

GABA- LOADED HALLOYSITE NANOTUBE SYSTEM: RELEASE CHARACTERISTICS and EFFECT on SEIZURE PARAMETERS in EPILEPTIC RATS

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OUTLINE



EXPERIMENTAL

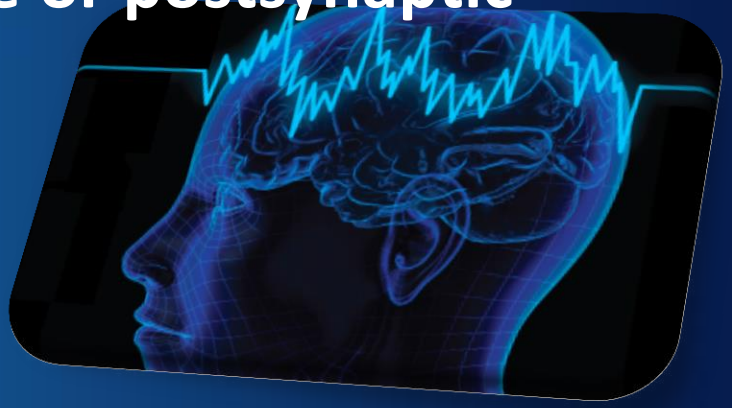
- Methods
- Characterization
- In vitro study
- In vivo study

RESULTS and DISCUSSION

EPILEPSY

- ‘epilepsy’
  generalized or paroxysmal focal reaction of brain

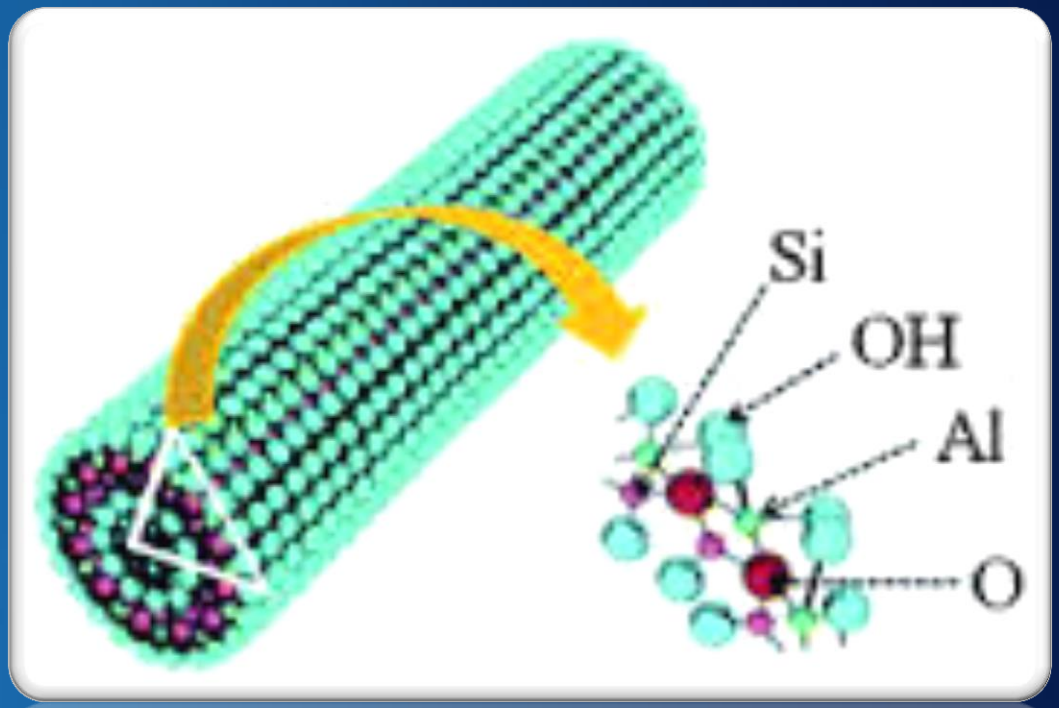
- convulsion provoked by inhibition of GABA synthesis, blockage of release or postsynaptic reaction



γ-Aminobutyric Acid (GABA)

- Major inhibitor neurotransmitter in central nervous system
- -COOH and $–NH_2$ groups
- Regulation of impulse transmission, induction of hypotensive, diuretic and tranquilizer effects
- Changes in GABA metabolism may play an important role in the origin and spreading of seizure activity in epilepsy

HALLOYSITE NANOTUBE (HNT)



- Tubular structure
- Good biocompatibility and very low cytotoxicity
- Two-layered aluminosilicate
- Outer diameter 50-100 nm, length 0.5-2 µm

