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Thyrotropin-Releasing Hormone Loaded and Chitosan Engineered Polymeric Nanoparticles: Towards Effective Delivery of Neuropeptides

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Thyrotropin-Releasing Hormone (TRH), a tripeptide amide with molecular formula L-pGlu-L-His-L-Pro-NH_2, is used in the treatment of brain/spinal injury and certain central nervous system (CNS) disorders, including schizophrenia, Alzheimer's disease, epilepsy, depression, shock and ischemia due to its profound effects on the CNS. However, TRH's therapeutic activity is severely hampered because of instability and hydrophilicity owing to its peptidic nature which results into ineffective penetration into the blood brain barrier. In the present study, we report the synthesis and stability studies of novel chitosan engineered TRH encapsulated poly(lactide-co-glycolide) (PLGA) based nanoformulation. The aim of such an encapsulation is to allow effective delivery of TRH in biological systems as the peptidase degrade naked TRH. The synthesis of TRH was carried out manually in solution phase followed by its encapsulation using PLGA to form polymeric nanoparticles (NPs) via nanoprecipitation technique. Different parameters such as type of organic phase, concentration of stabilizer, ratio of organic phase and aqueous phase, rate of addition of organic phase were optimized, tested and evaluated for particle size, encapsulation efficiency, and stability of NPs. The TRH-PLGA NPs were then surface modified with chitosan to achieve positive surface charge rendering them potential membrane penetrating agents. PLGA, PLGA-TRH, Chitosan-PLGA and Chitosan-PLGA-TRH NPs were characterized and analyzed using Dynamic Light Scattering (DLS), Transmissiom Electron Microscopy (TEM) and Infra-red spectroscopic techniques.

Keywords: Polymeric Nanoparticles, Thyrotropin-Releasing Hormone, Poly(lactide-co-glycolide) PLGA, Chitosan, Neuropeptides, Nanoformulation.

1. INTRODUCTION

Neurological disorders are increasingly becoming one of the major causes of diseases leading to fatal consequences across the globe. As per a recent report by World Health Organization (W.H.O.), neurological-based stroke kills nearly 6.2 million people each year, out of which more than 80% die in third world countries.[1] It is estimated that there are more than 50 million people that suffer from epilepsy worldwide.[2] The role of neuropeptides in the treatment of neurological diseases has become an important area of research over the past two decades.[3] This includes the use of neuropeptides as primary pharmacological agents in neurological disorders such as epilepsy, making this approach an attractive proposition due to enhanced potency and low toxicity of metabolites.[4]

Thyrotropin-releasing hormone (TRH), which is a simple neuropeptide with molecular formula L-pGlu-L-His-L-Pro-NH_2 (Fig. 1), is synthesized in the hypothalamus[5] and acts in the anterior pituitary to control levels of thyroid-stimulating hormone (TSH, thyrotropin) and prolactin.

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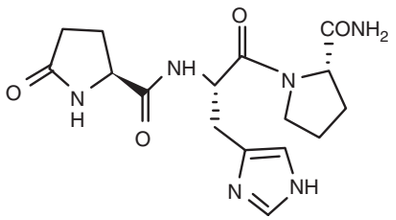


Figure 1. Chemical structure of thyrotropin-releasing hormone (TRH).

Apart from the neuroendocrine action of TRH on the anterior pituitary, TRH exerts profound effects on the central nervous system (CNS).[6] As a result of these effects, TRH has been shown to alter neuronal excitability,[7] enhance transmitter release and turnover,[8] increase CNS arousal, increase blood pressure,[9] body temperature[10, 11] and respiration rate,[12, 13] alter body water and food intake, enhance locomotor activity, and produce antinociception.[14, 15]

Most of the potential therapeutic applications of TRH are based on its broad spectrum of stimulatory actions within the CNS. Moreover, TRH has been found beneficial in the treatment of Alzheimer's disease,[16] depression,[17, 18] epilepsy,[19–21] spinal trauma[22] etc. The use of TRH as a CNS-active agent is, however, hampered by factors such as its short half-life that does not allow its effective penetration into blood brain barrier.[23] Also TRH's endocrine effects are usually manifested at doses causing significant cognitive improvement.[24]

To address these issues, there have been attempts to produce TRH-like compounds[25, 26] for separation of its CNS and hormonal effects and to increase the drug availability at the target site. However, the therapeutic efficacy of TRH and similar compounds is compromised by instability and hydrophilic nature owing to its peptidic nature. The access of TRH to the CNS following oral or parenteral administration is limited because of rapid degradation by enzymatic action through proteolytic cleavage. These factors lead to administration of high dosage of peptide for obtaining neuropharmacological effects often resulting in adverse side effects due to the endocrine actions of TRH.[27]

