Modified Chitosan nanoparticles: a trojan horse for crossing BBB

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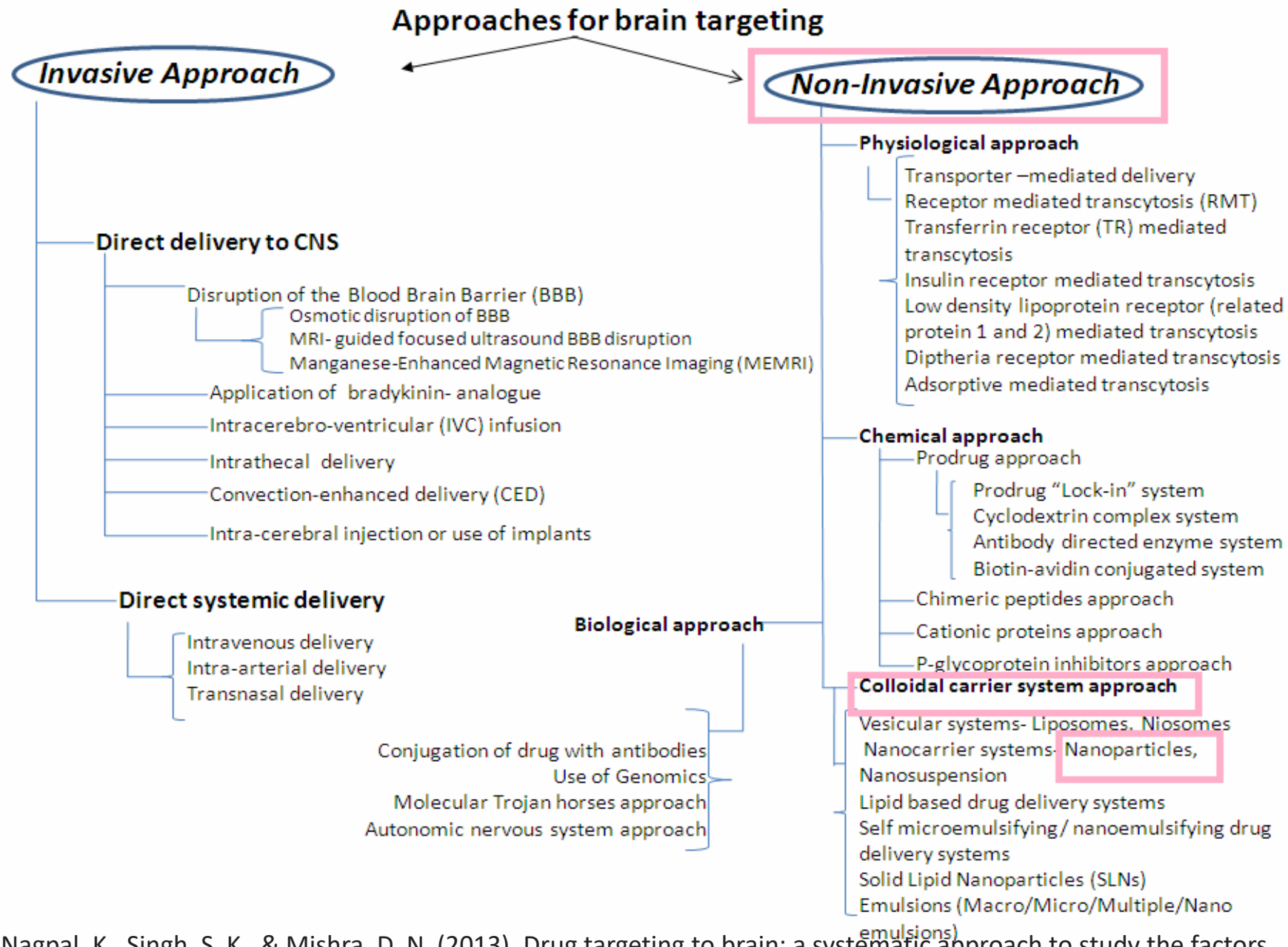
INTRODUCTION