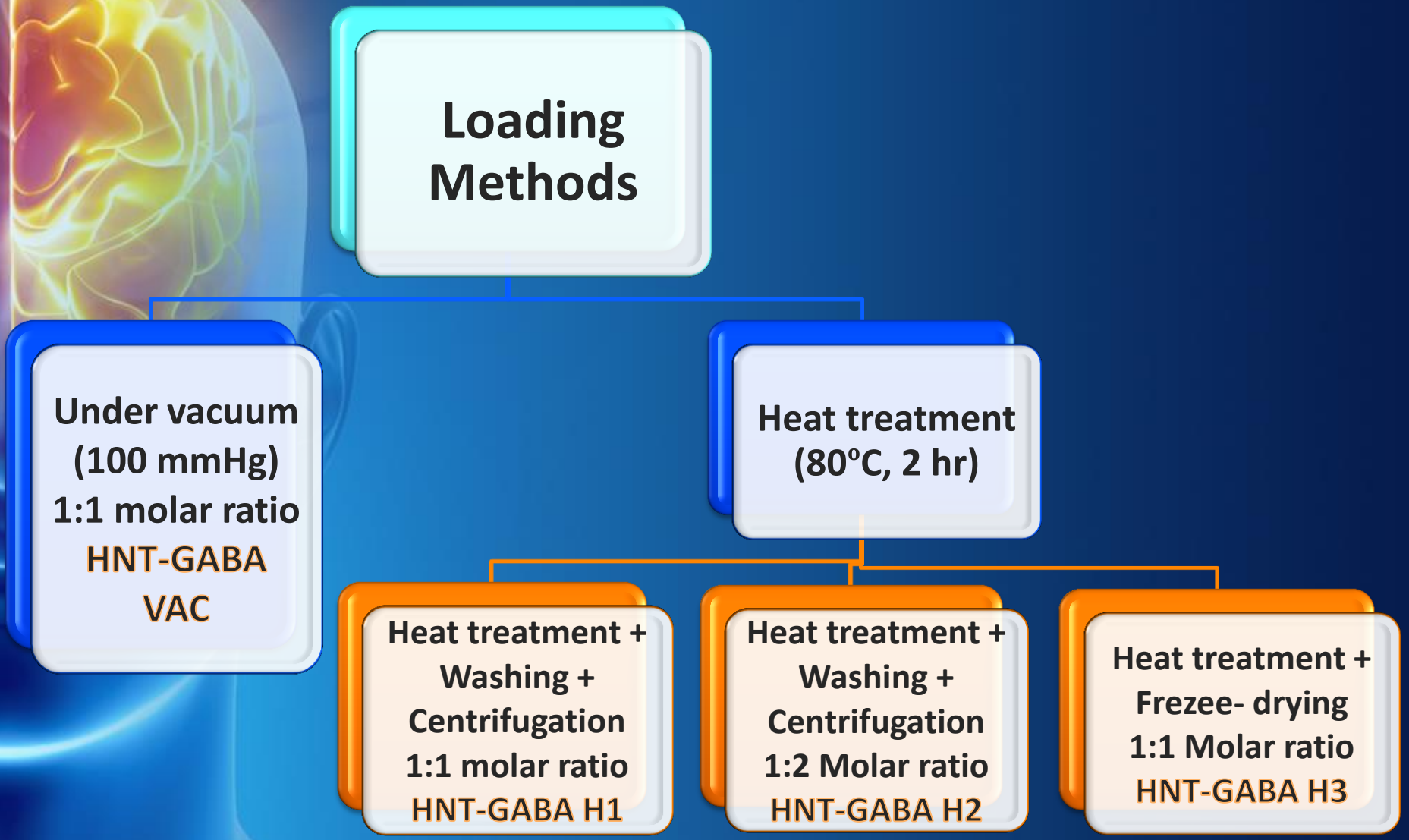


AIMS

- Incorporation of GABA into halloysite
  - nanosized
  - nontoxic
  - biocompatible
  - highly specific and high affinity formulations
- Providing GABA entrance into brain
- Modifying GABA release from formulations
- Minimizing serious side effects
- Enhancing patient compliance