Over the recent times, there has been an increased interest in nanosizing and particle manipulation of selected drugs. In particular, polymeric nanoparticle formulation has gained an increasing amount of attention in the fields of drug delivery and pharmaceutics in recent years.[28] There has been an immense focus on the industrial utilization of biodegradable polymer based nanoparticles as effective drug delivery agents because of their ability to prolong drug release, increase drug bioavailability, decrease drug degradation and reduce drug toxicity.[29] In this context, the nanoparticle drug formulation research has focused on the use of poly(lactic acid) (PLA), poly(D,L glycolide) (PLG), and poly(lactide-co-glycolide) (PLGA) based nanoparticles because of their tissue compatibility, low toxicity, and high rate of hydrolysis.[30] In the same context, the use of PLA for encapsulation of TRH has been recently utilized with good results.[31] The success of polymeric nanoparticle formulation encouraged us to explore the encapsulation of TRH using PLGA nanoparticles (PLGA NPs) and to study the effect of such modification on the physicochemical nature of the resulting formulation. Hence, in this paper we report a nanoparticle system that will also help in improving the stability as the polymeric nanoparticles (PLGA) protect the peptide drug from proteolytic cleavage by enzymes and thereby help in achieving low drug dosage regime.

Further, chitosan, a polysaccharide based on repeating units of randomly distributed *D*-glucosamine and *N*-acetyl-*D*-glucosamine, has been employed for the surface modification of PLGA NPs. The easy availability of free amino groups in chitosan provides enough positive charge that allows it to bind negatively charged surfaces/polymers and also undergo chelation with metal ions. Moreover, the electrostatic adhesion to PLGA takes effect as the chitosan surface is positively charged while the PLGA with carboxyl groups has a negative surface charge. This surface modification leads to reversal of surface charge as negatively charged PLGA becomes positively charged on account of the free amino groups of chitosan. In the present study, nano-precipitation technique has been evaluated for the physical encapsulation of TRH in PLGA NPs and further binding to chitosan (Fig. 2). Different variables involved in the nano-precipitation method for synthesis of nanoparticles have been optimized, tested and evaluated for particle size, encapsulation efficiency, and stability. To best of our knowledge, no studies have been conducted so far to examine or improve the physical loading of TRH in PLGA/chitosan-PLGA NPs.

2. EXPERIMENTAL SECTION

2.1. Materials

PLGA (75:25) [mol wt (66,000–107,000)], Kolliphor P 188, L-Prolinamide, L-Pyroglutamic acid, Boc-Histidine-OH, *N*,*N*-Diisopropylethylamine (DIPEA), *N*,*N*′-Diisopropylcarbodiimide (DIC), *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB), Acetone, Acetonitrile, Tetrahydrofuran (THF), Dichloromethane (DCM), and *N*,*N*-Dimethylformamide (DMF) were purchased from Sigma-Aldrich (India). Ultrapure type I water of 18 Ωm resistivity used for experiments was obtained from Millipore Direct Q3 system. All other chemicals used were of reagent grade.

2.2. Synthesis of Thyrotropin-Releasing Hormone (TRH)

Boc-L-His-OH (1.2 mmol) (**1**) was dissolved in about 10–12 mL of dry DMF followed by addition of 1.8 mmol (1.5 equiv.) of HONB and DIC each and the reaction mixture was stirred for 15 minutes (Scheme 1). This was followed by addition of 1.8 mmol (1.5 equiv.)