- Satisfactory treatment of central nervous system (CNS) disorders is almost non-existent due to insufficient permeability of therapeutic moieties through blood--brain barrier (BBB).
- 98% of small molecular weight drugs and almost all of the proteins and nucleic acid therapies excluded from the CNS due to delivery barriers (Pardridge, W.M., 2005)
- Poor Blood Brain Barrier penetration makes the drug uptake to brain highly pertinent
- Complexity of pharmaceutical formulations can be better understood by statistical tools such as factorial design optimization



Nagpal, K., Singh, S. K., & Mishra, D. N. (2013). Drug targeting to brain: a systematic approach to study the factors, parameters and approaches for prediction of permeability of drugs across BBB. *Expert opinion on drug delivery*, *10*(7), 927–955.

MAIN FOCUS

- To enhance brain uptake potential of the selected drugs
- Formulation development of nanoparticles (NPs) incorporating the selected drugs
- Characterization of the formulated NPs
- To optimize the formulation
- *In vitro* and *in vivo* evaluations

SELECTION OF BIOCOMPATIBLE POLYMER

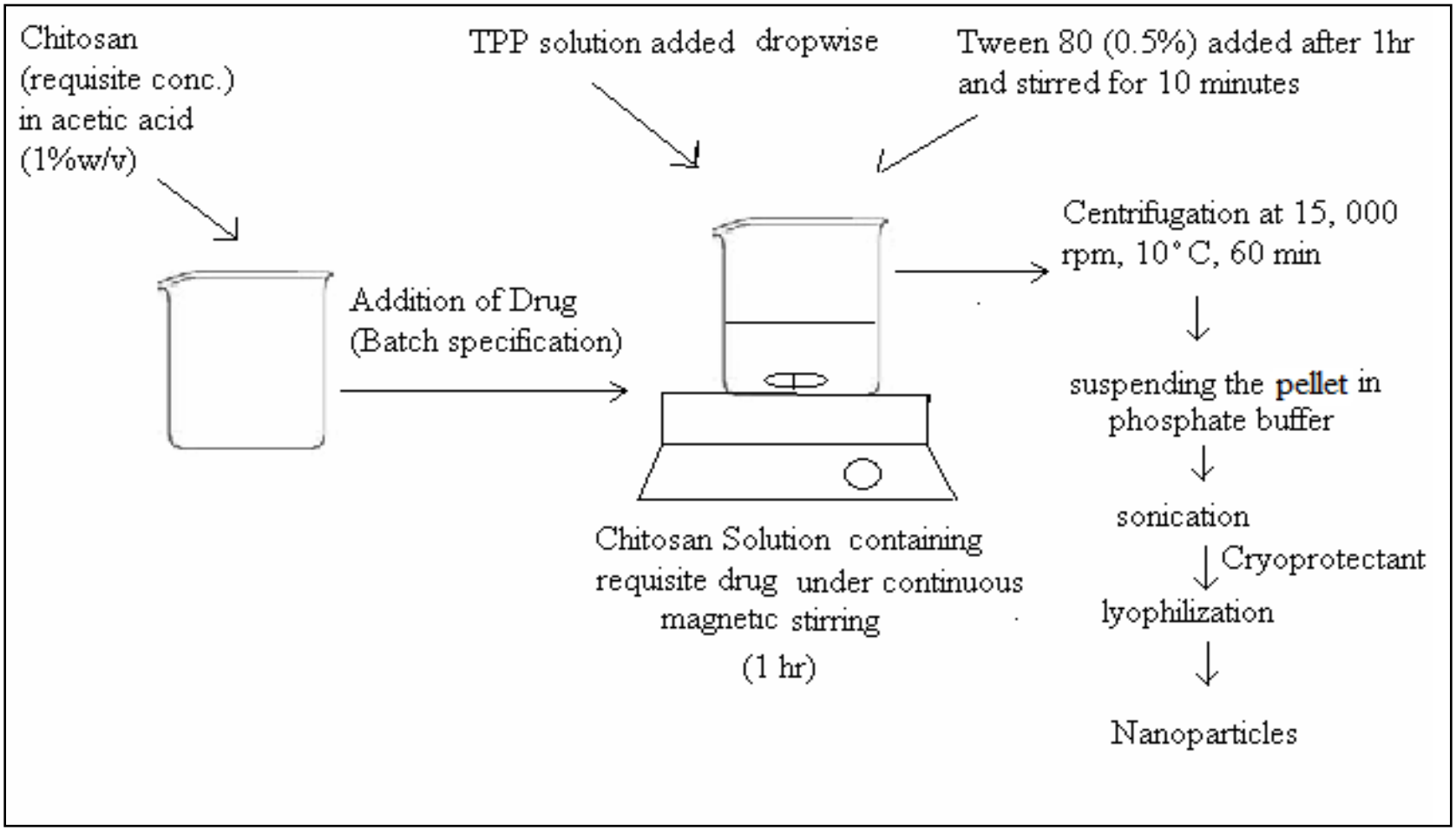
✓Chitosan was selected as a polymeric carrier for NPs formulation.