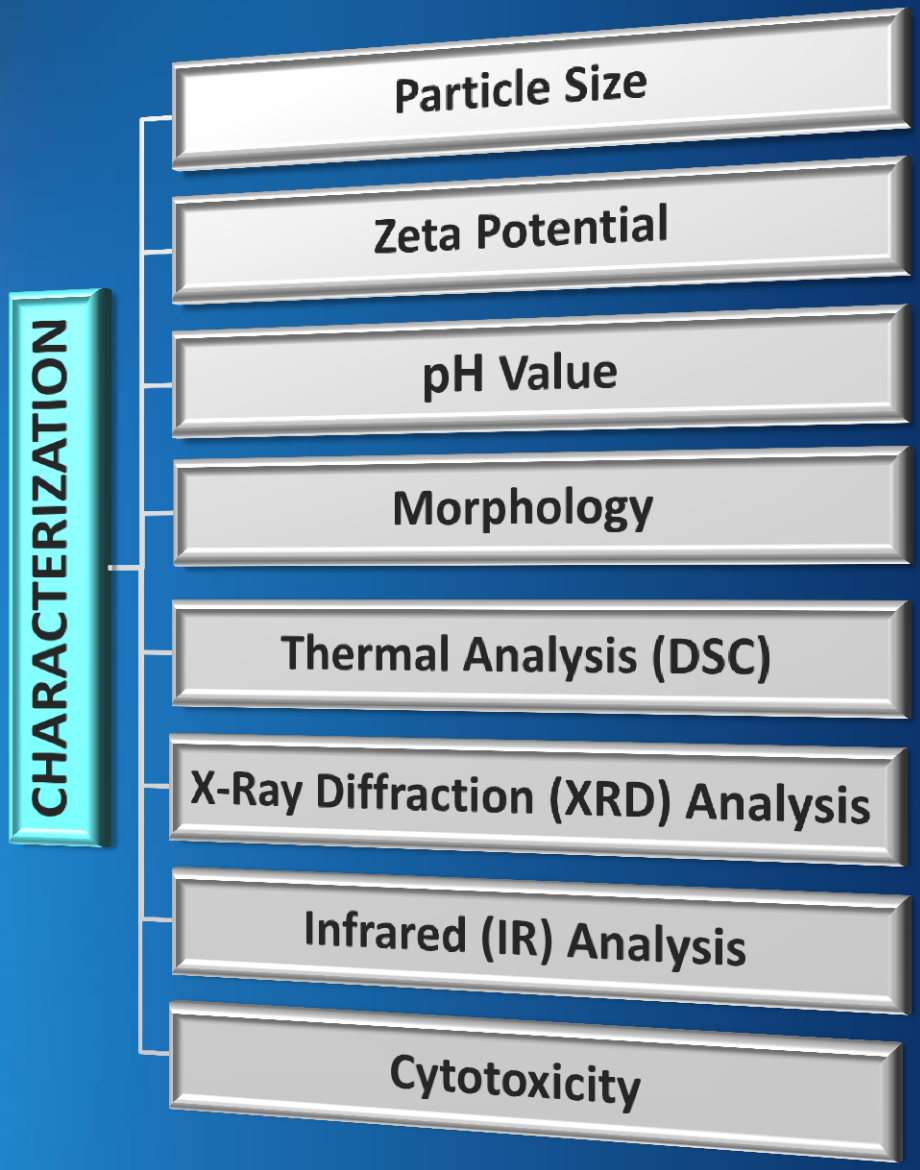

EXPERIMENTAL

PREPARATION of GABA-LOADED HNTs



CHARACTERIZATION



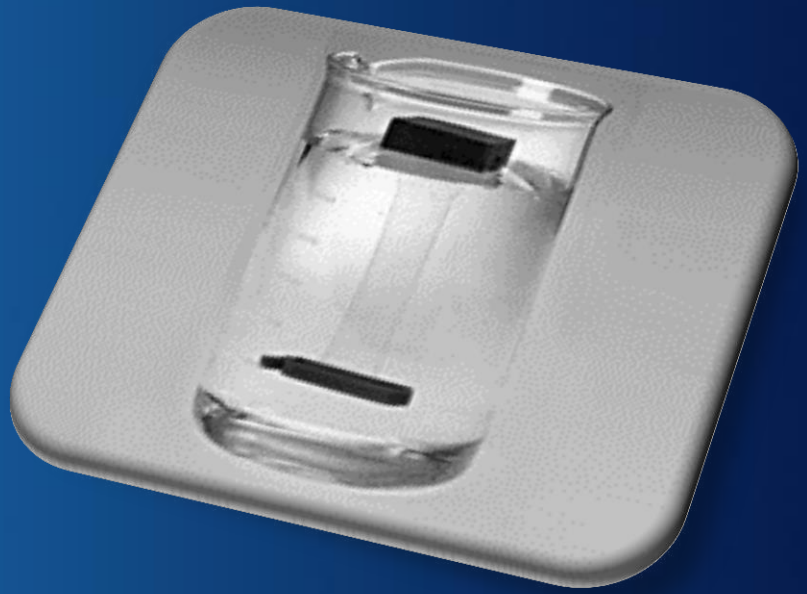


DETERMINATION of GABA in HNTS

Cihaz	Schimadzu- 20A
Column	4.6 x 100 mm, 3 µm C_{18} Inertsil CDS column, particle size 100 Å
Oven Temperature	30°C
Mobil Phase	Methanol:Na_2HPO_4 buffer (40:60) (pH 6.7)
Dedector	Fluorescence dedector
Wavelength	280 nm – 450 nm
Flow Rate	0.8 $mL.min^{-1}$
Injection vVlume	27 µL

In Vitro Drug Release

- Dialysis bag (3500-5000 Da)
- pH 7.4 phosphate buffer
- Validated HPLC method



IN VIVO STUDY PROTOCOL

Eskişehir Osmangazi University Scientific Ethical Committee
(Protocol No: 265/2012)

Wistar rats



Group Code	Treatment
Group 1	PBS (*ip*)
Group 2	GABA solution (100 $mg.kg^{-1}$) (*ip*)
Group 3	HNT-GABA H1 (100 $mg.kg^{-1}$ GABA) (*ip*)

IN VIVO STUDY PROTOCOL

- Pentylenetetrazole (70 mg/kg) induced epileptic model
- Latency to myoclonic jerks and incidence of generalized tonic clonic seizures with loss of righting reflex noted for 30 min
- Rats decapitated under ether anaesthesia
- Brains removed immediately and *Stratum corsatum* isolated
- GABA concentration in *Stratum corsatum* → ELISA kit

As a result of the analyses



RESULTS and DISCUSSION

CHARACTERIZATION

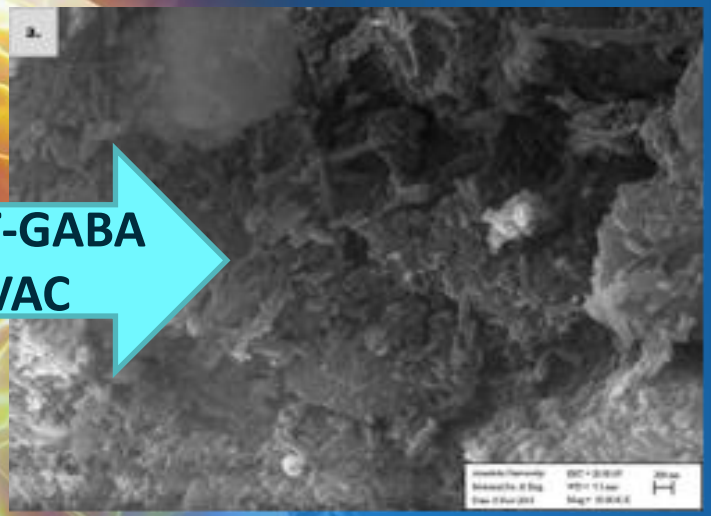
Particle Size, Zeta Potential, pH value

	HNT-GABA VAC	HNT-GABA H1	HNT-GABA H2	HNT-GABA H3	HNT (pure)
Particle size (nm)	554.20 ± 25.18	444.20 ± 2.36	530.70 ± 5.06	807.70 ± 10.72	446.80 ±23.71
PI	0.67 ± 0.02	0.27 ± 0.10	0.45 ± 0.07	0.68 ± 0.09	0.42 ± 0.06
Zeta potential (mV)	-32.70 ± 0.58	-28.80 ± 0.38	-25.30 ± 2.78	-30.20 ± 2.44	-22.30 ± 0.09
pH value	7.28 ± 0.01	7.42 ± 0.01	7.45 ± 0.00	7.29 ± 0.01	7.38 ± 0.00