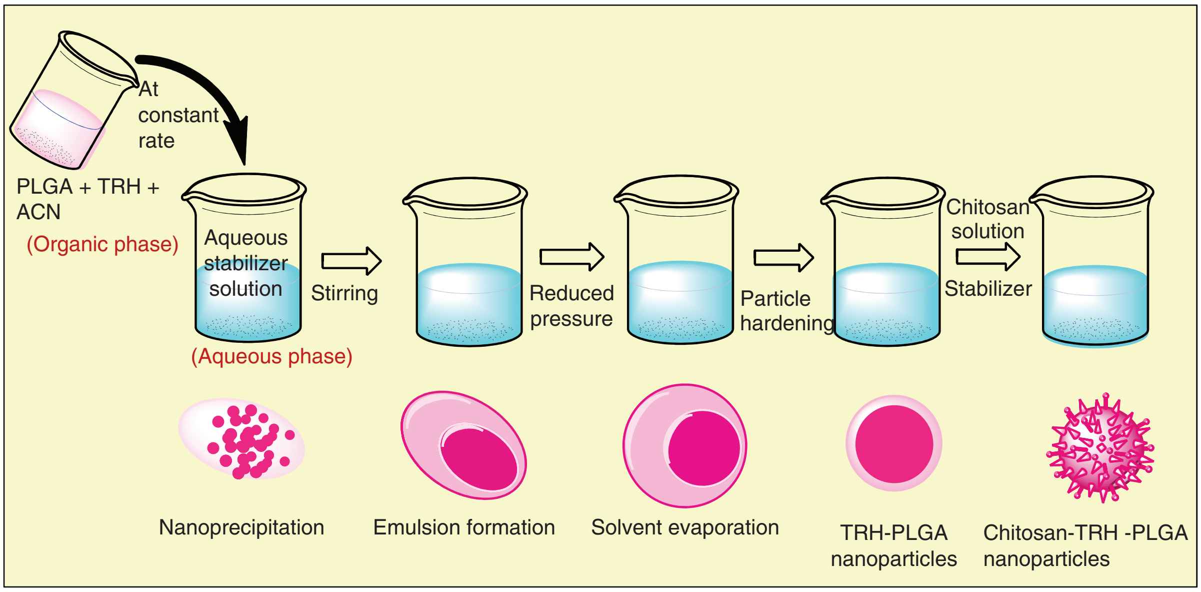
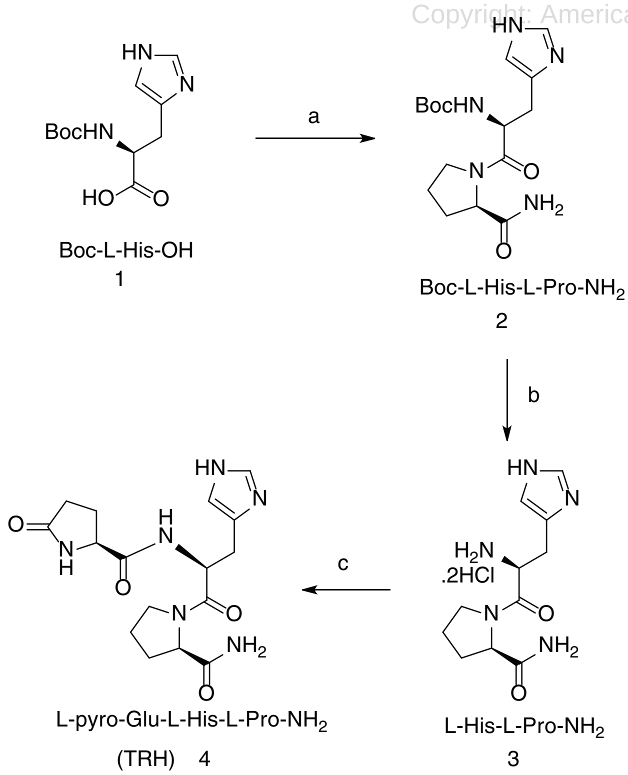


Figure 2. Schematic diagram showing various stages involved in the preparation of chitosan-TRH-PLGA nanoparticles using nanoprecipitation technique.

of L-Prolinamide and reaction mixture was stirred for 36 h at room temperature to afford Boc-L-His-L-Pro-NH_2 (**2**), which was purified using column chromatography. Further, the Boc group was deprotected using 10 mL of 7*N*-methanolic HCl and the resulting substrate was neutralized using 3 equiv. of DIPEA and then coupled with L-Pyro-Glu-OH in the presence of HONB (1.5 equiv.) and DIC (1.5 equiv.) under constant stirring for 36 hours at room temperature to obtain L-pGlu-L-His-L-Pro-NH_2 (**4**). The final product **4** was purified using column chromatography and all the synthesized compounds were analyzed and characterized at each stage using ^{1}H-NMR and mass spectrometry.



Scheme 1. Synthesis of TRH (a) DMF, DIC, HONB, Pro-NH_2, 48 h, rt (b) 7*N*-methanolic HCl (c) (i) DIPEA (3 equiv.) (ii) DIC (1.5 equiv.), HONB (1.5 equiv.), *p*-Glu-OH (1.5 equiv.), DMF, 24–36 h, rt.

2.3. Synthesis of Polymeric Nanoparticles

To synthesize the polymeric nanoparticles, the nanoprecipitation technique with necessary modifications was employed.[32]

2.3.1. Synthesis of TRH-PLGA and PLGA NPs

For TRH-PLGA NPs, the polymer (PLGA) and drug (TRH) were dissolved in a suitable organic solvent at a concentration of 2.5 mg/mL and at various concentrations (0.5–2 mg/mL), respectively to form the diffusing phase. Further, 4 mL of this phase was then added to the dispersing phase (10 mL) at a specific rate by means of a peristaltic pump positioned with the output tip directly in the medium under moderate magnetic stirring. The dispersing phase was constituted from a liquid in which the polymer is insoluble [here water—the non-solvent (NS) containing a surfactant (Kolliphor P 188)] at various concentrations followed by stirring for 3–4 h, which allowed complete evaporation of organic solvent. Finally, traces of organic solvent were eliminated under reduced pressure at 40 °C for 30 min. For PLGA NPs, TRH was omitted in the first step with other steps followed as such in the above procedure.

