Group	Reported Frequency (cm^{-1})	Observed Frequency (cm^{-1})
1	O-H Stretching	4000-3000	3624.06
2	Aromatic -H stretching	2000-1650	1808.02
3	=CH or C=C Stretch	1650-1566	1572.0
4	– C=O stretching	2000-1650	1656.91
5	C-O-C group of acid stretch	1400-1000	1265.35

Formulation development (Error and Trial Method):
I. Homotaurine

F. Code	Homotaurine (mg)	PLGA 50:50(mg)	Soya lecithin(mg)	PEG - 6000(mg)	Acetone (ml)	Ethanol (ml)
F1	50	50	100	50	10	5
F2	50	100	200	100	10	5

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F3	50	150	300	150	10	5
F4	50	200	400	200	10	5
F5	50	250	500	250	10	5
F6	50	300	600	300	10	5
F7	50	350	700	350	10	5
F8	50	400	800	400	10	5
F9	50	450	900	450	10	5

Table-5: Evaluation Studies of nanoparticles

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	8.4	72.93	-70.02
F2	9.5	73.27	-60.05
F3	11.3	78.23	-50.01
F4	13.0	80.65	-40.3
F5	13.3	88.93	-21.3
F6	14.9	86.31	-10.8
F7	17.0	75.25	+20.04
F8	19.6	78.90	+30.01
F9	22.9	82.38	+30.9

In vitro **Homotaurine drug release studies**

Table 6: In vitro drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	**0**	0	0	0	0
1	9.63	10.56	12.53	13.45	**14.21**	12.29	13.23	12.20	11.56
2	22.54	25.86	23.22	25.98	**26.78**	28.32	27.98	24.93	25.62
3	34.38	33.58	36.45	35.37	**37.67**	36.35	35.80	32.25	34.18
4	43.57	46.23	45.42	44.89	**48.49**	42.72	42.19	44.10	45.23
5	55.79	54.58	62.50	62.58	**65.31**	60.98	55.86	52.31	55.18
6	68.22	65.50	74.55	75.82	**78.36**	76.54	66.22	68.28	70.12
7	75.92	78.63	85.60	87.21	**88.45**	82.32	80.21	76.25	81.29
8	89.92	91.58	93.63	94.89	**96.85**	92.28	90.84	90.13	95.38
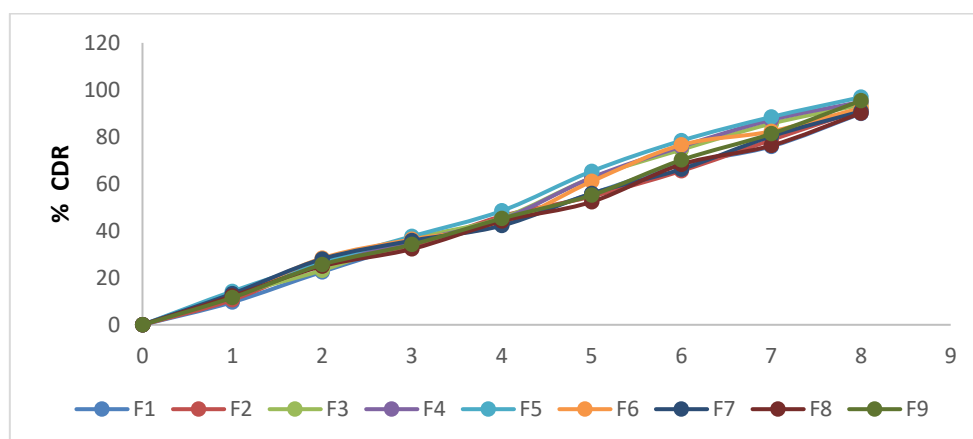
Figure 7: In vitro Homotaurine drug release studies of all formulations

II. Luteolin:

F. Code	Luteolin (mg)	PLGA 50:50(mg)	Soya lecithin(mg)	PEG - 6000(mg)	Acetone (ml)	Ethanol (ml)
F1	5	50	100	50	10	5
F2	5	100	200	100	10	5
F3	5	150	300	150	10	5
F4	5	200	400	200	10	5
F5	5	250	500	250	10	5
F6	5	300	600	300	10	5
F7	5	350	700	350	10	5