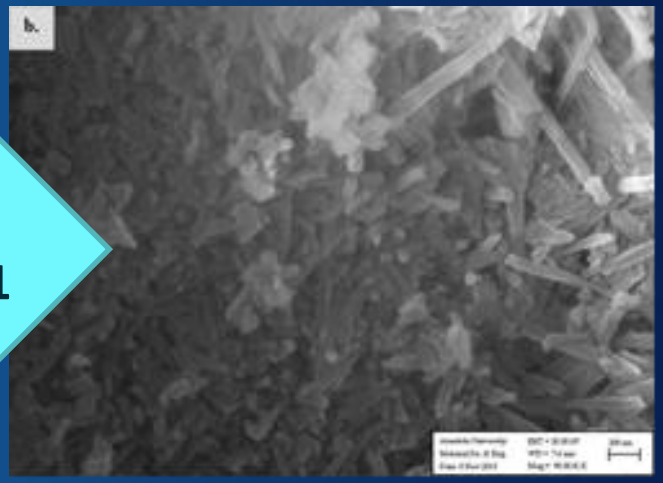
PI: Polydispersity index, SE: Standard error

CHARACTERIZATION- Morphology

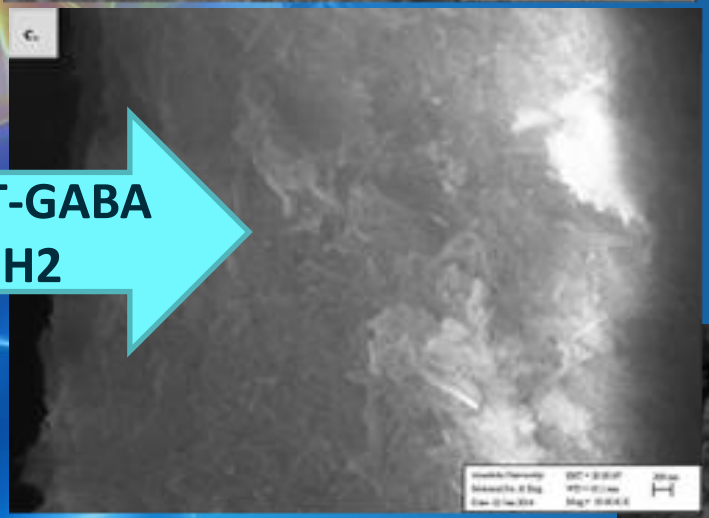
HNT-GABA VAC



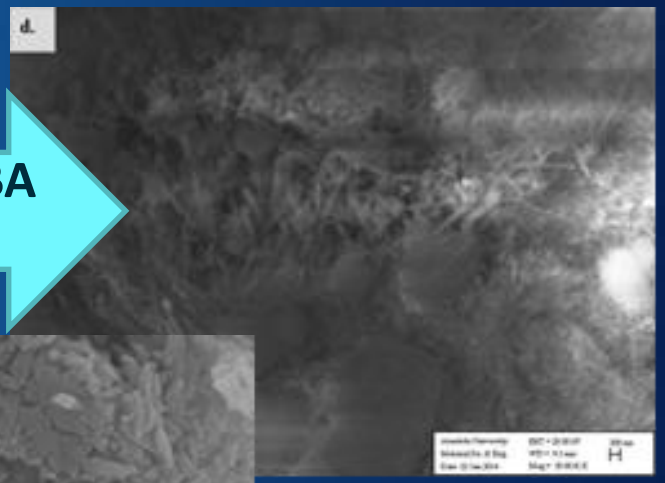