2.3.2. Synthesis of TRH-PLGA-Chitosan NPs

A similar protocol, as reported in last section, was followed for the preparation of chitosan coated PLGA nanoperticles.[33] Initially, chitosan solution (1 mg/mL) was prepared by dissolving chitosan in 0.25 M HCl containing stabilizer at pH 5 as it was only sparingly soluble in water at normal pH. Then, 4 mL of chitosan solution so prepared was added to 10 mL of PLGA nanoparticles solution and the reaction mixture was incubated for 3–4 hours to get chitosan coated PLGA (PLGA–CS) nanoparticles. The freshly prepared nanoparticles were then centrifuged four times for 15-min cycles at 6000 rpm and washed with distilled water, in order to remove the unbound chitosan.

2.4. Entrapment Efficiency and Drug Loading of TRH-Loaded Nanoparticles

The amount of TRH present inside the nanoparticles was estimated by using reverse phase high performance liquid chromatography (RP-HPLC) (Waters 515HPLC model). Briefly, 10 mL of prepared nanoparticles formulation was centrifuged at 10,000 rpm. The supernatant collected was analyzed using HPLC to determine amount of untrapped TRH. 20 μl of each sample was injected manually in the injection port and was analyzed using a mobile phase of acetonitrile-water-triflouroacetic acid mixture in the ratio 15:85:0.1. TRH level was quantified by UV detection at 215 nm (photodiode array detector) with flow rate of 1 mL/min using HPLC pump (Waters 2996). The amount of TRH in nanoparticles was determined from the peak area correlated with the standard curve prepared under identical conditions. The encapsulation efficiency (E.E.%) was calculated by subtracting the amount of free drug in the clear supernatant from the total amount of TRH added to the nanosuspension.

2.5. Particle Size Analysis

Mean particle size of the nanoparticles was determined by photon correlation spectroscopy (PCS)/Dynamic light scattering (DLS) using a Zeta Sizer (Nano S-90, Malvern Instruments, UK). For size measurements, various formulations of nanoparticles were further diluted with water to 50% and measured for a minimum of 120 s. All the samples were maintained at a constant temperature of 25.0 ± 0.1 °C, in all experiments and each batch was analyzed in triplicates.

2.6. Transmission Electron Microscopy (TEM) Studies

Particle morphology was examined by transmission electron microscopy (TEM) using Hitachi (Model H-7500) transmission electron microscope operating in accelerating voltage of 120 kV. Samples (nanoparticulate suspensions) were dropped onto carbon-coated copper grids, and after complete drying, the samples were stained using 1% w/v phosphotungstic acid.

2.7. Zeta Potential Determination

The Zeta potential of nanoparticles was determined by using Zeta Sizer (Nano S-90, Malvern Instruments, UK). The samples were placed in an electrophoretic cell, where a potential of ±150 mV was established and analyzed. The samples were maintained at a constant temperature of 25.0 ± 0.1 °C, in all experiments.

2.8. Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR transmission spectra of various samples (chitosan, PLGA, TRH-PLGA-CS nanoparticles) were obtained using a Thermo Scientific (Nicolet iS50 FT-IR) spectrophotometer. TRH-PLGA-chitosan nanoparticles were lyophilized to yield white powder, which was used as such for FTIR analysis. PLGA, chitosan and TRH were also analyzed in powder form. Each sample was then scanned over wave number region of 400–4000 cm^{-1} and the characteristic peaks for different samples were recorded.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Thyrotropin-Releasing Hormone (TRH)

The solution phase based chemical synthesis of the TRH was carried out in three steps. Initially, Boc-His-OH (**1**) was coupled with L-prolinamide to obtain protected dipeptide (**2**), which was subjected to Boc-deprotection step leading to the formation of dipeptide (**3**) followed by coupling with pyroglutamic acid to yield TRH (**4**) (Scheme 1).

3.2. Synthesis of Polymeric Nanoparticles: Investigation of Parameters Influencing Nanoprecipitation

The polymeric nanoparticle preparation for effective uptake by cells requires modulation of various parameters. Size is one of the most important and effective parameter in determining the cellular uptake and biological functions of colloidal systems with general assumption that a reduced size will usually result in significantly better uptake of NPs by cells[34] or better passage through membrane. Moreover, it has been earlier demonstrated that emulsion fabrication is a critical step during the preparation of NPs as the size of emulsion droplets is directly relevant to final nanoparticle size.[35, 36] Various other factors that may affect the size of polymeric NPs such as PLGA NPs include the choice and concentration of stabilizer used as well as the type of organic solvent used.[37] Keeping these facts in mind, synthetic and physicochemical studies were carried out for generating a polymeric nanoparticle system based on PLGA for the improved physical loading of TRH thereby augmenting the stability of peptide drug and achieving low drug dosage regime.