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F8	5	400	800	400	10	5
F9	5	450	900	450	10	5

Table-7: Evaluation Studies of nanoparticles

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	8.8	78.96	-50.15
F2	14.7	80.15	-40.6
F3	62.1	89.47	-31.6
F4	22.0	86.35	-30.95
F5	31.0	87.25	-20.6
F6	42.2	86.44	-10.2
F7	56.9	78.65	+0.05
F8	78.2	81.96	+0.01
F9	129.9	85.89	+10.0

***In vitro* Luteolin drug release studies**

Table 8: In vitro drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	**0**	0	0	0	0	0	0
1	10.2	8.93	**10.56**	10.23	9.86	11.38	13.28	11.24	12.84
2	21.25	25.85	**28.80**	20.68	23.78	24.94	26.89	25.63	24.89
3	36.93	35.15	**38.92**	35.89	38.93	35.32	32.48	30.28	35.43
4	43.98	48.90	**46.75**	42.86	48.49	43.93	42.19	43.97	46.17
5	56.90	52.96	**63.56**	63.58	66.93	59.21	56.82	53.89	56.89
6	68.22	64.12	**75.89**	74.86	79.25	75.93	69.86	70.17	72.14
7	78.61	75.93	**89.51**	88.93	82.18	82.32	79.82	77.90	82.84
8	91.25	92.35	**97.62**	95.82	90.32	95.86	91.56	92.14	94.25
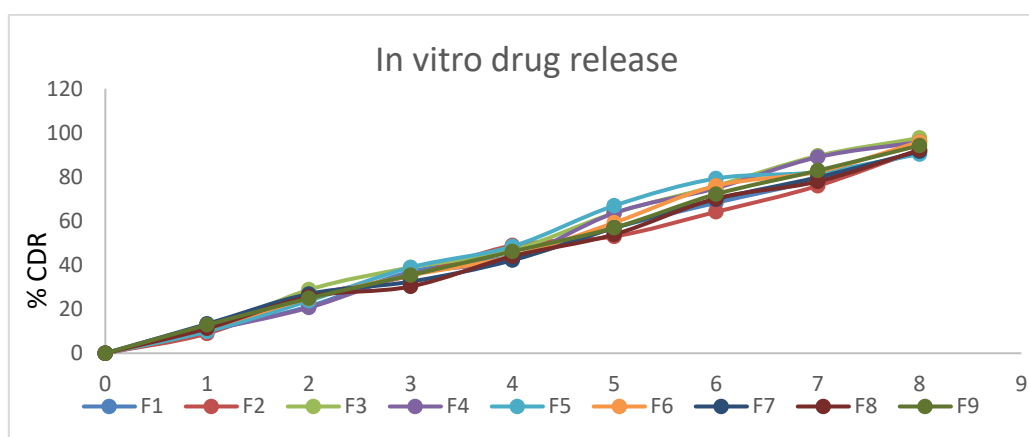
Figure 8: In vitro Luteolin drug release studies of all formulations

III. Combination of Tramiprosate and Luteolin results:

| F. Code	Combination of Homotaurine and Luteolin (mg)	PLGA 50:50 (mg)	Soya Lecithin (mg)	PEG-6000 (mg)	Acetone (ml)	Ethanol (ml)
F1	1:1	1:1	1:2	1:1	10	5
F2	1:1	1:2	1:4	1:2	10	5
F3	1:1	1:3	1:6	1:3	10	5
F4	1:1	1:4	1:8	1:4	10	5
F5	1:1	1:5	1:10	1:5	10	5
F6	1:1	1:6	1:12	1:6	10	5
F7	1:1	1:7	1:14	1:7	10	5
F8	1:1	1:8	1:16	1:8	10	5
F9	1:1	1:9	1:18	1:9	10	5

Table 9: Evaluation Studies of nanoparticles

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	4	80.85	-60.0
F2	4.5	79.21	-50.0
F3	5.4	83.84	-40.2
F4	12.0	78.52	-30.8
F5	12.5	90.20	-25.5
F6	19.9	86.25	-20.9
F7	28.6	75.24	-10.3
F8	38.9	86.32	+0.05
F9	51.3	82.50	+10.01