HNT-GABA-H1



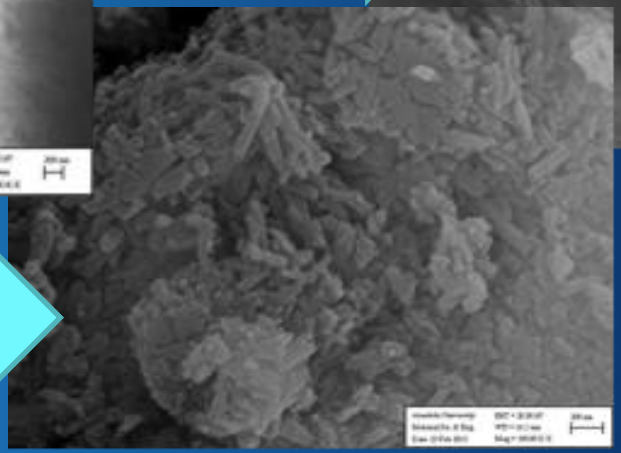
HNT-GABA H2



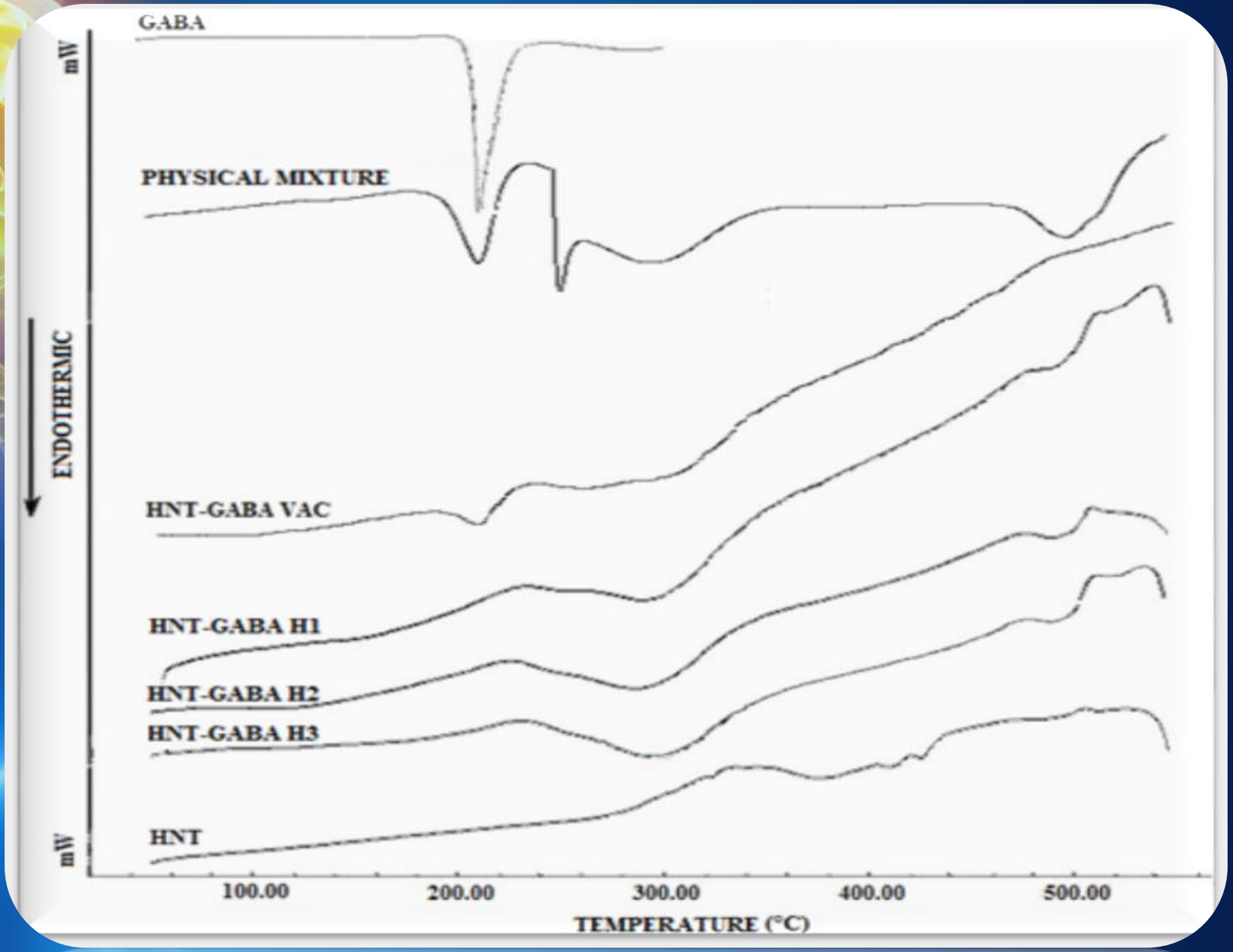
HNT-GABA H3



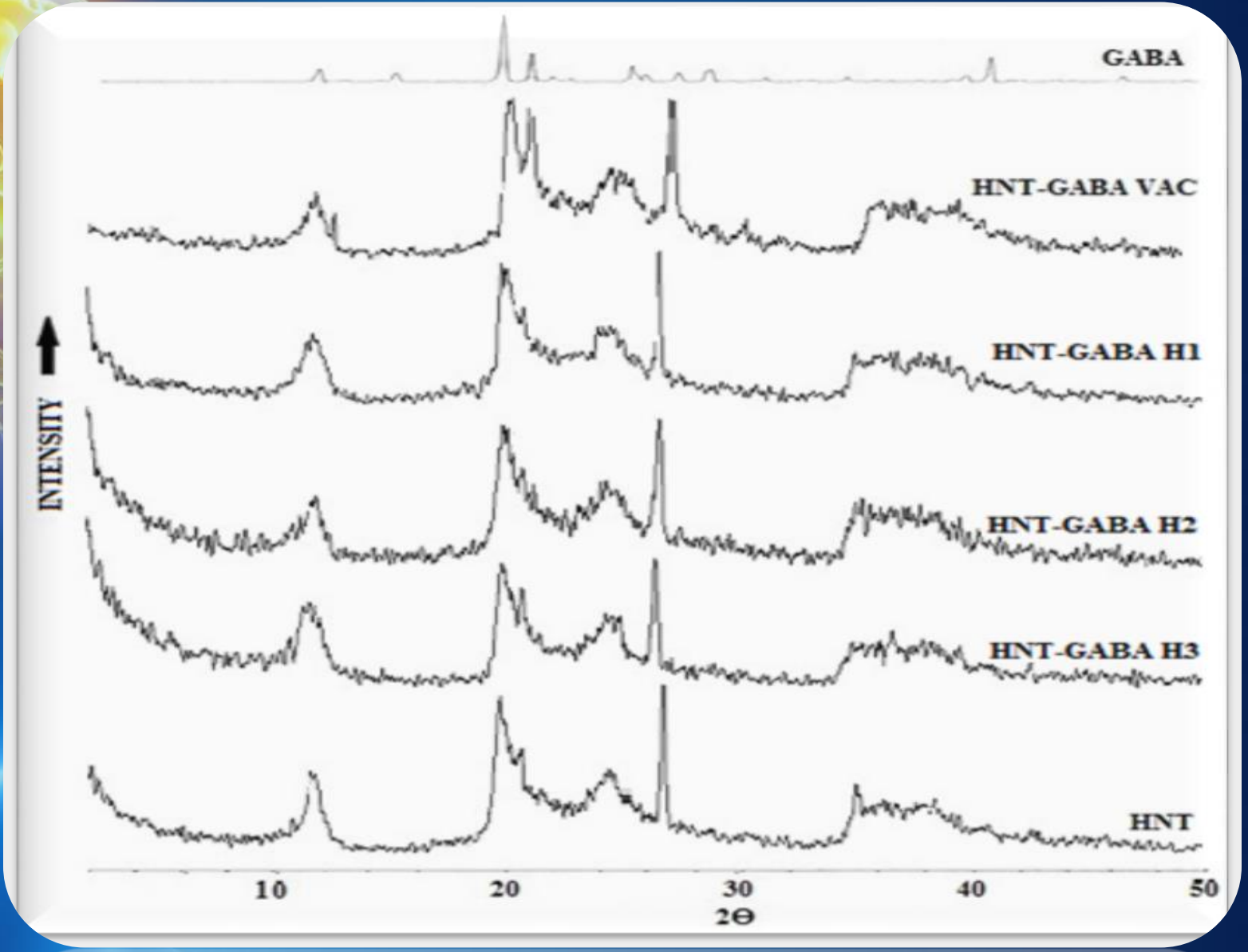
HNT (PURE)