For the synthesis of PLGA NPs, PLGA was dissolved in various volatile organic solvents. The organic phase was

then added to the buffer solution with continuous stirring at room temperature for 3–4 h to allow complete evaporation of the organic solvent followed by evaporation at reduced pressure. Similarly, the preparation of TRH-loaded nanoparticles was carried out in the same manner by adding TRH along with PLGA. In order to achieve the optimum particle size and high drug entrapment efficiency during synthesis of polymeric NPs, effect of following parameters was studied.

3.2.1. Effect of Organic Phase

To develop a successful formulation system comprising of biodegradable NPs containing a pharmaceutical agent, the choice of organic solvent becomes a critical step. While choosing the suitable organic solvent, the parameters that are most importantly considered include the ability of organic solvent to dissolve polymer as well as drug and its miscibility with water (for oil in water emulsion). Toxicity of the solvent chosen is also an important factor for consideration as a trace amount of solvent is always left in the final nanoparticle formulation prepared. Generally, organic solvents that have low or no solubility in water are considered to yield good quality spherical NPs with non-porous and thus non-leaky surfaces. In addition, size distribution of NPs as well as encapsulation efficiency is closely affected by the ability of solvent to dissolve large amount of polymer.[38–40]

Thus, we envisioned a study in which different organic solvents (i.e., acetone, acetonitrile, tetrahydrofuran and dichloromethane) were used as the organic phase to analyze the effect of solvent on particle size during NPs formation. The bar graph shows the effect of different solvents on particle size evaluated using dynamic light scattering (DLS) (Fig. 3(A)). Although THF is considered relatively non-toxic among solvents under consideration but the size of NPs obtained using THF was very large as seen in the plot graph (Fig. 3(A)). On the other hand acetonitrile,[41] which also exhibits negligible toxicity in trace amount, displayed minimum size of NPs in formulation and hence it was chosen as a solvent of choice for further studies.

3.2.2. Effect of Ratio of Organic Phase to Aqueous Phase on Particle Size

Ratio of organic solvent with respect to aqueous phase plays an important role in nanoparticle formation. Using acetonitrile as organic solvent, different organic to aqueous phase ratios (1:10, 3:10, 5:10, 7:10, 9:10) were studied to optimize particle size (Fig. 3(B)). The general observation was that an increase in the organic phase to aqueous phase ratio led to a slight decrease in mean diameter of nanoparticles as the ratio was increased from 1:10 to 5:10. This trend was followed till the ratio 5:10, beyond which the particle size started to increase. Hence it was evident from the plot that the particle size was least in case of 5:10 ratio

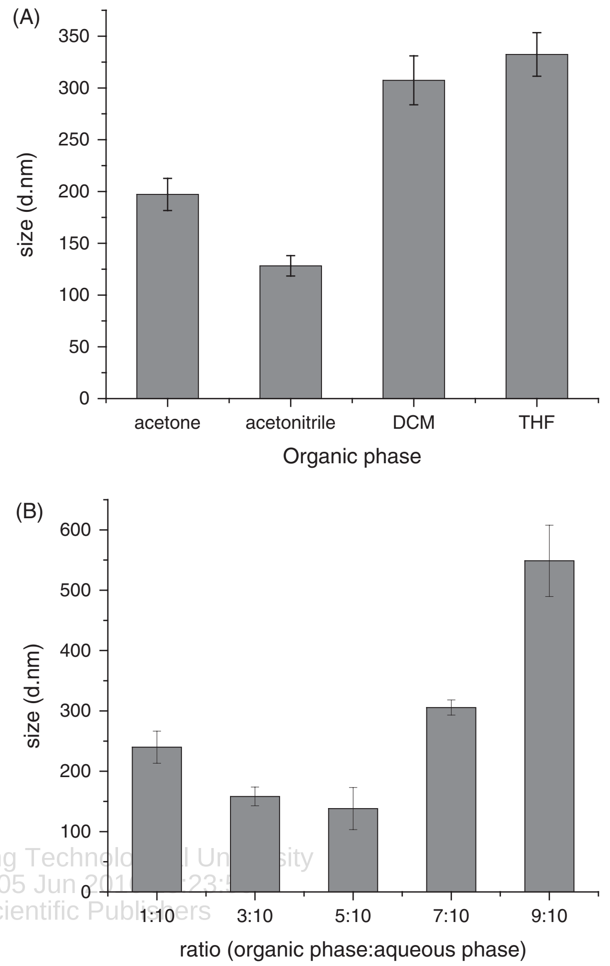


Figure 3. Bar graph showing the variation in size (diameter in nm) of PLGA nanoparticles with change of organic solvent (A) and variation in size (diameter in nm) of PLGA nanoparticles with change of ratio of organic phase to aqueous phase used (B) as obtained using particle size analyser.

of organic phase to aqueous phase (Fig. 3(B)). Therefore, this concentration was chosen as the optimized condition for further studies.