In vitro **Homotaurine and Luteolin drugs release studies**

Table 10: In vitro drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	**0**	0	0	0	0
1	8.50	9.32	10.23	9.55	**12.20**	11.50	10.22	9.86	10.55
2	22.12	23.85	24.89	19.58	**30.85**	25.25	24.12	28.22	25.12
3	35.21	34.58	39.11	33.50	**40.24**	38.50	30.15	35.21	35.20
4	42.85	49.10	47.85	45.66	**46.12**	42.75	44.50	48.92	45.12
5	57.82	53.84	65.80	62.11	**65.86**	60.55	57.85	64.85	50.34
6	66.80	65.13	70.12	71.55	**76.12**	68.89	65.82	72.22	65.12
7	75.82	74.11	80.22	80.52	**81.22**	79.82	72.58	78.92	70.28
8	90.22	88.92	92.14	92.80	**94.15**	93.50	93.15	93.11	93.89
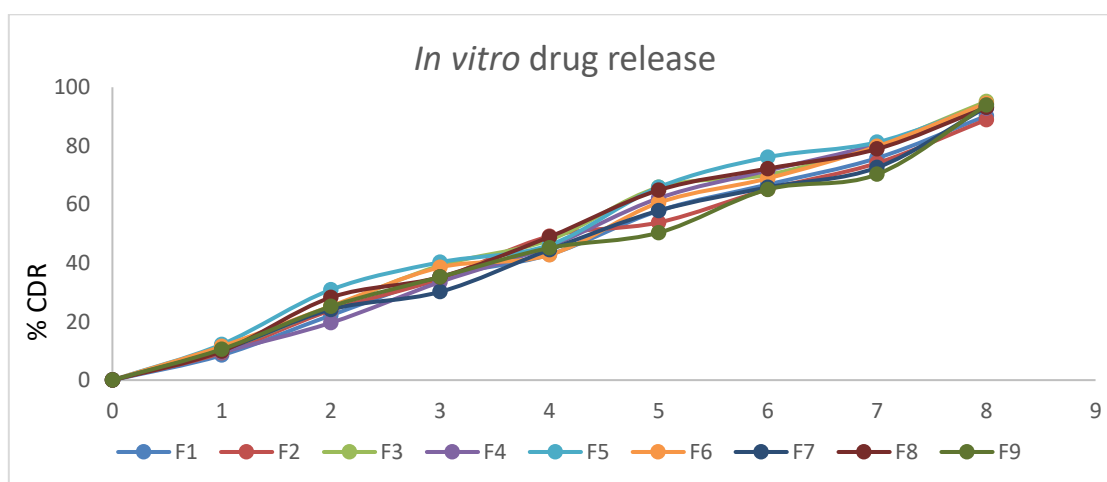
Figure 9: In vitro Homotaurine and Luteolin drugs release studies of all formulations

LPHNP Formulation Optimization by CCD:

A thorough understanding of the process parameters is necessary to create nano formulations with the desired qualities. We applied CCD to examine the relationship between PLGA-50:50 concentration, PEG-6000 quantity, and soya lecithin amount. There are 15 different experimental groups in total in this experimental design.

The responses, which are prepared by various levels and parameters, are shown in Table 11. Table 11 shows that the LPHNP particle size ranges from 186.34 to 314.2 nm, the EE ranges from 72.93 to 89.47 percent, the zeta potential ranges from 3.5 to 301 mV, and the PDI ranges from 0.027 to 0.351.

Table 11: Experimental design and response of LPHNP.

R. O	Factors and Level			Responses			
	X1: PLGA (mg)	X2: Lecithin (mg)	X3: PEG (mg)	Y1: P.S (nm)	Y2: Z.P (mv)	Y3: EE(%)	Y4: PDI
1	450	100	450	186.34	199.4	80.85	0.2
2	250	0	250	254.6	8.8	79.21	0.265
3	250	500	0	230.1	8.3	83.84	0.093
4	50	900	50	263.5	253.5	78.52	0.216
5	250	1172.72	250	210.3	301	89.2	0.224

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6	250	500	586	208.3	36.2	86.25	0.287
7	0	250	500	227.8	13.2	75.24	0.351
8	250	500	250	202.9	93.2	86.32	0.162
9	450	900	450	196.97	20.5	82.5	0.216
10	586	500	250	231.8	205	78.96	0.232
11	50	900	450	314.2	54.2	80.15	0.215
12	50	100	50	204.9	3.5	89.47	0.027
13	50	100	450	226.8	47.9	72.93	0.278
14	450	100	50	263.8	232.7	73.27	0.273
15	450	900	50	195.45	162	78.23	0.29

R.O= Run order, P.S= Particle size, Z.P= Zeta potential, EE= Entrapment efficiency, PDI= Poly dispersity index.