✓Chitosan has better stability, low toxicity, simple and reproducible preparation methods, providing versatile routes of administration

✓Also avoids the use of hazardous organic solvents during fabrication since they are soluble in aqueous acidic solution.

Chitosan has been obtained as a gift sample from Central Institute of Fisheries Technology, Kochi, India.

SELECTION OF APPROPRIATE PROCESS



✓**Ionotropic gelation method is the simplest, feasible and appropriate method for formulation of chitosan NPs.**

DRUG 1: RIVASTIGMINE HYDROGEN TARTARATE

DRUG 2: GALLIC ACID

DRUG 3: MINOCYCLINE HYDROCHLORIDE

RESPONSE SURFACE METHODOLOGY OPTIMIZATION

- ✓ Identification of variables and the design of experiments for optimization
- ✓ Formulation of brain targeting polymeric nanoparticles incorporating the selected drug
- ✓ Characterization of formulated nanoparticles
  - Particle size, particle size distribution, zeta potential, polydispersity index
  - Surface morphology using TEM
  - Differential Scanning Calorimetry
- ✓ Quantification of dependent variables (Responses)
- ✓ Generation of polynomial equation through linear regression analysis for defining the relationship between dependent and independent variables
- ✓ Generation of response surfaces/ contour plots and formulation optimization
- ✓ *In vitro* drug release study from the optimized batch of nanoparticles

EXPERIMENTAL DESIGN

Design Employed :	**Central Composite Design**
Independent Variables :	**Chitosan concentration, % (X1)**
	Tween 80 concentration, % (X2)
Response Variables:	**Particle size (nm) ↓**
	Zeta Potential (mV) ↑
	Drug Entrapment efficiency (%) ↑

Particle size, PDI, zeta potential, and DEE of RT encapsulated NPs prepared as per experimental design

Batch	Particle Size (nm)	PDI	Zeta Potential (mV)	DEE (%)
1	278.2	0.169	35	94.41
2	183.4	0.175	29.3	93.65
3	165.9	0.177	0.00524	96.24
4	171.7	0.187	7.45	96.73
5	188.6	0.200	30.6	93.39
6	189.3	0.218	31.7	93.27
7	361.2	0.206	38	85.76
8	191.2	0.139	32.8	93.01
9	176.4	0.147	15.2	91.65
10	153.7	0.177	-5.25	95.71
11	540.8	0.289	45.8	89.17
12	398.2	0.271	35.6	83.65
13	186.4	0.180	32.5	92.13

ANOVA – Influence of formulation variables on the response factors

Respose factor	Model F-value	Prob>F	Lack of fit F-value	Prob>F
Particle Size (nm)	51.02	< 0.0001	0.97	0.3802
Zeta Potential (mV)	51.53	<0.0001	0.99	0.4835
Entrapment efficiency (%)	149.90	0.0002	0.33	0.8081