3.2.3. Effect of Rate of Addition of Organic Phase

During optimization studies, it was found out that even the rate of addition of PLGA dissolved in organic phase to aqueous phase had significant effect on the particle size. Keeping this in mind, experiments were performed in which variation in size of NPs with change of rate of addition of organic phase was studied and the corresponding results have been illustrated in a bar graph (Fig. 4(A)). It is clearly evident from these results that at a lower rate of addition of organic solvent phase to dispersion phase (aqueous phase) leads to formation of smaller and uniform particles in nano-precipitation process. The rate 0.5 ml/min was found out to be appropriate for synthesis of small sized and monodispersed NPs.

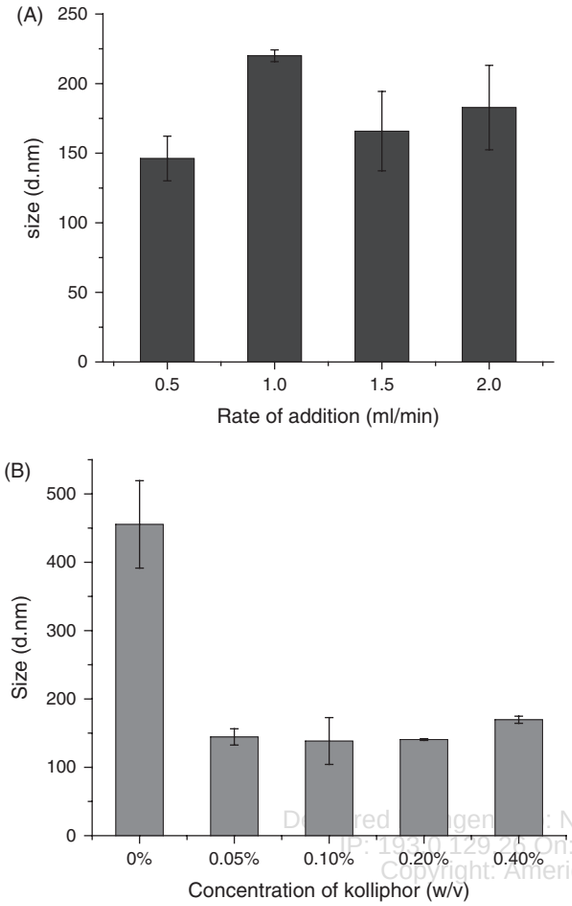


Figure 4. Bar graph showing the variation in size (diameter in nm) of PLGA nanoparticles with change of rate of addition of organic phase (A) and variation in size (diameter in nm) of PLGA nanoparticles with change of concentration of stabilizer (B) as obtained using particle size analyser.

3.2.4. Effect of Stabilizer on Properties of Synthesized Nanoparticles

The amount of stabilizer plays an important role in the prevention of agglomeration of particles.[42,43] NPs were formulated with five different concentrations of buffer solution (also acting as the stabilizer) i.e., 0%, 0.05%, 0.1%, 0.2% and 0.4% to study the effect of stabilizer in properties of NPs. In the present study, an increase in concentration of the stabilizer (Kolliphor P188) from 0 to 0.05% w/v made a significant decrease in the size of the NPs prepared with the synthesized polymer (Fig. 4(B)). The results exhibited the variation of particle size with increasing concentration of stabilizer and it was observed that 0.05% concentration of stabilizer resulted in small and more stable NPs. As the concentration of stabilizer is increased to higher values the particles size gradually begins to increase. This might be due to the fact that at higher concentrations, the increased viscosity of the aqueous phase might cause a hindrance to the energy input used for the size reduction of the droplets resulting in bigger organic phase droplets, which consequently led to increased particle size. Thus, it was found that the stabilizer concentration is crucial in controlling the particle size of synthesized NPs.

3.3. Nanoparticle Shape and Surface Morphology

Shape, size and surface morphology of unloaded PLGA NPs as well as TRH-loaded PLGA NPs were examined with the use of Transmission Electron Microscope (TEM) (Fig. 5). Initially, the NPs were purified by repeated redispersion cycles and then centrifuged at 2000 rpm followed by examination using TEM at 80 kV. The particle size was found out to be approximately 60 nm for unloaded PLGA NPs (Fig. 5(A)) and approximately 65 nm for TRH-loaded PLGA NPs (Fig. 5(B)).

In comparison to unloaded and TRH-loaded PLGA NPs, chitosan coated NPs showed increase in size as the average diameter for these NPs was found out to be about 90 nm (Figs. 5(C and D)). This increase in size of NPs also confirms the successful surface coating of chitosan layer over PLGA NPs. The hydrodyanamic diameter obtained using DLS of TRH-PLGA NPs and TRH-PLGA chitosan NPs displayed the similar trend of increasing size. However, sizes of the NPs observed using DLS were found to be much larger as compared to sizes observed in TEM images (Table I). This may be due to the large polymeric nature of NPs, which leads to much larger virtual hydrodynamic circumference around the nanoparticles in solution form.