The Effect on Particle Size (Y1)

In this study, particle size is significant and has a significant impact on how LPHNP is characterised. To understand the association between LPHNP variables and responses, the study used ANOVA statistical study and structure model. The p-values for X1 (the amount of PLGA) and $X1^2$ are below 0.05 in this model, signifying that they would have an effect on the calculated particle size. In Figure 10A, the particle size of LPHNP significantly reduces as the amount of PLGA decreases, whereas the concentration of lecithin has no discernible impact on the particle size of LPHNP. According to earlier research by Song et al.[15], the inclusion of PLGA would produce the organic phase more viscous and prevent it from dispersing into the aqueous phase, which would cause the nanoparticles to have larger particle sizes.

The Effect on Zeta Potential (Y2)

Evidence regarding the stability of LPHNP dispersion is provided by the zeta potential. Due to electrostatic repulsion, the stability of LPHNP performs better the higher the absolute value of this response. The p-value of $X1^2$ (square of PLGA amount) for zeta potential is less than 0.05, showing that the amount of PLGA significantly affects LPHNP. In Figure 10B, the addition of PLGA caused the zeta potential to first increase and subsequently decrease, while the concentration of lecithin had no effect. The presence of PLGA's terminal carboxylic groups contributed to LPHNP's negative charge. When a result, the surface charge became increasingly negative as PLGA was applied more and more. The dissociation of carboxyl groups on the surface of the PLGA nanoparticle during preparation results in -45 mV zeta potential[16]. Because the concentration of lecithin in our study is less, ranging from 0.295 percent to 1.705 percent, the variation in zeta potential is minimal.
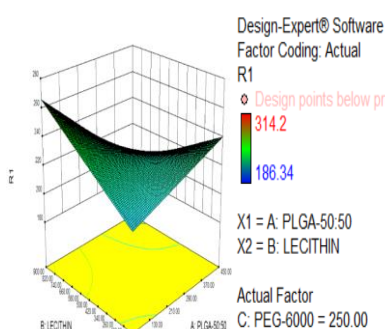
The Effect on Encapsulation Efficiency (Y3)

The preparation effectiveness of this study would be shown by EE. The p-values for X1 (the amount of PLGA), X2 (the concentration of lecithin), X1X2, $X1^2$, and $X2^2$ for EE are all less than 0.05, indicating that these variables

significantly affect the EE response. Encapsulation effectiveness may decrease with more lecithin content and less PLGA amount, as shown in Figure 10C. Feczkó et al.[17] used the Box-Behnken experimental design, one DOE model, to encapsulate bovine serum albumin (BSA) in PLGA nanoparticles and observed that the concentration of PLGA would have a beneficial impact on EE. Because of the increased viscosity, EE for PLGA nanoparticles with flavonoids loaded increased when PLGA concentrations raised from 5 to 40 mg/mL in some studies[18]. Our investigation shows a pattern that is consistent with other research because the concentration of PLGA varies from 1.67 mg/mL to 38.3 mg/mL.
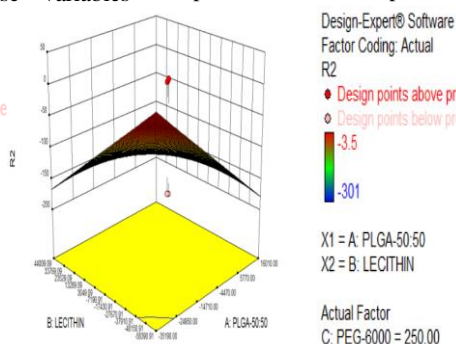
The Effect on PDI (Y4)

For a homogeneous population of nanocarriers, the PDI (poly dispersity index) is typically represented as being smaller than 0.3 [19]. The p-values of variables X1 (PLGA amount), X1X2, and $X2^2$ are less than 0.05 after ANOVA analysis, indicating that these variables significantly affect PDI. The amount of PLGA has an impact on the size distribution in Figure 10D. Low Lecithin concentration, however, may evidently raise PDI value and cause uneven formation, which lowers the efficacy of drug delivery.