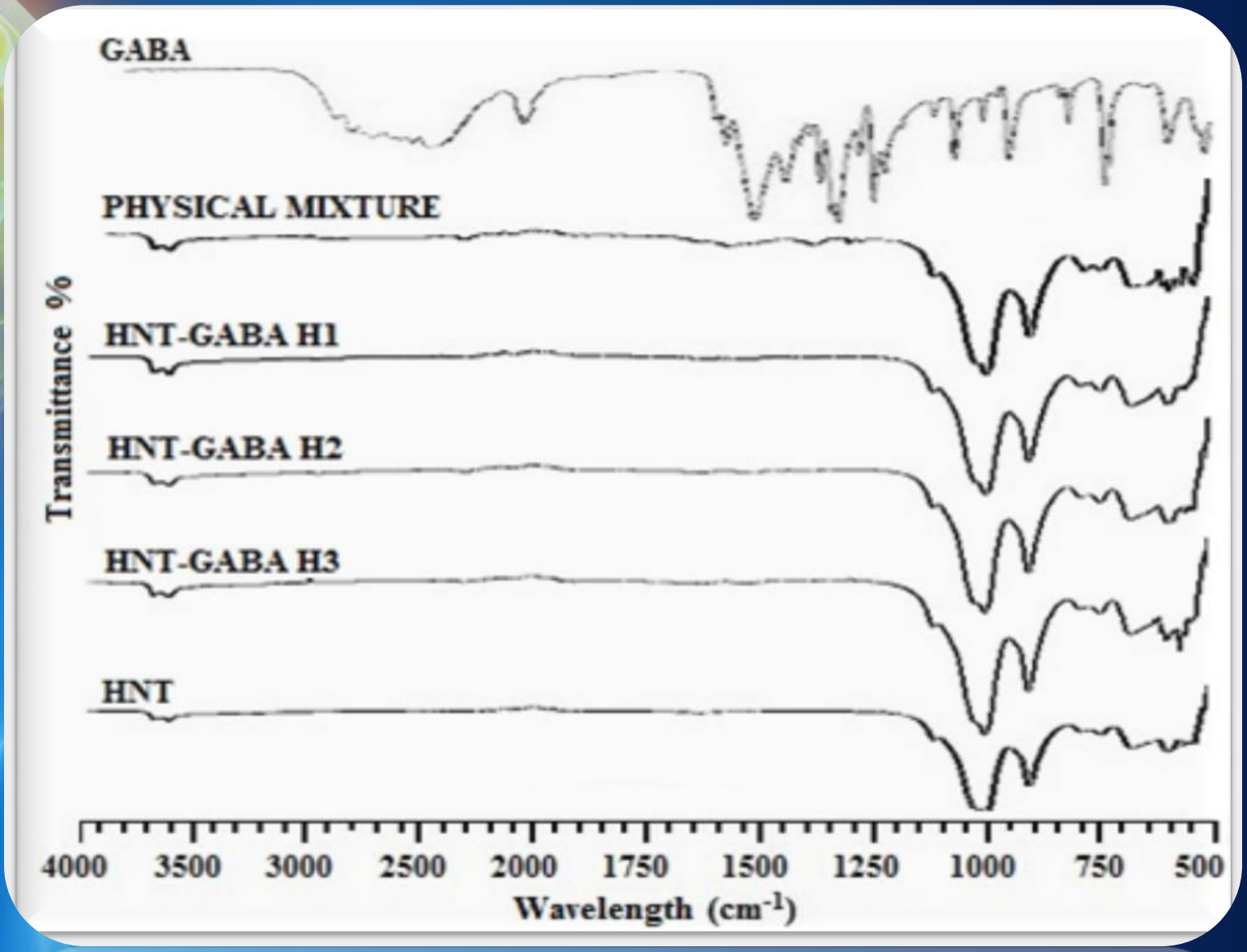
CHARACTERIZATION- Thermal Analysis



CHARACTERIZATION- XRD Analysis



CHARACTERIZATION- FT-IR Analysis



GABA loading of nanotube formulations is shown in the Table.