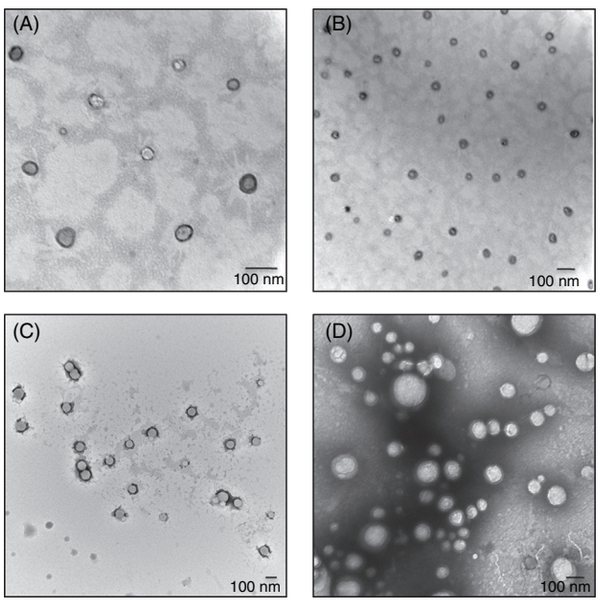


Figure 5. TEM images of unloaded PLGA nanoparticles (A), TRH-loaded PLGA nanoparticles (B), unloaded PLGA nanoparticles surface coated with chitosan (C) and TRH loaded PLGA nanoparticles surface coated with chitosan (D) prepared by nanoprecipitation technique.

Table I. Physicochemical characterization parameter values of TRH Loaded NPs.

	Hydrodynamic diameter (nm) (DLS)	Polydispersity index (PDI)	Size (nm) (TEM)	Zeta potential (mV)	Entrapment efficiency (EE) (%)	Drug loading (DL) (μg/mg)
PLGA NPs	128.9	0.178	60	−23.5	–	–
TRH-PLGA NPs	133.3	0.103	65	−10.3	30.73	2.56
TRH-PLGA-Chitosan NPs	269.1	0.227	90	+19.9	29.71	2.01

3.4. Physicochemical Characterization of PLGA, TRH-PLGA and TRH-PLGA-Chitosan Nanoparticles

The encapsulation efficiencies of TRH loaded PLGA NPs and PLGA-CS NPs were determined by using High Performance Liquid Chromatography (HPLC). Table I shows the encapsulation efficiency at various types of nanoparticles synthesized. All the encapsulation efficiency (EE) values were calculated using formulas mentioned below and standard calibration curve was plotted using known quantities of TRH employing area under the peak values obtained from HPLC. The encapsulation efficiency (EE) and drug loading (DL) were calculated as follows:

$$\text{EE\%} = (\text{Total amount of TRH used} - \text{Amount of TRH in supernatant } (\mu\text{g})) \times (\text{Total amount of TRH used (mg)})^{-1} \times 100$$

$$\text{DL (w/w)} = \frac{\text{Amount of TRH in nanoparticles } (\mu\text{g})}{\text{Amount of PLGA (mg)}}$$

The amount of TRH used in the nanoparticle preparation was also first optimized to get maximum efficiency. It can be noted that EE of 30.73% was observed in TRH-PLGA loaded NPs when amount of TRH used was 2 mg per 6 mL of total NPs prepared and the drug loading was found out to be 2.56 μg/mg of PLGA. Whereas in case of chitosan coated PLGA NPs, the encapsulation efficiency was 29.71%, which is equivalent to TRH-PLGA NPs (Table I). So it can safely interpreted that surface modification does not hamper the encapsulation ability of NPs formulation. Even drug loading in TRH-PLGA-Chitosan NPs is quite similar to TRH-PLGA NPs (Table I).

3.5. Surface Charge Analysis of Synthesised Nanoparticles

The charge on the surface of PLGA, PLGA-TRH and chitosan-TRH-PLGA NPs was analyzed by determining their zeta potential values. The results indicated that PLGA NPs as well as TRH-PLGA NPs demonstrated negative zeta potential of −23.5 mV and −10.3 mV respectively, while chitosan capped TRH-PLGA NPs exhibited positive surface charge (+19.9 mV) (Table I). The amino groups present on chitosan are responsible for the inversion of charge. Thus, the zeta potential values confirm the association of positively charged chitosan to the surface of negatively charged PLGA NPs.