According to the conclusions, the ideal formulation has a PLGA content of 75.3 mg and a lecithin concentration of 0.5 percent. This formulation was used to create LPHNP nanoparticles, and Table 11 shows the particle size, zeta potential, encapsulation effectiveness, and PDI. Following the experiment, the optimised LPHNP's particle size and EE are suited to values estimated by DOE with an error rate of less than 5%. Better stability and particle distribution result from the zeta potential and PDI values being lower than expected. According to Kadari et al. [20], PLGA nanoparticles were used to enclose the FST-cyclodextrin inclusion complex. The zeta potential was -8.71 ± 0.03 mV, EE was 79 percent, and the particle diameter was 87.27 ± 0.10 nm. The EE was significantly reduced to 47.3% without the use of cyclodextrin. In our study, we suggested a simple procedure that only used PLGA to produce LPHNP with improved EE and the optimal zeta potential (-20 mV).
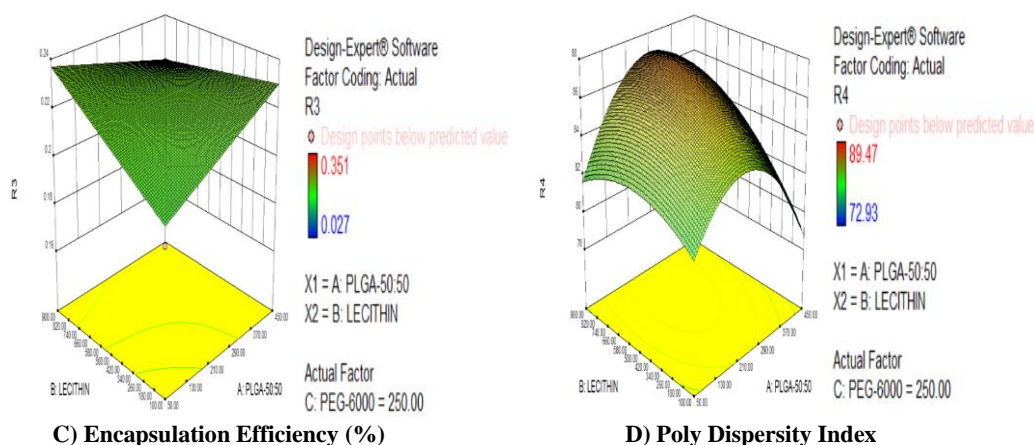

A) Particle size (nm)

B) Zeta potential (mV)

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C) Encapsulation Efficiency (%)

D) Poly Dispersity Index

Figure 10. Response surface plots of amount of PLGA 50:50 (X1) and concentration of Lecithin (X2) for (**A**) particle size (Y1), (**B**) zeta potential (Y2), (**C**) Encapsulation efficiency analysis (Y3), and (**D**) PDI (Y4).

CONCLUSION

To order to enhance the biopharmaceutical properties of LPHNP for nasal delivery, this study suggested a useful nanoparticle-based method. The LPHNP was effectively prepared using a simple single-step nanoprecipitation process, and the preparation parameters were improved by the RSM based on CCD to obtain the desired features. This study examined the effects of formulation factors such as particle size, zeta potential, PDI, and EE of LPHNP. To order to analyze the effects of the variables and to improve the manufacturing process conditions, a CCD experimental design was created.

The optimized LPHNP formulation consists of 450 mg PEG-6000, 9% (w/v) Soya lecithin, and 450 mg of PLGA-50:50, resulting in particles with an average size of 196.97 nm, PDI of 0.232, negative surface charge -20.5 mV, and EE of 78.96%. According to the above mentioned findings, the LPHNP has a great deal of potential to be developed as a nasal carrier for effectively delivering homotaurine and luteolin with enhanced biopharmaceutical capabilities.

Finally, a Central Composite experimental design was employed successfully to produce LPH nanoparticles with optimal properties.

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COMPLIANCE AND ETHICAL STANDARD

I.Ethical Approval
Not Applicable
Consent For Publication
All the authors have approved the manuscript and given consent for publication.

Competing Interests
The authors affirm that here be situated not any conflict of comforts concerning the journal of this object.

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