The Design Expert software suggested equation for the quadratic model:-

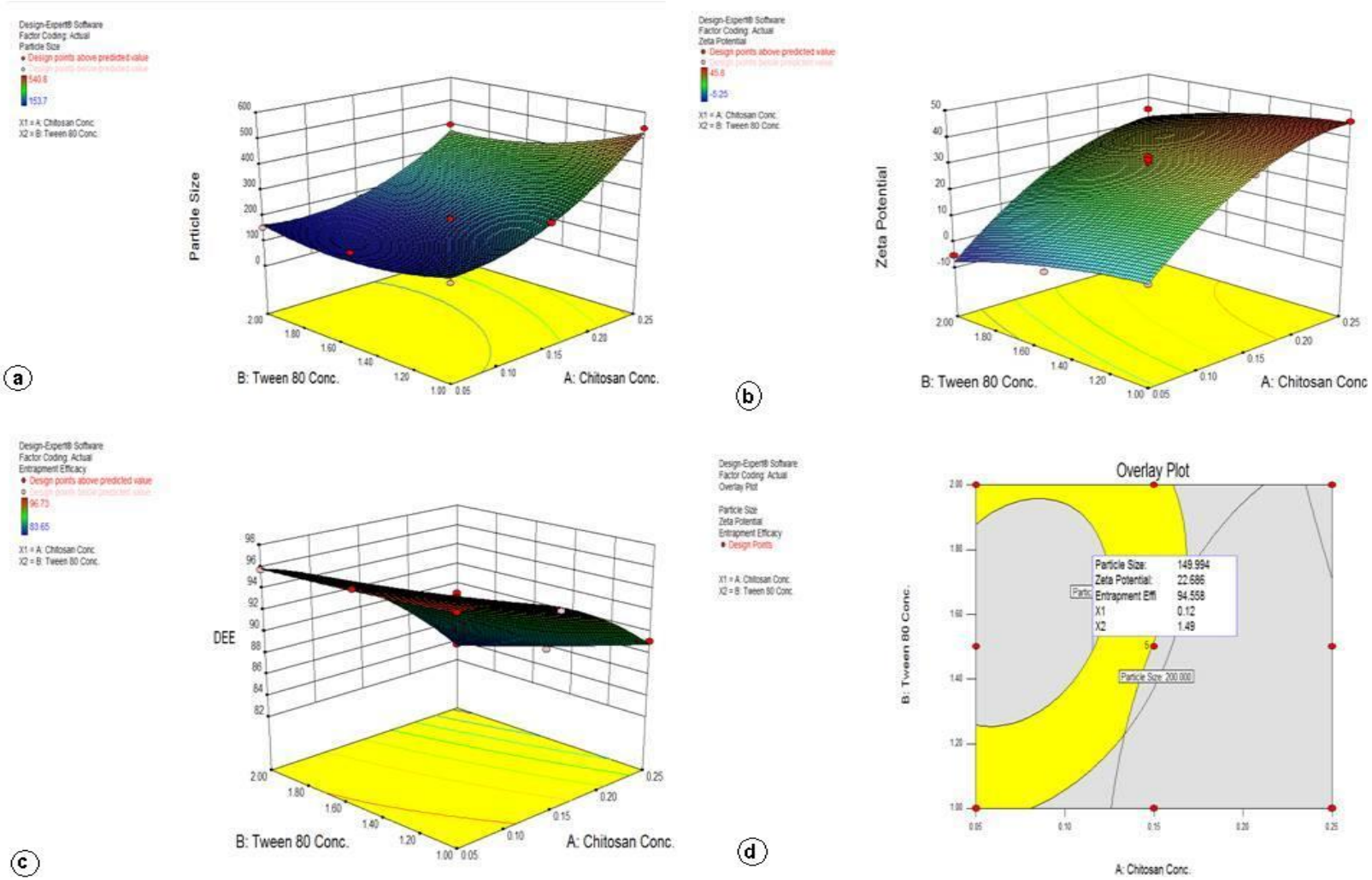
Particle Size = $571.71 - 179.14X1 - 543.74X2 - 623X1X2 + 8206.03X1^2 + 183.24X2^2$

Zeta Potential = $-14.84 + 429.46\ X1 + 11.88\ X2 + 12.5\ X1X2 - 842.97\ X1^2 + -9.33\ X2^2$

Entrapment Efficiency = $96.94 + 40.85\ X1 - 1.18\ X2 - 22.5\ X1X2 - 190.89\ X1^2 + 0.48\ X2^2$

Overlay plot suggested- using 0.12% chitosan and 1.49% Tween 80 should yield- NPs with particle size 149.994 nm ;zeta potential 22.686mV; and DEE of 94.558%.