3.6. Characterization of Synthesised Nanoparticles Using FTIR

To confirm the existence of chitosan on PLGA NPs and TRH in PLGA-chitosan nanoparticles, FTIR measurements of the NPs were carried out (Fig. 6). A characteristic band at 3285 cm^{-1} is attributed to $-NH_2$ and –OH groups stretching vibration in the chitosan matrix and the band for amide at ∼1636 cm^{-1} is seen in the infrared spectrum of native chitosan (Fig. 6(A)). Moreover, characteristic peaks at ∼1454 cm^{-1} and ∼2883 cm^{-1} due to amine functionality from chitosan was also found in case of chitosan coated PLGA NPs suggesting a cationic modification of the PLGA NPs (Fig. 6(D)). The FTIR spectrum of PLGA (Fig. 6(B)) shows strong peak at 1746 cm^{-1} which corresponds to the stretching vibrations of repeated ester bonds, band at ∼2937 cm^{-1} due to C–H streching, 1456, 1380 cm^{-1} for –CH3, 1180, 1080, 882 (C–O stretch) cm^{-1} while the band on 3100–3600 cm^{-1} belongs to the OH group of the water.

The FTIR spectrum of TRH (Fig. 6(C)) shows broad band between 3200–3600 cm^{-1} due to N–H stretching, peak at 2966, 2923 cm^{-1} due C–H stretching, a prevalent peak at 2852 cm^{-1} due C=O of amide bonds, peaks at 1733, 1679 cm^{-1} due to C=O stretching, peaks between 1400 to 800 cm^{-1} due C–H bending and rocking.

By comparing the IR spectra for PLGA, TRH and TRH-PLGA-chitosan NPs, it is confirmed that the synthesized NPs are composed of PLGA and TRH. Besides, the presence of characteristic groups for chitosan confirms the coating of chitosan on the TRH-PLGA NPs.

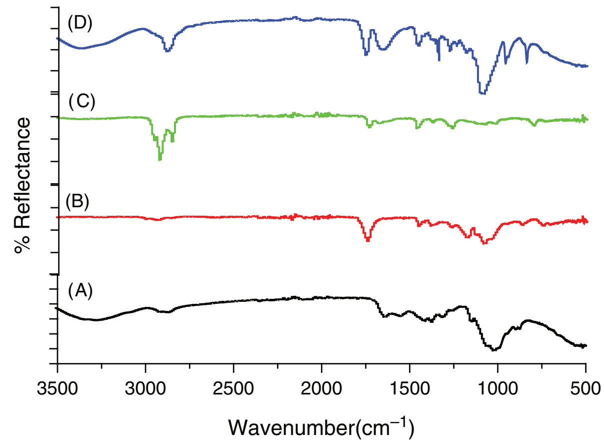


Figure 6. FTIR spectra of chitosan (A), PLGA (B), TRH (C) and TRH-PLGA-chitosan (D).

4. CONCLUSIONS

As the therapeutic efficacy of TRH is compromised by its instability due to proteolytic cleavage by the enzymes and hydrophilic nature, the incorporation of TRH into NPs might be a promising approach since colloidal formulations have been shown to protect similar drugs from the degrading. Polymeric NPs, particularly PLGA, have immense potential for application in therapeutics due to attractive properties as bio-compatibility and biodegradability. However, the polymeric micelles encounter a major limitation of relatively low loading capacity for water-soluble drugs; thereby necessitating the need of studies to be carried out to enhance the nanoparticle recovery and drug content for better efficiency and cost effectiveness. It is also quite well known that the formation parameters play an important role in deciding the size of particles formed and eventually their properties. Hence, we have tried to optimize conditions for maximal efficiency of encapsulation of TRH with the minimum particle size possible.

In this context, our emphasis has been on the synthesis of PLGA NPs using the nanoprecipitation technique. Various optimization studies were carried out which led to the inference that the particle size was minimum when

(i) acetonitrile was used as organic solvent,
(ii) 0.1% kolliphor solution was used as the stabilizer,
(iii) organic to aqueous phase ratio was 5:10 and
(iv) the rate of addition was taken as 0.5 mL/min.

Making use of these results, TRH was encapsulated into the PLGA leading to the formation of PLGA-TRH NPs. The aim of such an encapsulation is to allow effective delivery of TRH in biological systems as the peptidase enzymes degrade naked TRH. Further, surface modification studies were carried out via conjugation to the chitosan. This conjugation attributed to the reversal of the surface charge. PLGA, PLGA-TRH, chitosan-PLGA and chitosan-PLGA-TRH NPs were characterized and analyzed using DLS and TEM. The composition of nanoparticles was further confirmed by IR spectroscopy and particles have been shown to have good encapsulation efficiency as well as drug loading as per HPLC analysis. Finally, it can be safely concluded that the approach adopted for the encapsulation of TRH using chitosan-PLGA/PLGA NPs in the present study can be effectively used for delivery of such neuropeptides across blood brain barrier via intra-nasal route.

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