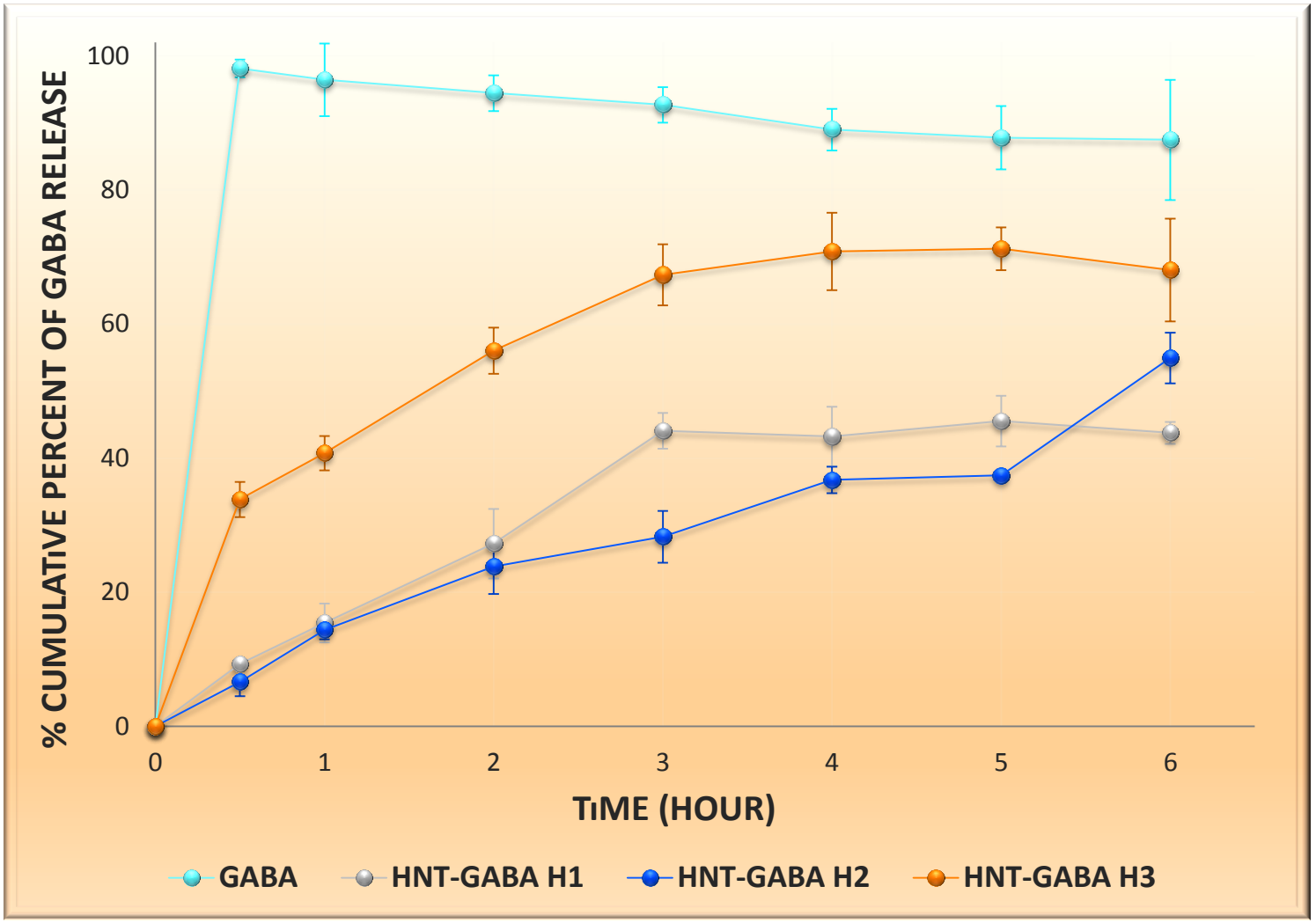
DETERMINATION of GABA in HNTS

	% GABA Loading ± SE
HNT-GABA H1	1.720 ± 0.030
HNT-GABA H2	0.733 ± 0.007
HNT-GABA H3	8.543 ± 0.106

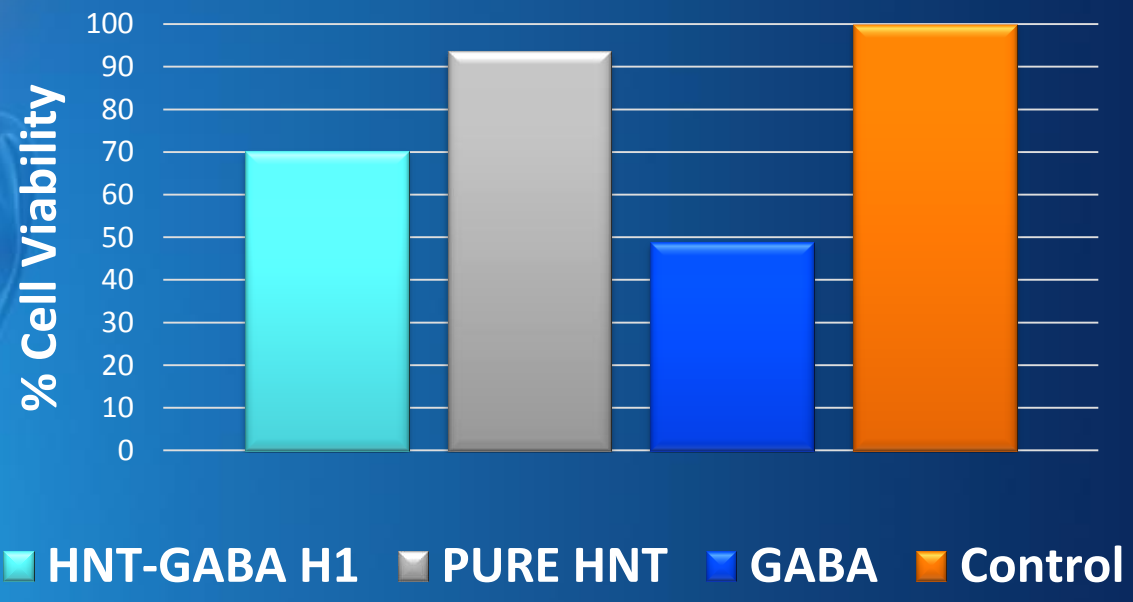


IN VITRO RELEASE STUDY

Dialysis Bag



CYTOTOXICITY





IN VIVO STUDIES

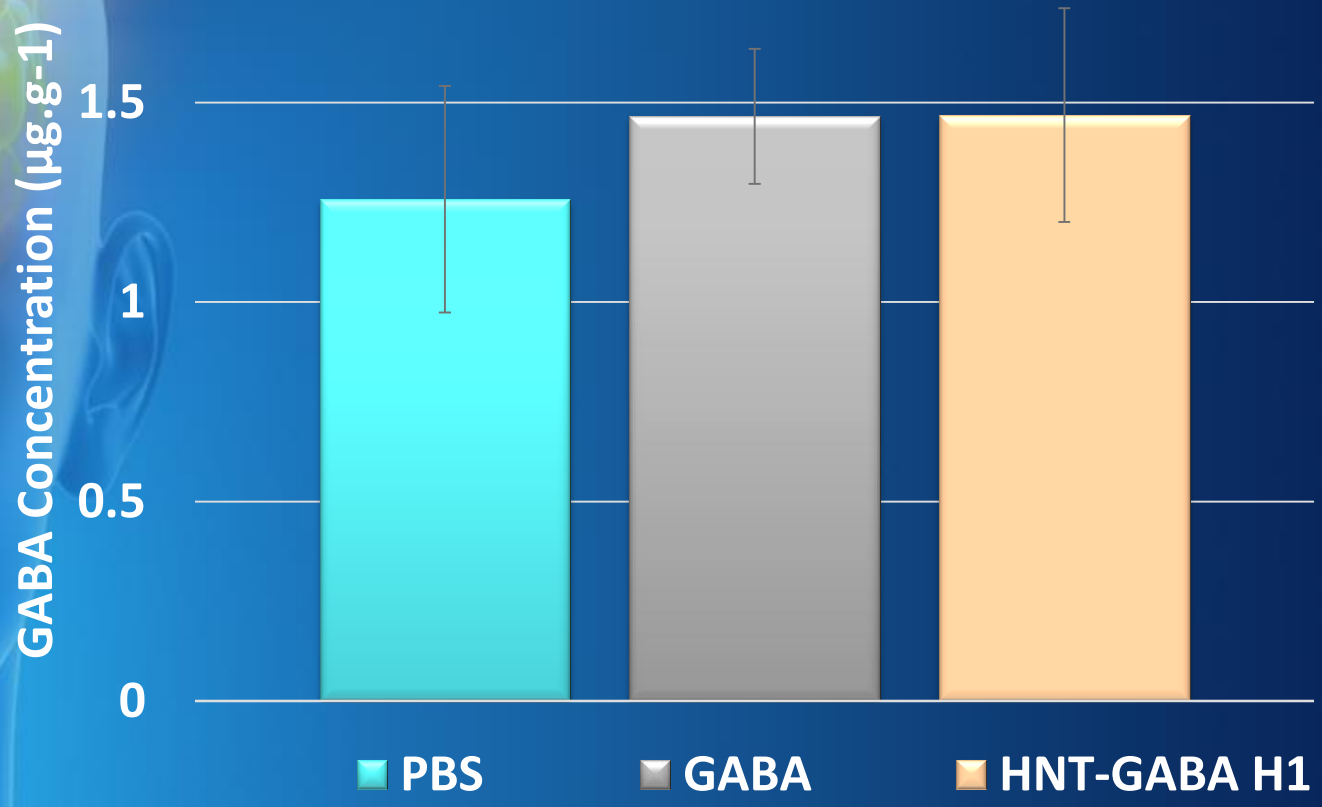
Seizure parameters	GABA Solution	PBS (pH 7.4)	HNT-GABA H1
Latency ± SD (min)	01.13 ± 00.13	01.12 ± 00.18	01.31 ± 00.48
Ending time of the convulsion ± SD (immobility) (min)	25.35 ± 04.43	21.21 ± 08.51	17.59 ± 09.36
Duration of severe convulsion ± SD (min)	02.37 ± 01.34	04.25 ± 01.57	01.35 ± 01.02
Time of death ± SD (min)	10.58	12:42	-
Mortality ratio (%)	4/7 (57.1)	3/7 (42.8)	0/7 (0)

PBS: Phosphate buffer saline, SD: Standard deviation

	HNT-GABA H1		
Groups	Latency ± SD (min)	Ending time of the convulsion ± SD (immobility) (min)	Duration of severe convulsion ± SD (min)
PBS	*	*	**
GABA solution	*	**	**

SD: Standart deviation, $p > 0.05$: No significant difference, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

GABA AMOUNT in Stratum Corsatum





CONCLUSION



HNT-GABA H1

- Nanosize
- Tubular shape
- Succesful incorporation of GABA
- Sustained release manner
- Low cytotoxicity

PTZ induced epileptic rat model

- Brain delivery and seizure inhibition
- Retardation of latency time
- Decrease in ending time of convulsion
- Decrease of duration of severe convulsion
- Reduction of mortality ratio



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