RESPONSE SURFACES

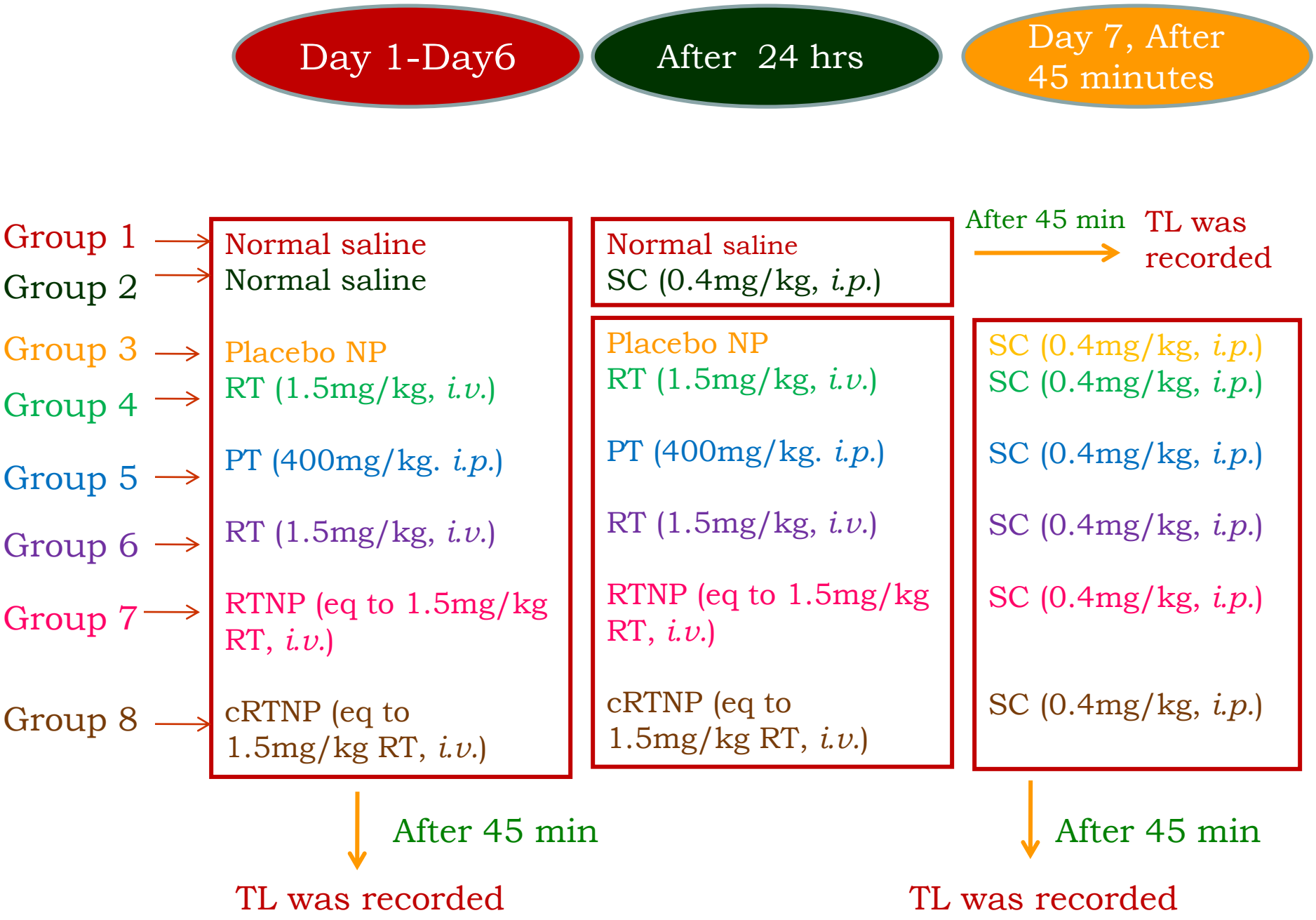


The effect of chitosan and Tween 80® on (a) particle size (b) on zeta potential (c) on DEE of RT encapsulated nanoparticles. (d) Overlay plot showing the location of optimized formulation.

Comparison of experimentally observed responses of the optimized nanoparticles with that of predicted responses

Response parameters	Goal	Observed values	Predicted values	Percent Error of mean(%)
Particle Size (nm)	Minimize	154.06±2.14	149.99	2.64%
Zeta Potential (mV)	Maximize	26.60±0.90	22.69	14.7%
Encapsulation efficiency (%)	Maximize	96.36±1.09	94.56	1.88%

Pharmacodynamic activity:
For Rivastigmine Hydrogen Tartarate & Formulations
Evaluation of Reversal of Scopolamine induced amnesia


Day 1-Day6
After 24 hrs
Day 7, After 45 minutes
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Normal saline
Normal saline
Placebo NP
RT (1.5mg/kg, i.v.)
PT (400mg/kg. i.p.)
RT (1.5mg/kg, i.v.)
RTNP (eq to 1.5mg/kg RT, i.v.)
cRTNP (eq to 1.5mg/kg RT, i.v.)
Normal saline
SC (0.4mg/kg, i.p.)
Placebo NP
RT (1.5mg/kg, i.v.)
PT (400mg/kg. i.p.)
RT (1.5mg/kg, i.v.)
RTNP (eq to 1.5mg/kg RT, i.v.)
cRTNP (eq to 1.5mg/kg RT, i.v.)
After 45 min
TL was recorded
SC (0.4mg/kg, i.p.)
SC (0.4mg/kg, i.p.)
SC (0.4mg/kg, i.p.)
SC (0.4mg/kg, i.p.)
SC (0.4mg/kg, i.p.)
SC (0.4mg/kg, i.p.)
After 45 min
TL was recorded
After 45 min
TL was recorded

Rivastigmine Hydrogen Tartarate & Formulations

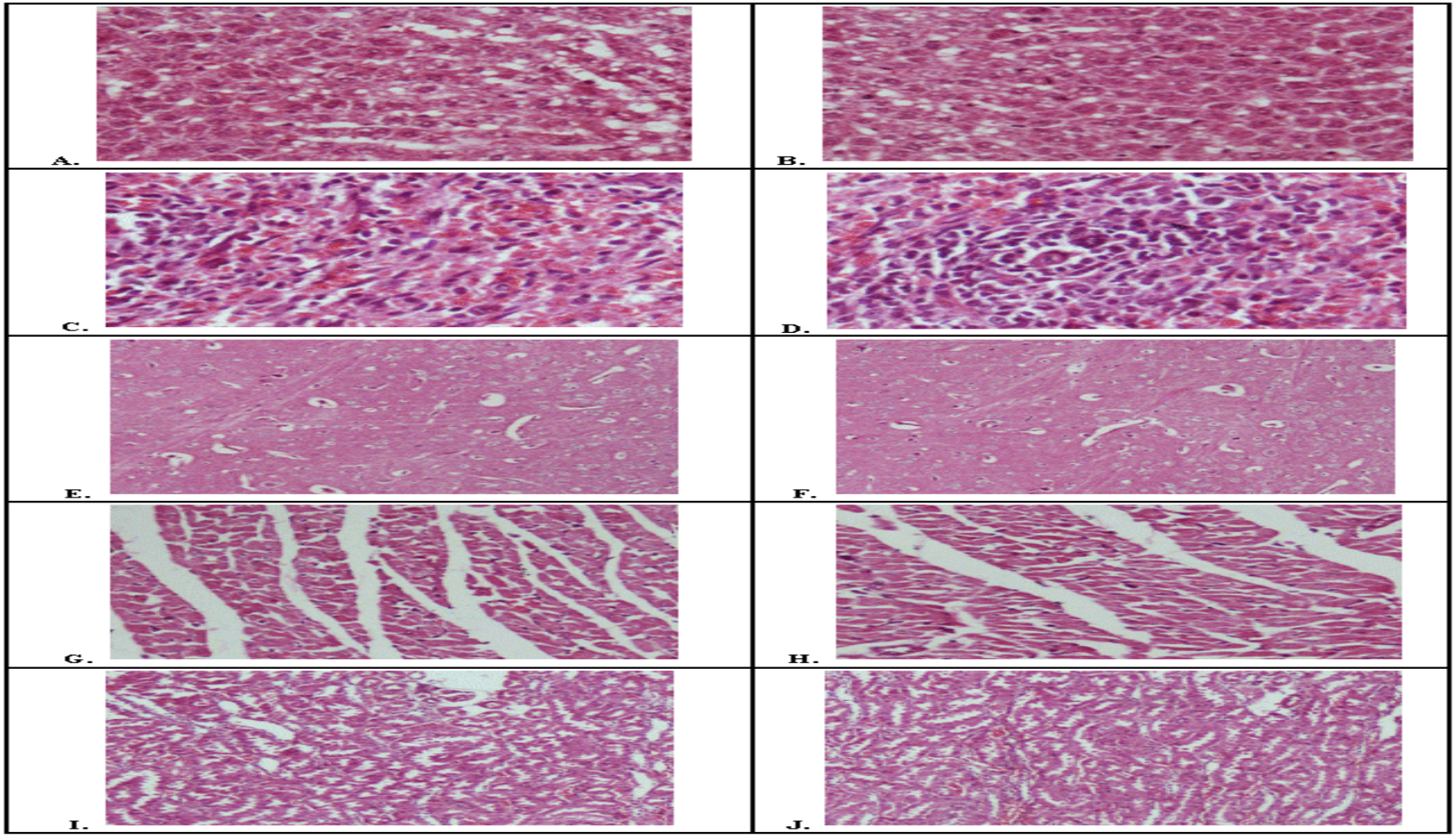
*Maximum Tolerable Dose (MTD) study**

***Nagpal K, Singh SK, Mishra DN. Optimization of brain targeted chitosan nanoparticles of Rivastigmine for improved efficacy and safety. *International Journal of Biological Macromolecules* 2013;59:72– 83**

STUDY PROTOCOL

- Group 1 (Control): Rats were orally administered normal saline for 28 successive days.
- Group 2 (RT 1.5): Rats were administered RT (1.5mg/kg, orally) for 28 successive days.
- Group 3 (RT 2.0): Rats were administered RT (2mg/kg, orally) for 28 successive days.
- Group 4 (RT 2.5): Rats were administered RT (2.5mg/kg, orally) for 28 successive days.
- Group 5 (cRTNP 1.5): Rats were administered cRTNP (equivalent to 1.5mg/kg RT, orally) for 28 successive days.
- Group 6 (cRTNP 2.0): Rats were administered cRTNP (equivalent to 2mg/kg, orally) for 28 successive days.
- Group 7 (cRTNP 2.5): Rats were administered cRTNP (equivalent to 2.5mg/kg, orally) for 28 successive days.

HISTOLOGY STUDY



Liver histology after Treatment: RT at 2.0 mg/kg (A) and cRTNP at 2.5 mg/kg (B).

Spleen histology after Treatment: RT at 2.0 mg/kg (C) and cRTNP at 2.5 mg/kg (D).

Cerebrum Histology after Treatment: RT at 2.0 mg/kg (E) and cRTNP at 2.5 mg/kg (F).

Myocardium histology after Treatment: RT at 2.0 mg/kg (G) and cRTNP at 2.5 mg/kg (H).

Kidney histology after Treatment: GA at RT at 2.0 mg/kg (I) and cRTNP at 2.5 mg/kg (J).

Conclusion

- ✓ **Tween 80 coated chitosan nanoparticles can be successfully utilized for enhanced brain uptake**
- ✓ **With formulation optimization, maximum efficacy of the dosage form can be successfully achieved both in terms of formulation behavior as well as pharmacodynamic activity**
- ✓ **The surfactant coated nanoparticulate drug delivery system holds immense potential as a platform technology to target therapeutic moieties to brain for improving their efficacy in various CNS disorders**

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